2009

Summer best-sellers…

Body fluids

Launch Binder

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Annexes competitors evaluations
Annexes competitors FDA files
Annexes flyers & brochures

Introduction

The Count Body Fluids mode (CBF) is available on the:

ABX Pentra DX 120 with **ABX Pentra ML**.

Clinical information

1. Punctures

Type of fluid		Clinic procedure
Cerebro Spinal Fluid		
Synovial fluid		
Serous	Peritoneal fluid	
	Pleural fluid	
	Pericardial fluid	

2. Therapists needs

- Presence or not of cells in the body fluids?
  neutrophils / lymphocytes or not?
  eosinophils (pleural liquid) or not?
  metastatic cells or not?
  RBC or not?

  } Cytology

- Protein total?
- Glucose?

  } Clinical chemistry

3. Interpretation

<table>
<tr><th>Characteristics</th><th colspan="3">Possible interpretation</th></tr>
<tr><td>Glucose normal
Protein normal</td><td colspan="3">Exudation: increased pressure: external cause, example cardiac problem</td></tr>
<tr><td>Glucose normal
Protein high</td><td colspan="3">Inflammation (if + Lymphocytes then Viral infectious suspicion?)</td></tr>
<tr><td rowspan="3">Glucose low
Protein high
Presence of cells</td><td rowspan="3">Infection</td><td>Neutrophils</td><td>Bacterial infectious suspicion?</td></tr>
<tr><td>Lymphocytes</td><td>Viral infectious suspicion?</td></tr>
<tr><td>Eosinophils (pleural)</td><td>Infectious, allergy, or presence of metastasis suspicion?</td></tr>
<tr><td>Metastasis cells</td><td colspan="3">Metastasis</td></tr>
<tr><td>RBC</td><td colspan="3">Local hemorrhage suspicion?</td></tr>
</table>

4. Normal values for the Cerebro Spinal Fluid

WBC

a) Adults = up to 5-10 WBC cells /uL
b) Newborns = up to 30 WBC cells /uL
c) Children (1 - 4 yrs) = up to 20 WBC cells /uL
Children (5 yrs - puberty) = up to 10 WBC cells

RBC

0 /L should be seen in normal CSF specimens, regardless of patient's age.

5. Manual procedure in the Cytology laboratories

- Count of the WBC and RBC
- Perform the differential = identify the cells

Manually, the counting is made on the microscope and the identification of the cells is made on a slide (cytospin in general) stained with MGG or other stain protocol.

Cells could be present in the biologic fluids:
All blood cells (neutro, eosino, baso, lympho, mono, blasts, atypical lympho, immatures cells…) but also other cells from tissues:

Name	Picture	Definition
Mesothelial cells		The mesothelium is a membrane that forms the lining of several body cavities: the pleura (thoracal cavity), peritoneum (abdominal cavity including the mesentery) and pericardium (heart sac).
Malignant or tumoral cells		
Macrophage cells		Macrophages are white blood cells within tissues, produced by the division of monocytes.
Epithelial cells		Epithelium is a tissue composed of cells that line the cavities and surfaces of structures throughout the body. In humans, epithelium is classified as a primary body tissue.

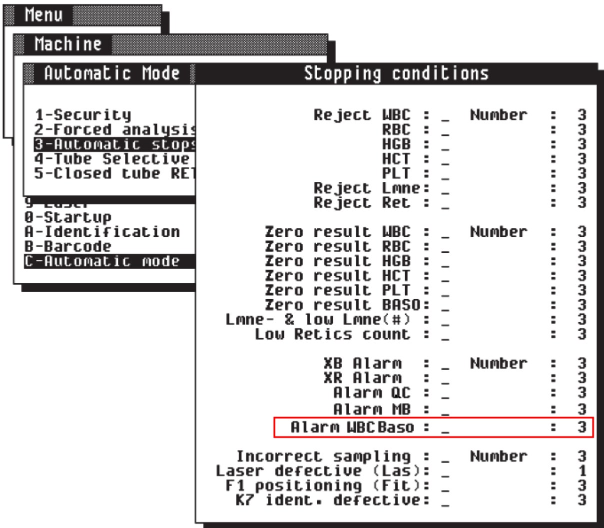
Count Body Fluids mode

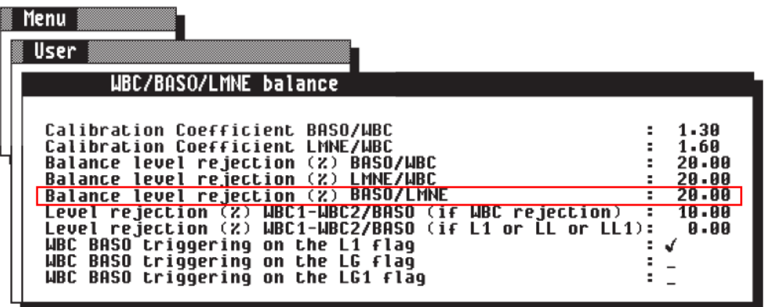
1. Software versions

The Body Fluids mode is separated in two parts: ABX Pentra DX 120 version 2.2.0 and ABX Pentra ML V7.

1.1 ABX Pentra DX 120 version 2.2.0

Technical note reference: RAN243D
Because two menus are differents in the V2.2.0, please follow the important recommendations:





Important recommendation:

The V2.2.0 ABX Pentra DX 120 / ABX Pentra DF 120 software version must be installed on instruments already equipped with V2.0.2 software version or above.

Before starting installation, you must print the following instrument settings. The values will be set by default during installation and will have to be restored after installation:

- User/ WBC/BASO/LMNE balance
- Machine/ Connection/ RS 232C
- Machine/ Connection/ Description Format/Numerical values
- Machine/ Automatic mode/ Forced analysis
- Machine/ Automatic mode/ Security
- Machine/ Automatic mode/ TSO Mode
- Machine/ Automatic mode/ Automatic stops

1.2 ABX Pentra ML V7

Important recommendation:

The algorithm contents the WBC and RBC's Pentra calibration coefficient.

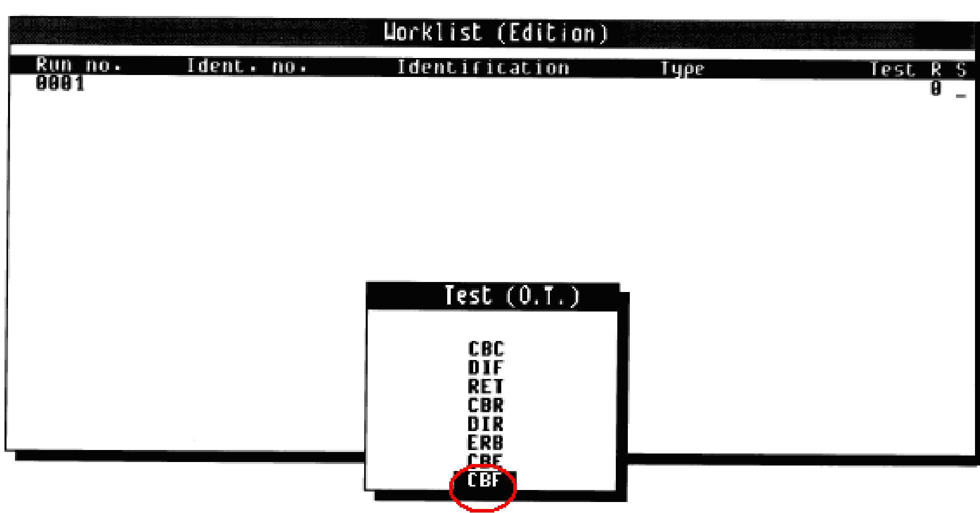
In the V7, the person in charge of the installation must introduce these coefficients in the algorithm rule.
For each new calibration, the modification must be updated directly on the algorithm.

In the future version of the ML, we plane to have a direct link for coefficient modifications by the user.

2. Specifications

2.1 Generalities

The Body fluid mode is presented as a new cycle: CBC, DIFF, RET, DIR... on the worklist.
The name of this cycle is **CBF = Count Body Fluids**



V2.1.0

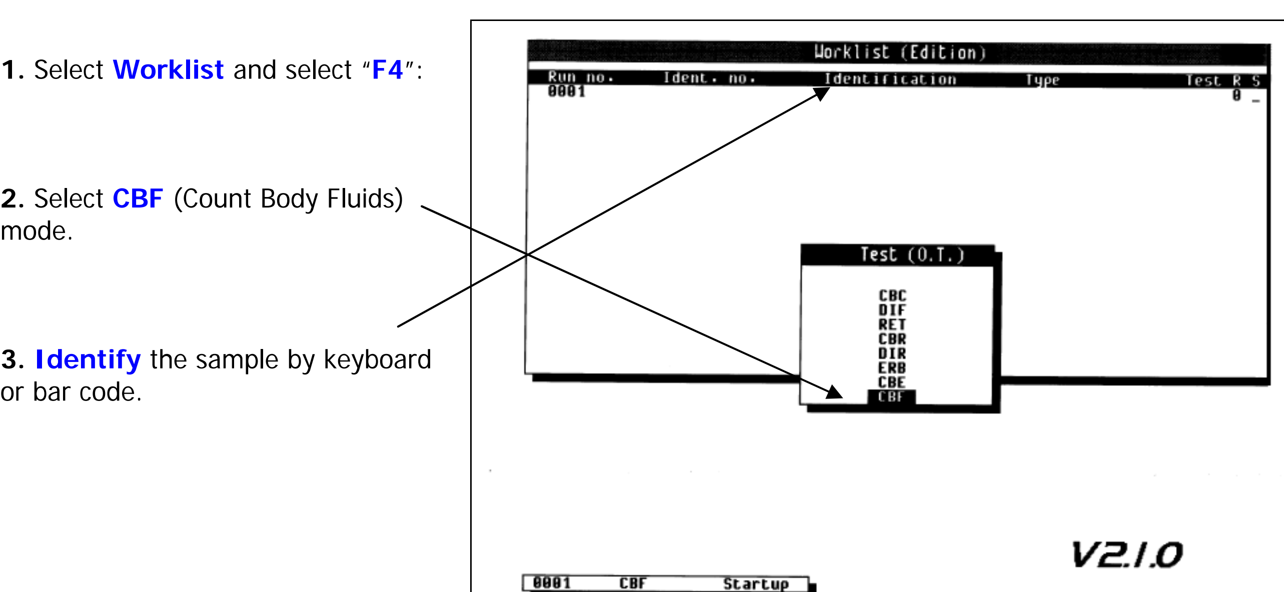
Content of the versions:

ABX Pentra DX 120:
- automatic blank cycle in DIR mode
- optical detection deselected
- aspiration during 4 seconds
- analyze in DIR mode

ABX Pentra ML:
- reception of the raw data
- calculation with the algorithm (LMNE for WBC, mononuclear, polynuclear and CBC/RET for RBC)
- display the results on the screen

Possible additional cycles order with a CBF:
- Init RET (initialization RET): if the open tube mode is DIFF
- Init DIR: if the open tube is CBC
- Autocontrol: if the first part of the CBF cycle is not correct (blank cycle)

2.2 Practice



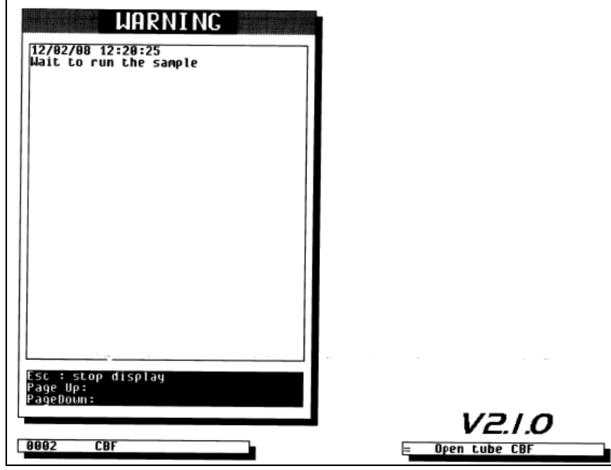
1. Select **Worklist** and select "**F4**":

2. Select **CBF** (Count Body Fluids) mode.

3. **Identify** the sample by keyboard or bar code.

4. Press **start** **without** presenting the tube and **wait**.

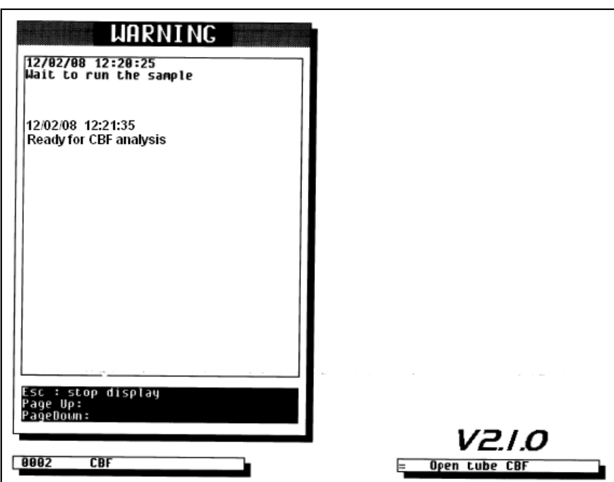
Message displayed:
"Wait to run the sample"



The led is red.

The analyzer performs a blank cycle.

Message displayed:
"Ready for CBF analysis"



5. Don't press the start key. Present the tube on the needle and wait.

You have 8 seconds to present the tube before the aspiration.

The aspiration starts when
- the led alternates red/green
- the sonorous detection rings

The aspiration stops when
- the led is red
- the volume of the sample is 640µl

The aspiration duration is 4 seconds

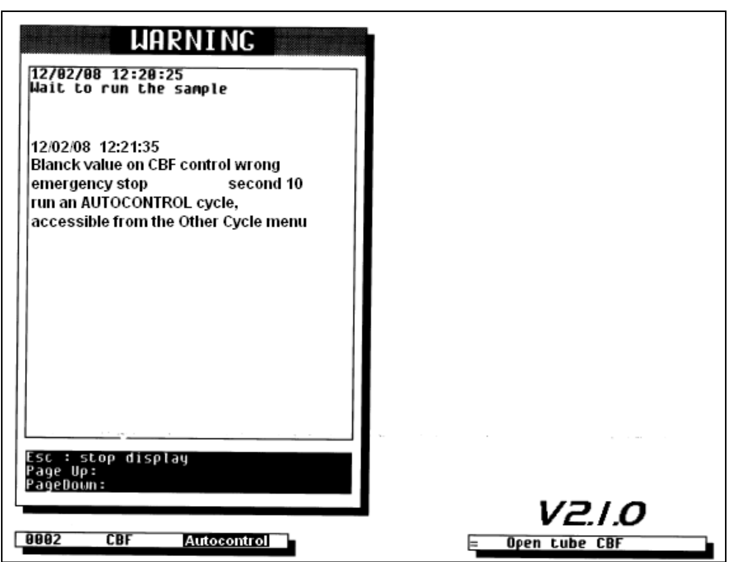
6. Wait for the results on the ABX Pentra ML station

Failures:

If the blank cycle is not correct, the system will stop automatically the analysis.
In this case, follow the procedure: perform an autocontrol cycle and repeat the CBF procedure.

Message displayed:
"BLANK VALUES ON CBF CONTROL WRONG

EMERGENCY STOP
Cycle second 10
run an AUTOCONTROL cycle,
accessible from the Other Cycles
menu"



2.3 Hardware (DX)

No modification.
The cycle DIR is using: CBC+DIFF+RET.

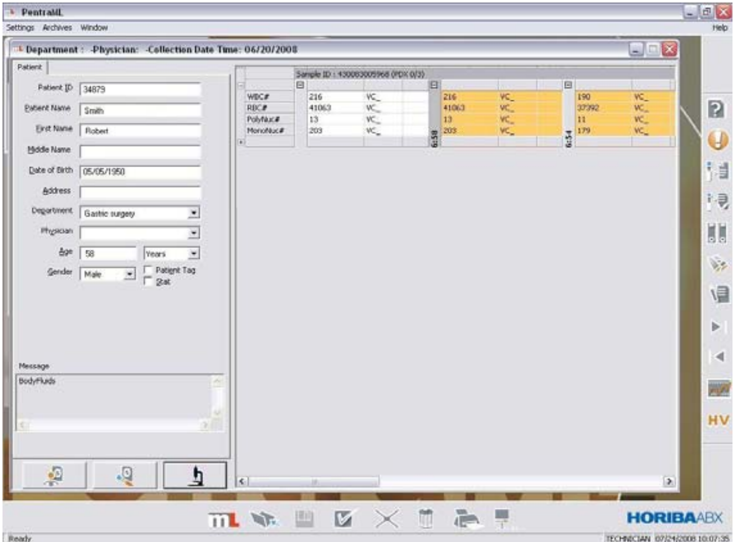
2.4 Software (ML)

Algorithm:

The WBC are counted on the LMNE channel:
WBC calculated from LYM + NEU + + MON + EOS + LIC populations
Mononuclear cells calculated from MON + LYM + LIC
Polynuclear cells calculated from NEU + EOS
The **RBC** are directly measured & counted on the RBC/RET channel.

Results:

The parameters are RUO (Research Use Only).



The result is the counting of the cells contained in the fluid:

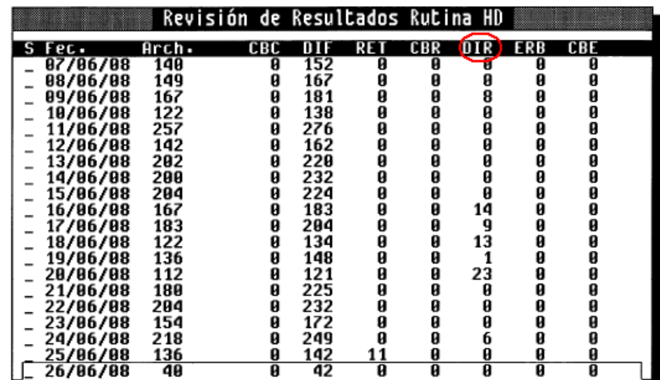
value of WBC in 10^6/L = WBC/mm^3
value of Polymorphonuclear cells in 10^6/L = PolyNuc/mm^3
value of Mononuclear in 10^6/L = MonoNuc/mm^3
value of RBC in 10^6/L = RBC/mm^3

If the result is lower than the linearity limit, the result is not displayed and the message "WBC < 25 cells" or "RBC < 10 000 cells" is presented on the message box.

The results are <u>only</u> available on the ML station, but the CBF cycles are saved in the Pentra memory.

It is absolutely forbidden to use the Pentra results!

You can use this memory only to transfer again a result from the Pentra to the ML.
On the ABX Pentra DX 120 memory menu, the CBF cycles are including in the DIR group:



Revisión de Resultados Rutina HD

S	Fec.	Arch.	CBC	DIF	RET	CBR	DIR	ERB	CBE
_	07/06/08	140	0	152	0	0	0	0	0
_	08/06/08	149	0	167	0	0	0	0	0
_	09/06/08	167	0	181	0	0	0	0	0
_	10/06/08	122	0	138	0	0	0	0	0
_	11/06/08	257	0	276	0	0	0	0	0
_	12/06/08	142	0	162	0	0	0	0	0
_	13/06/08	202	0	220	0	0	0	0	0
_	14/06/08	200	0	232	0	0	0	0	0
_	15/06/08	204	0	224	0	0	0	0	0
_	16/06/08	167	0	183	0	0	14	0	0
_	17/06/08	183	0	204	0	0	9	0	0
_	18/06/08	122	0	134	0	0	13	0	0
_	19/06/08	136	0	148	0	0	1	0	0
_	20/06/08	112	0	121	0	0	23	0	0
_	21/06/08	180	0	225	0	0	0	0	0
_	22/06/08	204	0	232	0	0	0	0	0
_	23/06/08	154	0	172	0	0	0	0	0
_	24/06/08	218	0	249	0	0	6	0	0
_	25/06/08	136	0	142	11	0	0	0	0
_	26/06/08	40	0	42	0	0	0	0	0

0002 DIF

V2.1.0

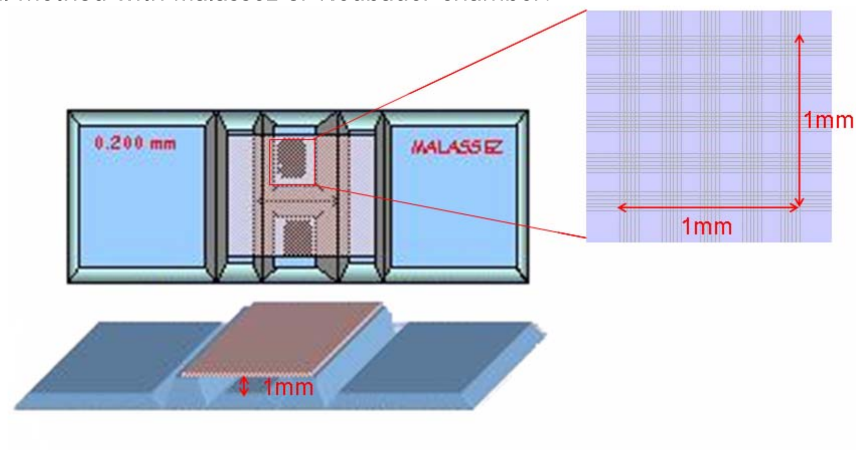
3. Performances

3.1 Units

- Unit conversion reminder:
  - 1000 mm^3 = 1 ml
  - 10^6 mm^3 = 1000 ml = 1 Liter
  - 10^6/L = 1/mm^3

1 /mm^3 = 1 /µl = 10^6 /L

- Manual method with Malassez or Neubauer chamber:



The number of cells counted on the microscope is given in the same unit as the ML.
Other chambers could be used: Fuchs-Rosenthal chamber.

3.2 Linearity

Parameter	Measure range	Tolerance
WBC	25 10^6/L - 150 000 10^6/L	25 - 100 : ± 50%
		100 - 250 : ± 40%
		> 250 : ± 20%
RBC	10 000 - 8 000 000 10^6/L	10 000 - 20 000 : ± 65%
		> 20 000 : ± 25%

3.3 Precision

Precision is given for 10 successive runs:

	Ranges (10^6/L)	Limit CV
WBC	25 - 200	CV ≤ 20 %
	200 - 1 000	CV ≤ 14 %
	1 000 - 4 000	CV ≤ 5 %
	4 000 - 10 000	CV ≤ 2 %
RBC	10 000 - 100 000	CV ≤ 10 %
	100 000 - 300 000	CV ≤ 5 %
	> 300 000	CV ≤ 2.5 %

3.4 Correlation vs manual method

		N	r²	Slope	Intercept
WBC	**All samples**	**62**	**0.85**	**1.22**	**-75**
	CSF	5	0.99	1.29	-38
	Ascite	45	0.88	1.03	5.2
	Pleural	10	0.97	1.84	611
Mononuclear %	CSF	5	0.51	0.47	
	Ascite + Pleural	45	0.78	0.93	3.52
Mononuclear #	CSF	5	0.99	0.55	0.48
	Ascite + Pleural	45	0.30	0.98	170
Polymorphonuclear %	CSF	5	0.51	0.47	50
	Ascite + Pleural	45	0.80	0.94	3.6
Polymorphonuclear #	CSF	5	0.98	1.82	-34
	Ascite + Pleural	45	0.86	1.28	188
RBC	**Ascite + pleural**	**18**	**0.83**	**0.86**	**8380**
	CSF	8	0.23	0.79	19701
	Ascite	14	0.56	0.73	7851
	Pleural	4	0.83	0.86	20790

4. Reagent consumption

Reagent consumption is given in ml. It has been calculated from an average on 100 cycles for the piercing and the manual modes.

Consumption (ml per cycle)	Diluent	Lyse	Leucodiff	Basolyse	Cleaner	Fluocyte
STARTUP (1 cycle DIR)	200.00	3.10	4.20	44.00	7.00	6.00
AUTOCONTROL	41.00	0.00	1.05	8.00	2.00	0.00
RET priming	14.00	0.00	0.00	0.00	0.00	4.00
DIR priming	14.00	0.60	2.10	6.00	0.20	4.00
Open tube DIR	46.00	0.60	1.05	7.00	2.20	2.60
RET priming	14.00	0.00	0.00	0.00	0.00	4.00
DIR priming	14.00	0.60	2.10	6.00	0.20	4.00

The CBF cycle corresponds to 2 "open tube DIR" cycle:

Consumption (ml per cycle)	Diluent	Lyse	Leucodiff	Basolyse	Cleaner	Fluocyte
CBF	92.00	1.20	2.10	14.00	4.40	5.20

5. References

5.1 Reagents

Reagent	Reference
ABX Diluent 20L	0901020
ABX Lysebio 1L	0906012
ABX Leucodiff 1L	0206013
ABX Basolyse 5L	0204050
ABX Fluocyte 0.5L	0904011
ABX Cleaner 1L	0903010

5.2 Blood controls

If the same instrument is being used for Complete Blood Cell (CBC) counting in peripheral blood, a separate control for body fluid cell counting is not necessary.

Control	Reference
ABX Difftrol : Vial Low Level: (1 x 3ml vial)	2062011
ABX Difftrol : Vial Normal Level (1 x 3ml vial)	2062012
ABX Difftrol : Vial High Level (1 x 3ml vial)	2062013
ABX Minotrol Retic Level 1 (1 x 3ml vial)	2072001
ABX Minotrol Retic Level 2 (1 x 3ml vial)	2072002
ABX Minotrol Retic Level 3 (1 x 3ml vial)	2072003

6. Evaluation references

- Professor Kaj Blennow Klin.Neurokemi, SU/Mölndal, 431 80 MÖLNDAL, **Sweden**
  November 2007 to January 2008.

- Jose Maria JOU, Servei d'Hemostàsia i Hemoteràpia. Core Laboratory, Hospital Clínico Universitario de Barcelona, C/ Villarroel n°170, 08036 Barcelona, **Spain**
  March 2008 to June 2008.

- Professor J.F. Schved, Hematology Laboratory, Hôpital Universitaire St-Eloi, Montpellier, **France**
  June 2008 to July 2008.

The results of the evaluations are compiled in the performance file, will be available in the documentation addendum.

7. Arguments

Time saving:
Manual counting = 10 to 20 min, including the cell sedimentation
CBF cycle = 3min 30 sec, including blank cycle.

Money savings:
No new reagent, no new control.

Competition

1. Competitors comparison

1.1 List of all the competitors able to perform the Body fluids testing

SIEMENS medical	Sysmex	BECKMAN COULTER	Abbott A Promise for Life	CELLAVISION
	XT-1800		CD3200	
	XT-2000i		CD3500	
ADVIA 120	XE 2100	LH750/LH755/780	CD4000	
ADVIA 2120	XE5000	DXH 800	SAPPHIRE	
	UF1000i			DM96

1.2 Technical comparison

Analyzer	FDA	Parameters	RUO parameters	Volume	Cycle	Throughput	Technology
ADVIA 120	2002	WBC RBC		155 µl open mode		120 CBC-DIFF/h	
ADVIA 2120		WBC RBC PMN MN	NEU EOS MON LYM	175 µl open mode	4 min pretreatment to fix and spherize the cells	120 CBC-DIFF/h	3 optical measurements: high-angle scatter, low-angle scatter, absorbance
XT 2000i	2006	WBC RBC		85 µl open mode		80 CBC-DIFF/h	
XT 1800	2006	WBC RBC		85 µl open mode		80 CBC-DIFF/h	
XE2100	2004	WBC RBC		130 µl open mode		150 CBC-DIFF/h	
XE5000	2007	WBC-BF RBC-BF MN PMN	HF-BF(high fluorescence count) % # TC-BF (total nucleated cells) #	130 µl open mode		38 CBF/h	4DIFFscattergram & the RBC distribution
UF100		WBC RBC		800 µl			light scatter (small-angle and wide-angle scattering) + fluorescence + impedance
LH750/755/780	2006	WBC RBC TNC (total nucleated cells)		200 µl open mode		105 CBC-DIFF/h	
DxH 800		TNC RBC		165 µl		100 CBC-DIFF/h	Flow Cytomteric Digital Morphology (FCDM)
CD 3200		WBC RBC				71 CBC-DIFF/h	
CD 3500		WBC		150-200 µl		90 CBC-DIFF/h	multiangle polarization scatter (WOC) + impedance (RBC)
CD 4000		WBC RBC		100 µl	CBC+C (extended counting cycle for WBC = + 32sec)	120 CBC-DIFF/h	
SAPPHIRRE		WBC RBC		100 µl	CBC+C (extended counting cycle for WBC = + 32sec)	120 CBC-DIFF/h CD analysis ≈ 8 min = 7 test/h	
DM96		NEU LYM EOS Macrophages Other cells				Up to 25 slides/h *(100 WBCs + 10X, based on a 6 mm sample area)*	
PENTRA DX			WBC RBC PMN MN	640 µl	DIR	28 CBF/h blank cycle included	DHSS cytochemistry + impedance

1.3 Analytical comparison: linearity

Analyzer	LINEARITY low limit		LINEARITY low limit	
	Unit used by competitor		Comparison with Horiba units	
	WBC	RBC	WBC	RBC
XE5000	WBC > 0.050 10^3/µl	RBC > 0.01 10^6/µl	50 10^6/L	10 000 10^6/L
XE2000i	WBC > 0.050 10^3/µl	RBC > 0.01 10^6/µl	50 10^6/L	10 000 10^6/L
XT 2000i	WBC > 0.050 10^3/µl	RBC > 0.01 10^6/µl	50 10^6/L	10 000 10^6/L
XT 1800	WBC > 0.050 10^3/µl	RBC > 0.01 10^6/µl	50 10^6/L	10 000 10^6/L
LH750/755/780	0.2 × 10^9/L	0.01 × 10^{12}/L	200 10^6/L	10 000 10^6/L
CD3500		1 000 10^6/L (display)		
CD4000	2/µl			
SAPPHIRE	100 /µl	0.001 10^{12}/L	100 10^6/L	1 000 10^6/L
ADVIA 120	0.150 10^9/L		150 10^6/L	
ADVIA 2120	5 100 WBC/µl	2 100 RBC /µl		
	20 WBC /µl	1 500 RBC /µl	20 10^6/L	1 500 10^6/L
Pentra DX 120	25 10^6/L	10 000 10^6/L	25 10^6/L	10 000 10^6/L

data source:
- flyer/brochure
- specification official web site
- evaluations

Graphic representation

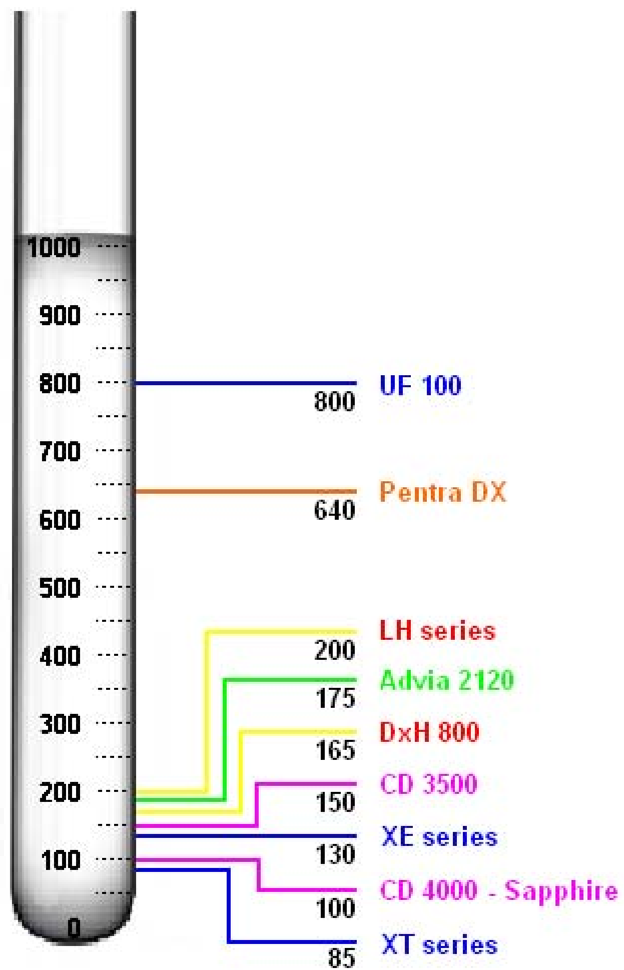
WBC low linearity (cell/mm³)

Number of cells	Instruments
0	
10	
20	Advia 2120
	Pentra DX
30	
40	
50	XE - XT series
60	
70	
80	
90	
100	Sapphire
110	
120	
130	
140	
150	
160	
170	
180	
190	
200	LH 750 / 755 / 780
210	

RBC low linearity (cell/mm³)

Number of cells	Instruments
0	
500	
1000	CDyn - Sapphire
1500	Advia 2120
2000	
2500	
3000	
3500	
4000	
4500	
5000	
5500	
6000	
6500	
7000	
7500	
8000	
8500	
9000	
9500	
10 000	LH series / XE - XT series / Pentra DX
10 500	

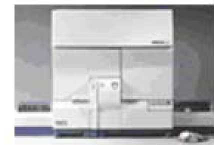
1.4 Sample volume comparison in µl



2. Competitors

SIEMENS
medical

Content



1. CSF from Siemens (Advia 2120)
2. Evaluation Advia 2120 (n°1)
3. Advia 120 FDA file
4. Evaluations Advia 120 (n°2-3)

1. CSF from Siemens (Advia 2120)

ADVIA 2120 CSF Assay
An Instant Hit
Another first, as Siemens Hematology is starting the FDA-clearance of an automated CSF assay on a routine analyzer.

Performance Specifications
Reportable Results

- Automated WBC on all samples
- Automated RBC on samples <1,500 RBC/µL
- Differential, PMN, and MN (absolute and %) on samples with >20 WBC/µL
- WBC Differential includes: Neutrophils, Lymphocytes, Monocytes, Eosinophils*

**For research use only.*

Linearity

Parameter	Range	Max. Deviation
WBC	0-50/µL	5 cells
	50-5,000/µL	10%
RBC	0-50/µL	5 cells
	50-1,500/µL	10%

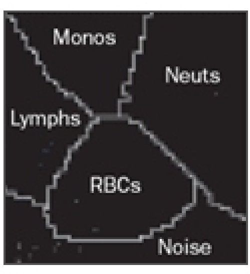
Within Run Precision and Accuracy

(Nominal 100 cells/µL; 150 samples, at least 50% abnormal)

Parameter	Stan. Dev./Bias	%CV/%Bias
WBC	15	15%
RBC	15	15%
#PMN	20	20%
#MN	20	20%

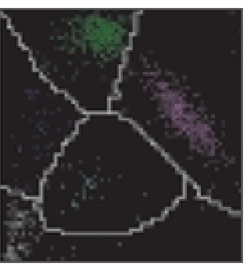
Sample Stability

The prepared sample is stable between 4 minutes and 4 hours when stored at 18°-30°C.



Normal

Viral Meningitis



Bacterial Meningitis

CSF Assay Kit Components

- **CSF Assay Reagent Kit**
  1 bottle of reagent
  - 25 aspirations
  - 1-year shelf life (unopened)
  - 30-day open bottle stability
  - store at room temperature, 18°-30°C

- **CSF Assay Control Kit**
  2 levels: Low and High
  - 1 vial of each level
  - 10 aspirations/vial
  - 105-day shelf life (unopened)
  - 10-day open vial stability
  - refrigerated storage, 2°-8°C

- **Summary of ADVIA 2120 CSF Assay:**
  - Reduces manual, labor-intensive procedure, optimizing efficiency, productivity, and resource management.
  - Improves turnaround time, providing critical results to clinicians for faster diagnosis and treatment.
  - Allows results to be available 24 hours per day, regardless of staffing constraints.
  - Increases consistency and confidence in results, minimizing technique variations between technologists.
  - Provides QC for previously uncontrolled method.
  - Expands platform for future development.

🕮 Complete file in **Competitor flyers & brochures** annex.

🕮 Flyer in French in **Competitor flyers & brochures** annex.

2. Evaluation Advia 2120

Evaluation n°1: Advia2120 vs Manual method

The ADVIA 2120 Hematology System: Flow Cytometry-Based Analysis of Blood and Body Fluids in the Routine Hematology Laboratory

Neil Harris, Jolanta Kunicka, Alexandre Kratz

University of Florida/Shands Hospital Core Laboratory, Gainesville, Florida; Bayer HealthCare LLC, Diagnostics Division,

Tarrytown, New York; Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA

- Complete article in **Competitor evaluations** annex.
- Article marketing points:

the importance of myeloperoxidase deficiency, a condition with an approximate prevalence in Caucasians of 1 in 2500 [52]. Severe myeloperoxidase deficiency is associated with the P0 pattern in the PANDA system. Although the condition is usually clinically benign, it is occasionally associated with an increased susceptibility to certain infections. An association between myeloperoxidase deficiency and a vulnerability to certain malignancies has also been proposed [53].

Activation Status of Neutrophils. The ADVIA 2120 Hematology System compares the location of the neutrophil cluster in the peroxidase cytogram to a standard cluster and uses this information to calculate the myeloperoxidase intracellular index (MPXI). This parameter can be used to diagnose partial and total myeloperoxidase deficiency [10]. The MPXI has also been shown to be a measure of systemic neutrophil activation. Several reports have shown its potential usefulness in determining neutrophil activation in patients with myocardial ischemia [54,55], in determining the therapeutic efficacy of recombinant granulocyte colony-stimulating factor [56], and in studying neutrophil degranulation patterns in lymphoma transplantation patients with bacteremia [57].

Cerebrospinal Fluid Analysis

The ADVIA 2120 CSF Assay is an automated method that uses direct cytometry to enumerate RBCs and WBCs and provide a WBC differential for cerebrospinal fluid (CSF) patient samples [58]. Before the CSF specimen is loaded on the analyzer, a 4-minute pretreatment with CSF reagent is required to fix and spherize the cells. The cells are differentiated and enumerated via 3 optical measurements: (1) high-angle scatter, (2) low-angle scatter, and (3) absorbance. The signals are digitized and used to construct the CSF cytogram (Figure 7). The automated differential parameters include the percentages of mononuclear cells, polymorphonuclear cells, neutrophils, lympho-

58 N. Harris et al

TABLE 5. Peroxidase and Nuclear Density Analysis (PANDA)*

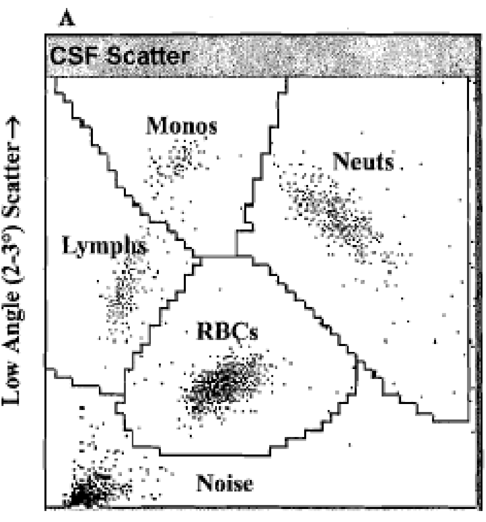
	P0	P1	P2	P3	P4	P5	P6
D0	CLL, PLL, HCL, ALL-L1, total MPO deficiency	Severe MPO deficiency	Partial MPO deficiency	CML	CML	AIDS, MDS	
D1	ALL (L1-L3), NHL, M0, M5a, M6, M7	M1, M5a, M2, M4	M2, M4, M5a, M5b, M1	M2, M4, CML-BC	M2, M3v, M4	M3v, atypical CML	M3
D2	Infectious mononucleosis, viral diseases						

*Adapted from [51] with permission from *Bloodline Reviews*. Copyright 2001, Carden Jennings Publishing Company, Ltd. CLL indicates chronic lymphocytic leukemia; PLL, prolymphocytic leukemia; HCL, hairy cell leukemia; ALL, acute lymphoblastic leukemia; MPO, myeloperoxidase; CML, chronic myelocytic leukemia; AIDS, acquired immunodeficiency syndrome; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; CML-BC, CML in blast crisis.

cytes, and monocytes. The cell counting is accurate up to 5100 WBCs/µL and 2100 RBCs/µL. If the RBC count exceeds 1500/µL, a specimen dilution to a maximum of 1:10 is recommended.

Studies by Aune et al and others have demonstrated excellent correlation between results with the standard manual CSF analysis and those with the automated method [58,59]. The slope and intercept were 0.87 and 8, respectively, for WBCs and 0.93 and 5 for RBCs. Precision was also very good. For low WBC counts (mean, 9/µL) and low RBC counts (mean, 22/µL), the between-run precision values were 20% and 19.5%. For high counts (WBC mean, 88/µL; RBC mean, 203/µL), the between-run precision values were 9.3% and 10.1%. Carryover was minimal. Hypochromic and microcytic specimens require a longer pretreatment than normochromic, normocytic CSF specimens.

The advantages of the automated CSF analysis over manual methods include a more reliable analysis, because more cells are counted than with the traditional chamber method,



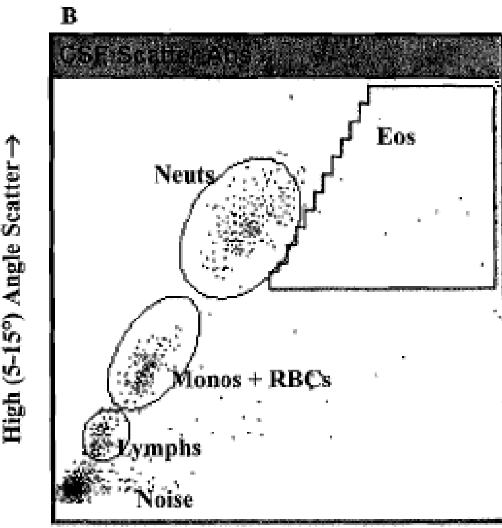


FIGURE 7. ADVIA 2120 CSF Assay cytograms. Three optical signals are measured for cerebrospinal fluid (CSF) analysis. Two signals represent low-angle (2°-3°) and high-angle (5°-15°) scatter signals. The third signal is an absorption measurement. After conversion to digital form, scatter and absorption signals are paired to generate CSF cytograms. High-angle scatter signals are paired with low-angle signals to form the CSF scatter/scatter cytogram (A), and low-angle signals are paired with absorption signals to form the CSF scatter/absorption cytogram (B). Both CSF cytograms have a resolution of 100 × 100 channels. Monos indicates monocytes; lymphs, lymphocytes; neuts, neutrophils; RBCs, red blood cells; Eos, eosinophils.

3. Advia 120 FDA file

📖 Complete file in **Competitor FDA** annex.

Bayer Receives FDA Clearance for the First Automated Test For Cerebrospinal Fluid Test for Quantifying and Differentiating Blood Cells Automated on the Bayer ADVIA® 120 Hematology System

November 19, 2002

TARRYTOWN, NY - November 19,2002: Bayer Diagnostics announced today the U.S. Food and Drug Administration (FDA) clearance for its Cerebrospinal Fluid (CSF) assay automated on the ADVIA 120 hematology system. This is first FDA cleared automated assay for spinal fluid analysis on a routine hematology analyzer. CSF testing provides results that may aid physicians in evaluating a variety of conditions including meningitis, encephalitis, neurological disorders, cerebral hemorrhage and some types of leukemia.

"Until now, CSF testing has been subject to manual variability and has been one of our most labor intensive tests," said Timothy Flaming, supervisor at St. Mary's Medical Center, Long Beach, CA. "In

addition to providing more precise test results, the availability of this automated test will allow us to deliver more timely results to physicians making critical therapy and disease management decisions."

Conventional hematological CSF analysis requires laboratories to use manual methods that are time and labor intensive, requiring 30-45 minutes to complete, and can be subject to technician variability. With the CSF test automated on the ADVIA 120 system, results are available in less than 5 minutes and eliminate hands-on sample management reducing the potential for operator error.

The FDA cleared assay provides an in vitro diagnostic, quantitative determination of blood cells in CSF specimens. In addition, the ADVIA 120 cerebrospinal fluid (CSF) method provides leukocyte (WBC) and erythrocyte (RBC) counts along with both absolute and proportional counts for the WBC differential.

"Bayer Diagnostics is committed to providing technology that enhances value to the laboratory and contributes to patient care," said Hans Hiller, senior vice president, Bayer Diagnostics' Laboratory Testing Segment. "The FDA clearance is good news for laboratories and physicians. Now laboratories can automate this important diagnostic test and provide physicians greater confidence in their results, leading to more timely disease management decisions."

4. Evaluations of the Advia 120

Evaluation n°2: Advia 120 vs Manual method

Automated Flow Cytometric Analysis of Blood Cells in Cerebrospinal Fluid: Analytic Performance

Marthe W. Aune,1 Joanne L. Becker, MD,2 Carlo Brugnara, MD,3 William Canfield, MA,4
David M. Dorfman, MD, PhD,5 W. Fiehn, Prof Dr,6 Gena Fischer, MT(ASCP),4
Patricia Fitzpatrick, MT(ASCP),5 Timothy H. Flaming, MT(ASCP),7 Hilde-Kristin Henriksen,8
Jolanta E. Kunicka, PhD,4 Karl J. Lackner, MD,9 Elaine Minchello, MT(ASCP),3
Patricia A. Mullenix, MT(ASCP)SH,10 Michael Myers,2 Annette Petersen,11 Wanda Ternstrom,12
Sandra J. Wilson, MT(ASCP)10

- Complete article in **Competitor evaluations** annex.
- Abstract :

Comment in:

- Am J Clin Pathol. 2005 Jan;123(1):154; author reply 154.

Automated flow cytometric analysis of blood cells in cerebrospinal fluid: analytic performance.

Aune MW, Becker JL, Brugnara C, Canfield W, Dorfman DM, Fiehn W, Fischer G, Fitzpatrick P, Flaming TH, Henriksen HK, Kunicka JE, Lackner KJ, Minchello E, Mullenix PA, Myers M, Petersen A, Ternstrom W, Wilson SJ.

St Olaf's University Hospital, Trondheim, Norway.

We compared the performance of an automated method for obtaining RBC and WBC counts and WBC differential counts in cerebrospinal fluid (CSF) samples with the reference manual method. Results from 325 samples from 10 worldwide clinical sites were used to demonstrate the accuracy, precision, and linearity of the method. Accuracy statistics for absolute cell counts showed a high correlation between methods, with correlation coefficients for all reportable absolute counts greater than 0.9. Linearity results demonstrated that the method provides accurate results throughout the reportable ranges, including clinical decision points for WBCs of 0 to 10/microL. Interassay precision and intra-assay precision for the ADVIA 120 (Bayer HealthCare, Tarrytown, NY) method were acceptable at all levels. The ADVIA 120 CSF Assay enumerates and differentiates cells via flow cytometry in a minimally diluted sample, improving the analysis of typically hypocellular CSF samples. Study results demonstrate that the automated ADVIA 120 CSF Assay is an acceptable alternative to the labor-intensive manual method.

Evaluation n°3: Advia 120 vs Manual method

Abstract marketing points:

1: Lab Hematol. 2003;9(4):214-24.

Use of the Advia 120 hematology analyzer in the differential cytologic analysis of biological fluids (cerebrospinal, peritoneal, pleural, pericardial, synovial, and others).

Aulesa C, Mainar I, Prieto M, Cobos N, Galimany R.

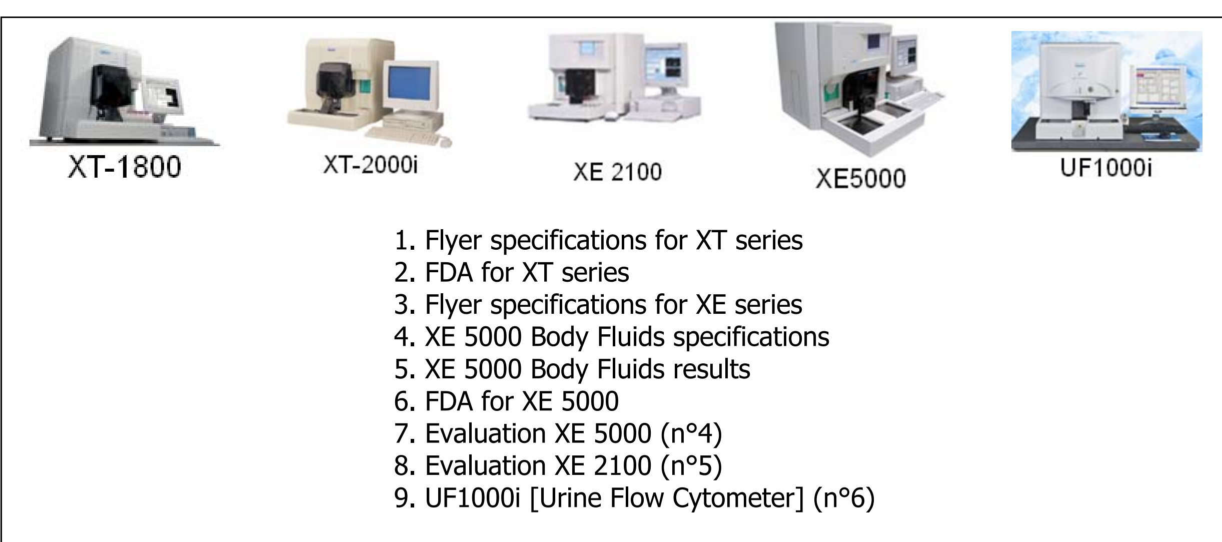
Unidad de Laboratorios, Ciudad Sanitaria Valle de Hebron, Barcelona, Spain. 27215cam@comb.es

The centralization of our laboratories and the demand for new parameters to measure have led to an increase in the number of biological fluid samples, which are generally sent for urgent analysis. Due to this they cannot be processed by manual methods. Meeting this increased demand for assistance is a challenge for the laboratory, and the challenge has been met by the automated hematology area. A study of the reliability of the Advia 120 hematology analyzer has been carried out through leukocyte and red blood cell counting of 179 biological fluids: cerebrospinal, peritoneal or ascitic, pleural, pericardial, synovial, and others. The automated leukocyte counts of cerebrospinal fluid samples containing up to 0.150 x 10(9) leukocytes/L are correlated with counts obtained with the manual reference method in a Neubauer counting chamber (r = 0.958; P = .0001). Applying Passing-Bablok regression analysis to these results indicates a slope p of 1.155 (95% confidence interval [CI], 0.915-1.347) and an ordinate intercept b of 0.0076 (95% CI, 0.012-0.034), showing the results to be perfectly interchangeable. In the comparison of the manual analysis of the leukocyte differential using the May-Grünwald-Giemsa staining method with the analysis using the automated method, the percentage of polymorphonuclear granulocytes of the Advia 120 basophil/lobularity method is significantly correlated (r = 0.844; P = .0001) with that obtained with the manual count. The results of Passing-Bablok regression analysis (p = 0.859 [95% CI, 0.58-1.190]; b = 8.8 [95% CI, -12.09-24.2]) indicate that these two counting methods are also perfectly interchangeable. Automated leukocyte and differential counts of peritoneal or ascitic fluids also show good correlations with the manual method, and the results are not statistically different. Pretreating synovial fluid samples with hyaluronidase enzyme allows their processing on the Advia 120; no significant differences were found between manual and automated methods with respect to leukocyte counts and differentials. Finally, results with pleural fluid samples indicated that leukocyte and differential counts obtained with the Advia 120 showed significant differences from results obtained with manual methods because of the high incidence of mesothelial, lymphoid, and other tumoral cells in this kind of fluid sample. This result shows that use of hematology analyzers is questionable for these kinds of samples, especially from oncology patients with tumors. A procedure is proposed for the processing of these pleural fluids.

PMID: 14649464 [PubMed - indexed for MEDLINE]

Sysmex

Content



1. Flyer specifications for XT series
2. FDA for XT series
3. Flyer specifications for XE series
4. XE 5000 Body Fluids specifications
5. XE 5000 Body Fluids results
6. FDA for XE 5000
7. Evaluation XE 5000 (n°4)
8. Evaluation XE 2100 (n°5)
9. UF1000i [Urine Flow Cytometer] (n°6)

1. Flyer specifications for XT series

🕮 Complete flyer in **Competitor flyers & brochures** annex.

Principles & Technologies	WBC: Fluorescent Flow Cytometry RBC: DC-sheath flow
Specimen Types	• Body Fluid Applications including CSF (cerebrospinal fluid), serous fluid, synovial fluid • Specimens collected in EDTA are acceptable
Precision	WBC: CV% <= 40% (WBC: 0.050-0.10 x 10^3/µL) RBC: CV% <= 40% (RBC: 0.01-0.20 x 10^6/µL)
Low Level of Linearity	WBC >=0.050 x 10^3/µL RBC >=0.01 x 10^6/µL
Carryover	WBC <=1% RBC <=1%
Sample Volumes	85 µL open mode
Quality Control (Total QC Management)	The same technology is used for both body fluid and complete blood cell (CBC) counting; therefore, a separate control is not necessary.

A logical step in blood cell analysis is the application of automated body fluid testing. The XT-Series analyzers with XT pro software now brings the power of fluorescent flow cytometry to body fluid analysis.

The XT-Series Body Fluid Application is a quantitative, automated procedure for analyzing cerebrospinal fluid, serous fluid and synovial fluid, providing WBC and RBC enumeration.

Increases Efficiency and Productivity

- Built on the robust fluorescent flow cytometry platform
- Reduces time consuming, labor intensive manual counts
- Accurate counts in the most clinically significant ranges

2. FDA for XT series

FDA Clears Body Fluids Analysis Application For Sysmex XT-Series
Main Category: Blood / Hematology
Also Included In: Medical Devices
Article Date: 26 Jul 2006 - 0:00 PST

Sysmex America, Inc. announced today that the Body Fluid application on the Sysmex XT-2000i(TM) and Sysmex XT-1800 (TM) Automated Hematology Analyzers has been cleared by the United States Food and Drug Administration (FDA) for the analysis of cerebrospinal fluid (CSF), serous and synovial fluids, said John Kershaw, President, Sysmex America, Inc. The XT-series analyzer is an instrument designed for mid volume laboratories. The Body Fluid application on the Sysmex(R) XE-Series Automated Hematology Analyzer, designed for high volume testing, was cleared by the FDA in 2004.

Body Fluid analysis consists of an automated white blood count (WBC) and red blood count (RBC). The WBC count is obtained on the XE and XT-Series analyzers from the Differential Channel, which uses Sysmex's patented Fluorescent Flow Cytometry technology to separate WBC's into distinct cell clusters. The RBC count is obtained using DC detection with Hydrodynamic focusing. No specimen preparation is needed before sample analysis.

3. Flyer specifications for XE series

🕮 Complete flyer in **Competitor flyers & brochures** annex.

Principles & Technologies	WBC: Fluorescent Flow Cytometry RBC: DC-sheath flow
Specimen Types	• Body Fluid Applications including CSF (cerebrospinal fluid), serous fluid, synovial fluid • Specimens collected in EDTA are acceptable
Precision	WBC: CV% <=30% (WBC: 0.050-0.10 x 10^3/µL) RBC: CV% <= 40% (RBC: 0.01-0.20 x 10^6/µL)
Low level of Linearity	WBC >=0.050 x 10^3/µL RBC >=0.01 x 10^6/µL
Carryover	WBC <=1% RBC <=1%
Sample Volumes	130 µL open mode
Quality Control (Total QC Management)	If the same instrument is being used for Complete Blood Cell (CBC) counting in peripheral blood, a separate control for body fluid cell counting is not necessary.

A logical step in body fluid analysis is the application of automated body fluid testing. The XE-Series analyzers with XE pro software now brings the power of fluorescent flow cytometry to body fluid analysis.
The XE-Series Body Fluid Application is a quantitative, automated procedure for analyzing cerebrospinal fluid, serous fluid and synovial fluid, providing WBC and RBC enumeration.

Increases Efficiency and Productivity

- Built on the robust fluorescent flow cytometry platform
- Reduces time consuming, labor intensive manual counts
- Accurate counts in the most clinically significant ranges

4. XE-5000 Body Fluids specifications

XE-5000

Body fluid mode specifications

Technologies Body fluid mode	■ fluorescence flow cytometry: WBC, DIFF ■ DC sheath flow method: RBC
Diagnostic parameters	WBC-BF, RBC-BF MN (%, #), PMN (%, #)
Histogram	RBC cell size distribution
Scattergram	WBC DIFF channel
Throughput	body fluid mode: 38 samples/h (max.), manual mode
Sample volume	130 µL (open manual mode)
Display data range	WBC-BF 0.000 – 999.999 x 10^9/L MN % 0.0 – 100% MN # 0.000 – 999.999 x 10^9/L PMN % 0.0 – 100 % PMN # 0.000 – 999.999 x 10^9/L RBC-BF 0.000 – 99.999 x 10^{12}/L
Reproducibility *	typical performance characteristics CV%: < 25% (WBC 10 – 15 cells/µL) CV%: < 20% (WBC 15 – 25 cells/µL) CV%: < 15% (WBC 25 – 50 cells/µL) CV%: < 10% (WBC > 50 cells/µL)
Carry over	WBC: ≤ 0,3% or less than or equal to 1 x 10^6/L RBC: ≤ 0,3% or less than or equal to 3 x 10^9/L
Data storage (IPU: information processing unit)	10,000 samples incl. graphics
Quality control	20 QC files, 300 data points, 51 parameter 1 XbarM file, 9 body fluid parameters IQAS ONLINE

Interfaces	serial (e.g. LIS, line printer) parallel (e.g. graphic printer) LAN (gigabit Ethernet – e.g. LIS, further SYSMEX components) USB (e.g. graphic printer, bar code reader, memory sticks) SUIT (SYSMEX UNIVERSAL INTERFACE) protocol for LIS connection
Dimensions/weights w x h x d [mm] / [kg]	main unit (MU): 706 x 711 x 535 / 81 MU incl. sampler: 706 x 711 x 912 / 93 pneumatic unit: 195 x 333 x 395 / 16 IPU: 338 x 101 x 379 / 8
Configurations	stand-alone or twin module of HST-N, XE-AMS or EXPERT LINE
Reportable research parameters (for laboratory use only)	high fluorescence count (HF-BF) (%, #) total nucleated cells (TC-BF) (#)

* *Typical performance characteristics for reproducibility were determined during evaluation on a typical XE-5000 instrument, using 14 body fluid samples which were counted 10 times consecutively in the body fluid mode and are based on the precision profile graph.*

Design and specifications may be subject to change due to further product development.

Fast analysis

- all body fluid parameters ready for review 1 min. after sample aspiration
- direct sampling of CSF and all other body fluids – no need for manual dilution and preincubation of the sample with specific reagents
- switching from whole blood mode to body fluid mode in less than 2 min.

Decisive results

- automatic rinse program to ensure a low WBC background count (0–1 cells/µL)
- newly designed count sequences with extended count volumes for increased precision in the very low cell concentration range
- a special algorithm to exclude mesothelial cells and macrophages from WBC and DIFF ← HF-BF cells ?

XE-technology made for body fluid analysis

- through fluorescence staining of RNA/DNA inside the white blood cells, effective separation from RBC
- XE-5000's body fluid channel shows no interference caused by hyaluronate from synovial fluid
- ascites, CAPD-, cerebrospinal-, pleural- and synovial fluid: all analysed in the same mode

Body fluid analysis – the economical and automated way

- cost per test is lower than that of a whole blood CBC+DIFF profile ← Because IMI channel is not used.
- SYSMEX e-CHECK (XE) control blood surveys all parameters of XE-5000's body fluid mode and whole blood mode at the same time
- ready-to-use analyser by robust mechanical and optical components requiring only a minimal daily operator's maintenance of 20 sec for aspiration of the cleaning solution

📖 Complete XE5000 brochure in **Competitor flyers & brochures** annex.

5. XE-5000 Body Fluids Results

FDA file extract:

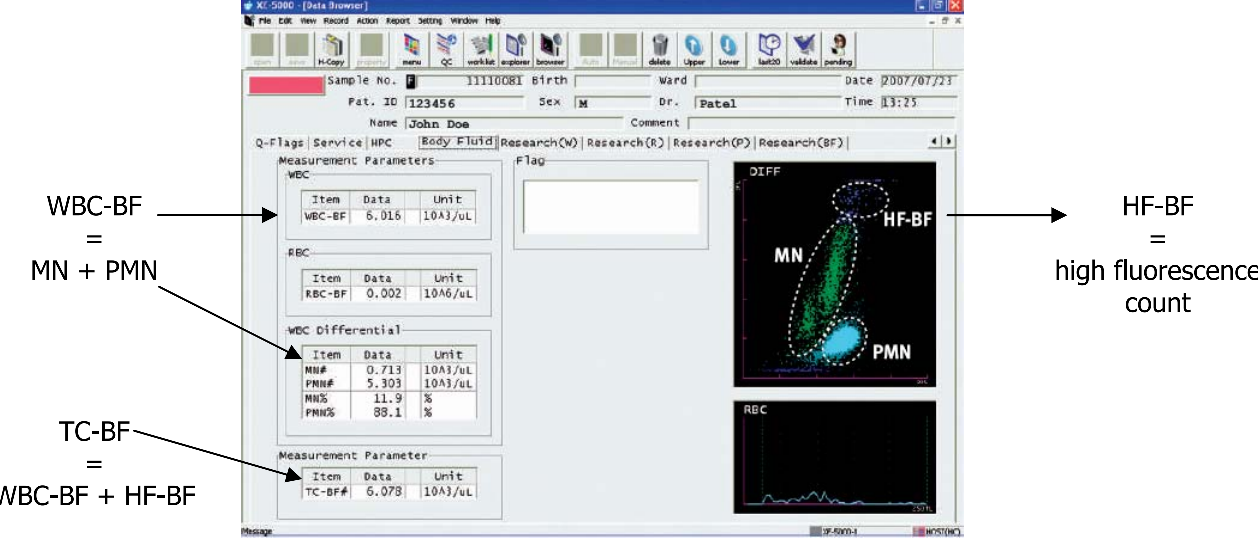
> The body fluid analysis mode of the XE-5000 uses the 4DIFF scattergram & the RBC distribution obtained from a specialized analysis sequence to calculate & display the WBC (WBC-BF) counts, mononuclear cell (MN) / polymorphonuclear cell (PMN) counts & percentages, TC-BF (Total Count) & RBC (RBC-BF) counts found in the body fluid.

XE-5000 Body Fluid Mode:

The XE-5000 analyzer includes a body fluid specific mode. This provides a reportable WBC, RBC, differential (polymorphonuclear and mononuclear) and a total count (TC-BF) for all common body fluid samples (CSF, synovial and serous).

The analyzer applies proven fluorescent flow cytometry technology and performs an automatic background check to produce accurate counts from a single sample analysis. No sample pre-treatment, reagents or additional quality control material are needed. WBC and RBC counts are reportable to 3-decimal places, providing expanded sensitivity and linearity.

The XE-5000 body fluid count and differential improve productivity and result turnaround time (TAT) and decreases manual technical intervention.



WBC-BF = WBC – body fluids
MN = mononuclear
PMN = polymorphonuclear
TC-BF = total count – body fluid
HF-BF = high fluorescence count – body fluid

6. FDA for XE-5000

Sysmex America Receives FDA Clearance for XE-5000 Hematology Analyzer

2007/12/11
Media Contact: Tammy Kutz, Manager, Media & Events
Sysmex America, Inc.
kutzt@sysmex.com

Mundelein, Illinois, December 11, 2007 - Sysmex America, Inc. today announced that its Sysmex XE-5000 Automated Hematology Analyzer has been cleared by the United States Food and Drug Administration (FDA) for sale in the U.S. The XE-5000 represents an enhanced, best-in-class, high throughput analyzer that provides 31 whole blood reportable parameters including advanced clinical parameters and a body fluid specific mode. The Sysmex XE-5000 will be available in the United States, in Latin America and in Canada beginning in December 2007.
Like its other X-Series counterparts, the XE-5000 uses Sysmex's patented fluorescent flow cytometry technology, providing both routine hematology testing and abnormal cell flagging within a single sample run. The body fluid specific mode is the first of its kind in the industry with reportable body fluid differential

parameters for all common body fluids (CSF, Serous and Synovial). Complete information within a single analysis reduces manual intervention and the errors inherent in manual counts. Hospital or university laboratories and clinics with renal, oncology and acute care patient populations will benefit from the XE-5000.

📖 510K in **Competitor FDA** annex.

FDA file extract:

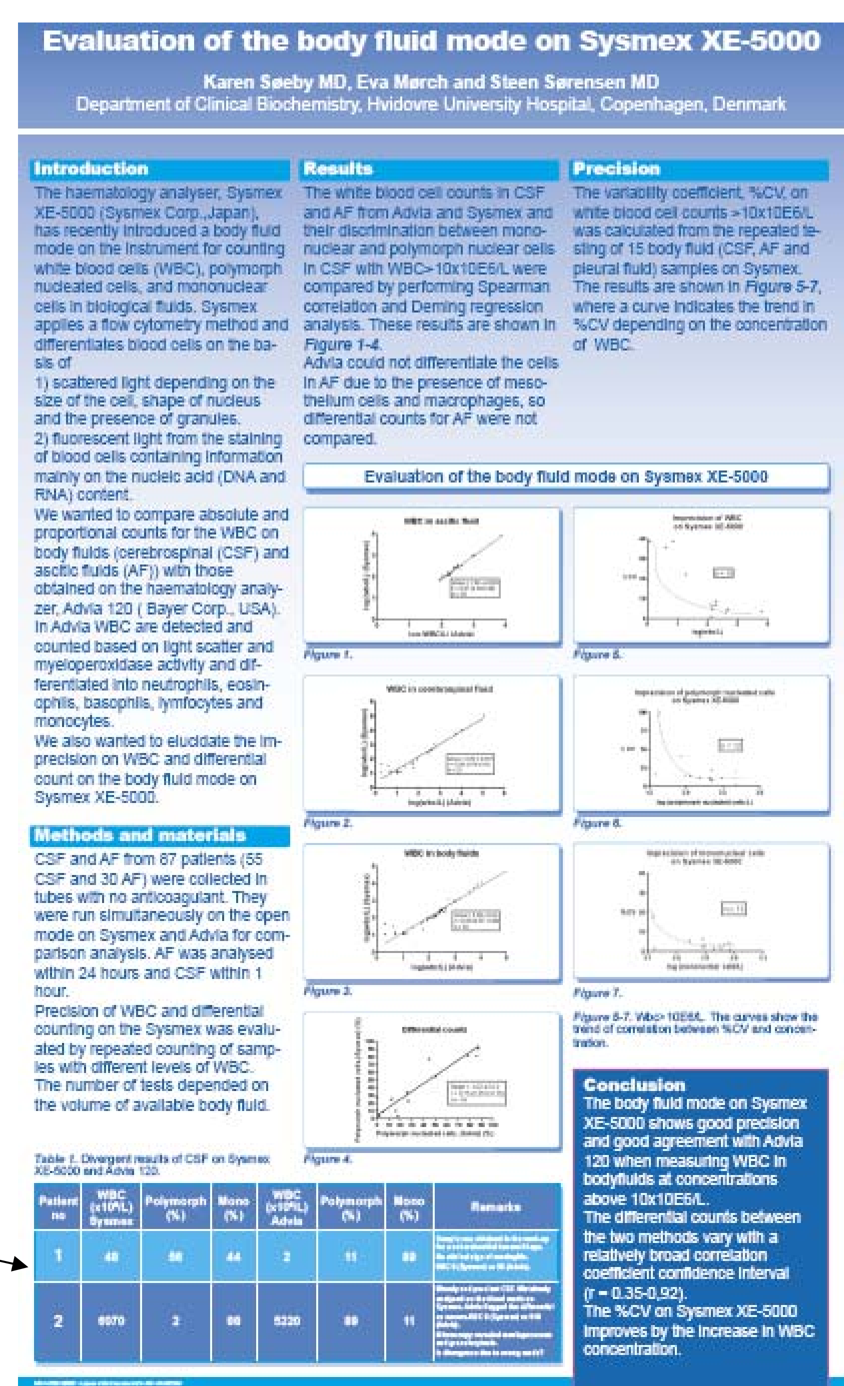
Table 1: Substantial Equivalence—Similarities and Difference to XE-2100

	Sysmex XE-2100 **Predicate**	**Sysmex XE-5000** **Modification of Predicate**	**Similarity/ Difference**
Intended Use	The Sysmex® XE-2100 Series Hematology Analyzer is a quantitative, automated hematology analyzer and leukocyte differential counter for *in vitro* diagnostic use in clinical laboratories. The body fluid application adds a quantitative, automated procedure for analyzing cerebrospinal fluid, serous fluid and synovial fluid. Body Fluid Parameters: WBC RBC	Sysmex® XE-5000 is an automated hematology analyzer for *in vitro* diagnostic use in screening patient populations found in clinical laboratories. The XE-5000 classifies and enumerates the same parameters as the XE-2100 using whole blood as described below, cord blood for HPC and has a body fluid mode for body fluids. The Body Fluid mode analyzes WBC-BF, RBC-BF, MN%/#, PMN%/# and TC-BF in body fluids (cerebrospinal fluids (CSF), serous fluids, and synovial fluids with EDTA, as needed). Body Fluid Parameters: WBC-BF RBC-BF MN%/ # PMN% / # TC-BF#	Both systems have the same intended use but the XE-5000 has additional capillary and body fluid parameters. 1) Body Fluid Mode has new differential parameters (MN%/# and PMN %/#) and detects WBC and RBC cells at a lower level than the XE-2100. The body fluid mode is used on body fluid samples with RBC counts greater than 0.003 x 10^6/ul, WBC counts greater than 0.01 x 10^3/ul for CSF and 0.030 x 10^3/ul for other body fluids and a WBC differential (MN%/# and PMN %/#) for samples with WBC counts. 2) Capillary mode on whole blood includes differential with NRBC#/% & IG#/%.

7. Evaluation XE-5000

Evaluation n°4: XE-5000 vs Advia 120

🕮 Poster in **Competitor evaluations** annex.



Evaluation of the body fluid mode on Sysmex XE-5000

Karen Søeby MD, Eva Mørch and Steen Sørensen MD
Department of Clinical Biochemistry, Hvidovre University Hospital, Copenhagen, Denmark

Introduction

The haematology analyser, Sysmex XE-5000 (Sysmex Corp.,Japan), has recently introduced a body fluid mode on the instrument for counting white blood cells (WBC), polymorph nucleated cells, and mononuclear cells in biological fluids. Sysmex applies a flow cytometry method and differentiates blood cells on the basis of
1) scattered light depending on the size of the cell, shape of nucleus and the presence of granules.
2) fluorescent light from the staining of blood cells containing information mainly on the nucleic acid (DNA and RNA) content.
We wanted to compare absolute and proportional counts for the WBC on body fluids (cerebrospinal (CSF) and ascitic fluids (AF)) with those obtained on the haematology analyzer, Advia 120 (Bayer Corp., USA).
In Advia WBC are detected and counted based on light scatter and myeloperoxidase activity and differentiated into neutrophils, eosinophils, basophils, lymfocytes and monocytes.
We also wanted to elucidate the imprecision on WBC and differential count on the body fluid mode on Sysmex XE-5000.

Methods and materials

CSF and AF from 87 patients (55 CSF and 30 AF) were collected in tubes with no anticoagulant. They were run simultaneously on the open mode on Sysmex and Advia for comparison analysis. AF was analysed within 24 hours and CSF within 1 hour.
Precision of WBC and differential counting on the Sysmex was evaluated by repeated counting of samples with different levels of WBC.
The number of tests depended on the volume of available body fluid.

Results

The white blood cell counts in CSF and AF from Advia and Sysmex and their discrimination between mononuclear and polymorph nuclear cells in CSF with WBC>10x10E6/L were compared by performing Spearman correlation and Deming regression analysis. These results are shown in Figure 1-4.
Advia could not differentiate the cells in AF due to the presence of mesothelium cells and macrophages, so differential counts for AF were not compared.

Precision

The variability coefficient, %CV, on white blood cell counts >10x10E6/L was calculated from the repeated testing of 15 body fluid (CSF, AF and pleural fluid) samples on Sysmex. The results are shown in Figure 5-7, where a curve indicates the trend in %CV depending on the concentration of WBC.

Evaluation of the body fluid mode on Sysmex XE-5000

Figure 1.

Figure 5.

Figure 2.

Figure 6.

Figure 3.

Figure 7.

Figure 4.

Figure 5-7. Wbc> 10E6/L. The curves show the trend of correlation between %CV and concentration.

Table 1. Divergent results of CSF on Sysmex XE-5000 and Advia 120.

Patient no	WBC ($x10^6/L$) Sysmex	Polymorph (%)	Mono (%)	WBC ($x10^6/L$) Advia	Polymorph (%)	Mono (%)	Remarks
1	48	56	44	2	11	89	[illegible]
2	6070	2	98	5220	89	11	[illegible]

Conclusion

The body fluid mode on Sysmex XE-5000 shows good precision and good agreement with Advia 120 when measuring WBC in bodyfluids at concentrations above 10x10E6/L.
The differential counts between the two methods vary with a relatively broad correlation coefficient confidence interval (r = 0.35-0,92).
The %CV on Sysmex XE-5000 improves by the increase in WBC concentration.

Two bad results:

1 false positive (meningitis)
1 false identification of the pathology (viral meningitis instead of bacterial meningitis)

8. Evaluation XE-2100

Evaluation n°5: XE-2100 vs Manual method

- Complete article in **Competitor evaluations** annex.
- Abstract:

1: Lab Hematol. 2005;11(1):24-30.

Performance evaluation of the application of body fluids on the Sysmex XE-2100 series automated hematology analyzer.

Kresie L, Benavides D, Bollinger P, Walters J, Pierson D, Richmond T, Issa-Dyer K, Fahs M.

Baylor University Medical Center, Dallas, Texas 75246, USA.

Body fluid analysis on the Sysmex XE-2100 series automated hematology analyzer was evaluated at 4 hospitals (Baylor University Medical Center, Dallas, TX; St. John's Mercy, St. Louis, MO; Carle Clinic, Urbana, IL; and ACL Laboratories, West Allis, WI, USA). The total nucleated cell and red blood cell (RBC) counts of 493 samples were obtained with the Sysmex XE-2100 automated hematology analyzer and compared with results obtained by manual chamber counting. Seventy-eight samples were not suitable for evaluation because of the presence of clots, crystals, error messages related to white blood cell (WBC) and RBC parameters, and so on. Pearson correlation coefficients for the WBC parameter were 0.99 for cerebrospinal fluid, 0.95 for serous fluid, 0.99 for synovial fluid, and 0.99 for samples of combined body fluids. Ninety-six samples were used to compare RBC counting methods because these samples had RBC counts greater than 0.01 10 6/mL. The Pearson correlation coefficients for the RBC parameter were 0.96 for cerebrospinal fluid, 0.97 for serous fluid, 0.97 for synovial fluid, and 0.97 for samples of combined body fluids. Carryover, precision, and linearity studies also performed for WBC and RBC counts yielded very good results.

PMID: 15790549 [PubMed - indexed for MEDLINE]

9. UF100 & UF1000i

- UF1000i flyer in **Competitor flyers & brochures** annex.
- UF series brochure in **Competitor flyers & brochures** annex.

Sysmex introduced the world's first fully automated urine cell analyzer in 1995, transforming urinalysis, a typically time-consuming and labour-intensive procedure.

In 2003, the company launched its simple-to-use UF series analyzers to perform routine urine formed element analysis.

Our advanced automated analyzers use laser-based fluorescent flow cytometry for results you can trust. And they operate unattended, freeing laboratory technologists to concentrate on more complex tasks.

Sysmex Urinalysis. Overview of the technology used in the Sysmex UF product line.

UF-1000i: A fully automated urine particle analyzer for the clinical lab.
The UF-1000i offers accurate and precise particle counts on uncentrifuged urine samples.
The UF-1000i enhances work flow efficiency in the clinical laboratory by offering true walk away capability, freeing the technologist from many of the labour-intensive steps of manual urine microscopy.
With the UF-Series, laboratories can deliver rapid, standardized results to clinicians.

Evaluation n°6: UF100 vs microscope & clinical chemistry

Automated Flow Cytometric Analysis of Cerebrospinal Fluid

Jos T. Van Acker[1], Joris R. Delanghe[a,1], Michel R. Langlois[1], Youri E. Taes[1], Marc L. De Buyzere[1] and Alain G. Verstraete[1]

[1] Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium.
[a] Address correspondence to this author at: Laboratory of Clinical Chemistry, Ghent University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium. Fax 32-9-240-49-85; e-mail joris.delanghe@rug.ac.be

Background: Recently, the UF-100 (Sysmex Corporation) flow cytometer was developed to automate urinalysis. We evaluated the use of flow cytometry in the analysis of cerebrospinal fluid (CSF).

Methods: UF-100 data were correlated with microscopy and biochemical data for 256 CSF samples. Microbiological analysis was performed in 144 suspected cases of meningitis.

Results: Good agreement was obtained between UF-100 and microscopy data for erythrocytes (r = 0.919) and leukocytes (r = 0.886). In some cases, however, incorrect classification of lymphocytes by the UF-100 led to underestimation of the leukocyte count. UF-100 bacterial count positively correlated (P <0.001) with UF-100 leukocyte count (r = 0.666), CSF total protein (r = 0.754), and CSF lactate concentrations (r = 0.641), and negatively correlated with CSF glucose concentration (r = -0.405; P <0.001). UF-100 bacterial counts were unreliable in hemorrhagic samples and in samples collected by ventricular drainage where interference by blood platelets and cell debris was observed. Another major problem was the UF-100 "bacterial" background signal in sterile CSF samples. *Cryptococcus neoformans* yeast cells and cholesterol crystals in craniopharyngioma were detected by the flow cytometer.

Conclusions: Flow cytometry of CSF with the UF-100 offers a rapid and reliable leukocytes and erythrocyte count. Additional settings offered by the instrument may be useful in the diagnosis of neurological disorders.

Evaluation n°10

Reference: Abbott CD3500 vs UF100, page 52.

UF100 result:

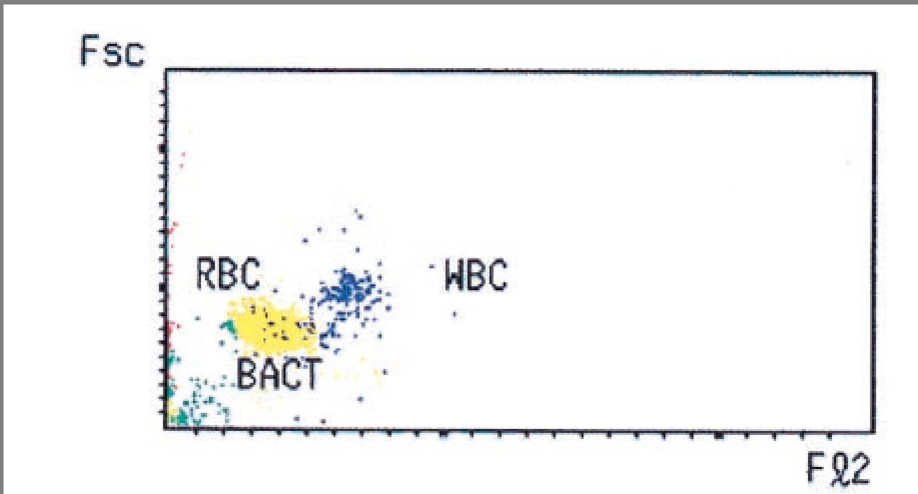


Fig. 4. UF-100 scattergram for a CSF cell sample with atypical lymphocyte characteristic.

Fsc, forward scattered light intensity; *Fl2*, fluorescent light intensity; *RBC*, erythrocytes (*red*); *WBC*, leukocytes (*blue*); *Bact*, bacteria (*green*). The cells in the *yellow clusters* are not allocable to any of the population defined in the UF scattergram.

Content

1. Body Fluids from Beckman Coulter
2. Recall for the Body Fluids
3. FDA for LH750 and equivalences
4. Evaluation of LH750 (n°7)

1. Body Fluids from Beckman Coulter

COULTER® LH 750 Body Fluids Application

Body Fluids Analysis Made Simple — and Automated

Simplify and automate your lab's body fluid analysis with the LH 750 Body Fluid Application. Available on all LH 750 hematology analyzers, this application allows you to efficiently and accurately test three categories of body fluids: Cerebrospinal fluid, Serous fluids (pleural, peritoneal, pericardial), and Synovial fluids that have been treated with Hyaluronidase.

Since our methods are adapted from routine hematology processes, your lab will sidestep any learning curve and experience the benefits of automated body fluid analysis right away.

Reporting Made Easy

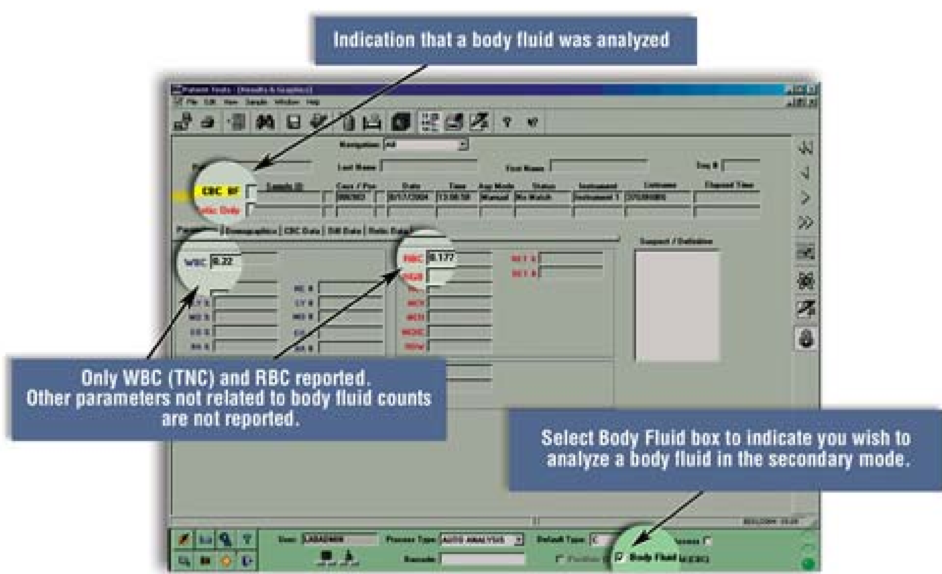
- Body fluids are reported in a format similar to whole blood.
- Results appear for Red Blood Cells (RBC) and White Blood Cells (WBC) / Total Nucleated Cells (TNC).
- All other parameters are suppressed to simplify reporting.

Automated Process Saves

- Technologists' Time, Lowers Cost.
- Maximize your resources by eliminating time-consuming manual processes.
- Lower costs by improving productivity.
- Use automation to decrease turnaround times for most body fluid samples.

Accuracy Climbs as Errors Decrease

- Calculations are done by the instrument and based on units used in complete blood counts.
- Standardized testing method evaluates and counts a larger number of cells within an established linearity range. This reduces the variability of low and high counts for WBC and RBC counts.
- Software displays only counts provided with appropriate flagging, and clearly indicates body fluid versus whole blood sample analysis.
- Fewer errors diluting the fluids, interpreting low counts and converting manual counts to reportable results.



Accuracy

TNC (WBC) $\geq$ 0.2 X 10^9/L									
Sample Type	Analyzed	Method	Mean	Min	Max	R^2	Slope	Intercept	P Value
Serous	82	Manual	3.87	0.20	34.10				
		LH750	3.37	0.25	27.19	0.895	0.768	0.395	0.88
Synovial	47	Manual	9.10	0,25	47.25				
		LH750	8.31	0.32	38.14	0.836	0.741	1.568	0.94
CSF	23	Manual	1.17	0.20	7.56				
		LH750	1.14	0.23	6.77	0.993	0.890	0.103	0.84

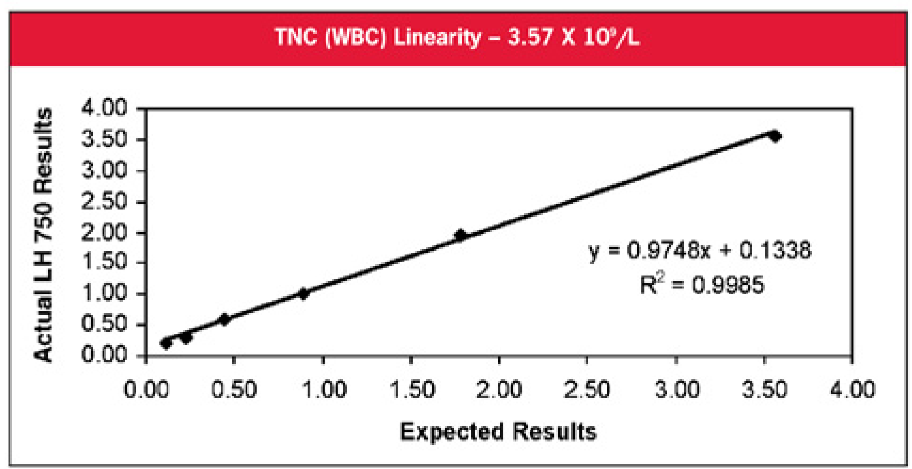
Carryover

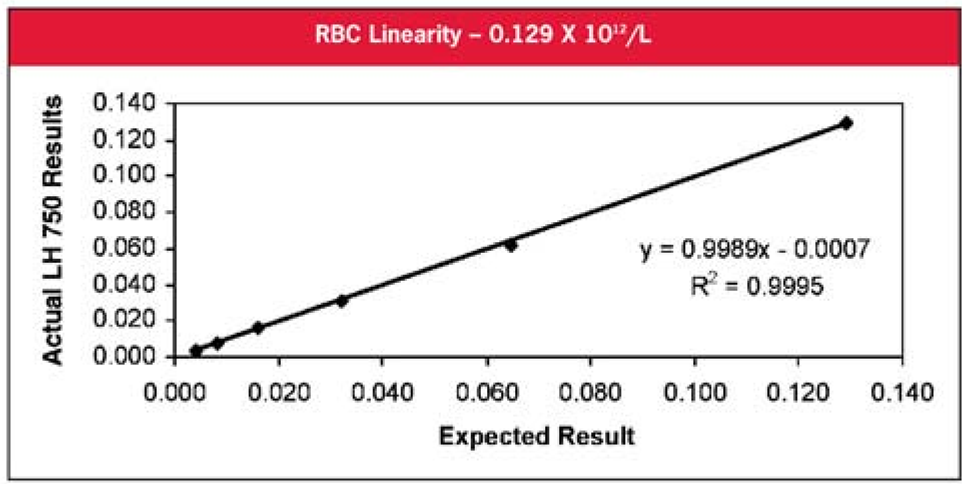
	High H3	Low L1	Low L3	Carryover
WBC	4.97	0.28	0.25	0.64%
RBC	1.803	0.005	0.000	0.28%

Precision

PARAMETER	Mean	2SD	CV%	Minimum	Maximum
TNC X 10^9/L	0.41	0.04	4.44	0.39	0.45
	2.13	0.11	2.55	1.9	2.18
	14.1	0.10	0.39	13.99	14.15
RBC X 10^{12}/L	0.015	0.002	6.586	0.014	0.017
	0.307	0.005	0.891	0.303	0.312
	0.988	0.015	0.747	0.977	1.001

Linearity





📖 LH700 brochure in **Competitor flyers & brochures** annex.

📖 LH780 brochure in **Competitor flyers & brochures** annex.

2. Recall for the Body Fluids

Trade Name / *Marque de commerce*	**Coulter LH750 Series System**
Recall Posting Date / *Date d'affichage du retrait*	2006-10-30
Manufacturer / *Fabricant*	Beckman Coulter Inc.
Recall Start Date / *Date de début du retrait*	2006-09-20
Recall Number / *Numéro du retrait*	32492
Hazard Classification / *Classification du risque*	Type III
Model or Catalog # / *Numéro de modèle ou de catalogue*	N/A
Lot or Serial # / *Numéro de série ou de lot*	All lot numbers. / *Tous les numéros de lot.*
Reason for Recall / *Raison du retrait*	There is a potential for erroneous results for body fluids on the LH750 when a cassette label fails to read and the subsequent sample is cycled in body fluid mode. / *Des résultats erronés peuvent se produire lorsque des liquides organiques sont analysés par l'appareil LH 750 : l'étiquette d'une cassette n'effectue pas la lecture et l'échantillon suivant est analysé en mode liquide organique.*

3. FDA for LH750 and equivalences

- 510K in **Competitor FDA** annex.
- DxH800 equivalence 510K in **Competitor FDA** annex.
- 510K equivalence marketing points:

1. Predicate device name(s):
   - Coulter® LH 750 Hematology Analyzer
   - Coulter® LH 780 Hematology Analyzer
2. Predicate 510(k) number(s):
   - K011342
   - K061616
3. Comparison with predicate:

Similarities			
Item	Device UniCel® DxH 800	Predicate Coulter® LH 780	Predicate Coulter® LH 750
Intended Use	The UniCel® DxH 800 Analyzer is a quantitative, automated hematology analyzer for *in vitro* diagnostic use in screening patient populations found in clinical laboratories. The UniCel® DxH 800 Analyzer provides: - a CBC, Leukocyte 5 Part Diff, Retic, and NRBC on whole blood - a TNC and RBC on Body Fluids	The Coulter LH 780 Hematology Analyzer is a quantitative, automated hematology analyzer and leukocyte differential counter for *in vitro* diagnostic use in clinical laboratories. The Coulter LH 780 Hematology Analyzer provides automated Retic analysis and enumeration of NRBCs as well as an automated method for enumeration of RBCs and WBCs in body fluids	The Coulter LH 750 Hematology Analyzer is a quantitative, automated hematology analyzer and leukocyte differential counter for *in vitro* diagnostic use in clinical laboratories. The Coulter LH 750 Hematology Analyzer provides automated Retic analysis and enumeration of NRBCs as well as an automated method for enumeration of RBCs and WBCs in body fluids
Principle of Measurement	- WBC, RBC, MCV, PLT, and TNC: Aperture impedance - HGB: Spectrophotometric	Same as DxH 800	Same as DxH 800

4. Evaluations of LH750

Evaluation n°7: LH750 vs Manual method

- Complete article in **Competitor evaluations** annex
- Abstract marketing points:

1: Lab Hematol. 2003;9(3):155-9.

Comment in:
Lab Hematol. 2003;9(3):153-4.

Validation of body fluid analysis on the Coulter LH 750.

Brown W, Keeney M, Chin-Yee I, Johnson K, Lantis K, Finn W, Wolfe N, Kaplan S.

London Health Sciences Centre, Ontario, Canada.

The role of the hematology laboratory in the analysis of body fluid has been to provide accurate enumeration of red blood cells (RBCs), total nucleated cells (TNCs), and differentials by manual analysis. Three hospitals (London Health Sciences Centre, University of Pittsburgh Medical Center, and University of Michigan Health System) participated in the assessment of the performance of automated analysis of body fluid by the Beckman Coulter LH 750, an impedance-based hematology analyzer. We evaluated the accuracy of analysis results for both the TNCs and RBCs of 372 samples (158 serous fluid, 148 cerebrospinal fluid [CSF], 66 synovial fluid) run on the LH 750 compared to results obtained from manual chamber counting. Of the 372 samples, 152 were suitable for evaluation of accuracy of the automated TNC. The remaining 220 samples were either flagged for interfering substances or the reference results were < 0.2 x 10(9)/L, below the background limit of the analyzer. Correlation coefficients for serous fluid were 0.895, P = .88; for CSF, 0.993, P = .84; and for synovial fluid, 0.836, P = .94. Of the 372 samples, 106 had RBC counts greater than 0.01 x 10(12)/L and were used for method comparison. Correlation coefficients for serous fluid were 0.957, P = .66; for CSF, 0.849, P = .55; and for synovial fluid, 0.667, P = .81. Linearity and precision studies showed excellent agreement for both TNC and RBC parameters. Low-level sensitivity excluded the majority of cerebrospinal (119) and a small number of peritoneal dialysate fluid samples (8), which require accurate enumeration at clinical decision points between 0 to 100 cells/microliter. In the case of synovial and serous fluids, however, most clinicians are interested in TNC counts above 0.2 x 10(9)/L, and RBC counts are relevant only if they are significantly increased (> or = 0.05 x 10(12)/L). Adopting the criteria of reporting TNC counts as < 0.2 x 10(9)/L or accurate enumeration on counts > or = 0.2 x 10(9)/L, clinically relevant results could be provided by automated analysis in 93.8% of serous fluids and 85.8% of synovial fluids.

PMID: 14521323 [PubMed - indexed for MEDLINE]

Article marketing points:

linearity, with an R^2 of 0.9995 (slope, 0.9989; intercept, 0.0007) (Figures 1 and 2).

Precision

Six separate body fluid samples representing different TNC and RBC ranges were evaluated. Each fluid sample was run 11 consecutive times, and the results of the first run were universally discarded. Table 5 represents the corresponding coefficients of variation for each precision sample.

Carryover

Carryover was evaluated by processing 3 consecutive high body fluid counts (H1-H3) followed by 3 consecutive low body fluid counts (L1-L3). Carryover was calculated as [(L1-L3)]/[(H1-L3)] × 100%. TNC carryover was 0.64% and RBC carryover was 0.28%.

DISCUSSION

Manual analysis of body fluids remains a problematic area for most laboratories. This procedure requires a high level of expertise in cell identification and is labor-intensive, and results are poorly reproducible even with experienced observers [1]. New automated cell counters such as the Beckman Coulter LH 750 offer an alternative to the labor-intensive, imprecise manual methods of body fluid analysis. The LH 750, which offers technological advances in cellular analysis, particularly in the presence of interference, provides laboratorians with more accurate results as well as improved efficiency.

In this study, we compared the accuracy of the TNC and RBC counts in 372 body fluid samples run on the LH 750 to manual chamber counting. Of the 372 samples, only 106 had RBC counts greater than 0.01×10^{12}/L, and 142 had TNCs greater than 0.2×10^9/L measurable by automated analysis and suitable for method comparison. Low-level sensitivity (TNC $<0.2 \times 10^9$/L and RBC $<0.01 \times 10^{12}$/L) limits the utility of the LH 750 for analysis of many cerebrospinal and peritoneal dialysate fluids, which require accurate enumeration at clinical decision points between 0 and 0.1×10^9/L. Cellular CSF and peritoneal dialysate fluid samples with counts above the background limit of the analyzer, however, are excellent candidates for automated analysis. In the case of synovial and other serous fluids, most clinicians are interested in TNC counts above 0.2×10^9/L, whereas RBC counts are of limited significance [7-10]. If the criteria of reporting TNC counts as $<0.2 \times 10^9$/L or accurate enumeration on counts $\geq 0.2 \times 10^9$/L are adopted, clinically relevant results could be provided by automated analysis in 93.8% of serous fluid specimens and 85.8% of synovial fluid specimens. Over this range of values for TNC and RBC counts, the LH 750 demonstrated high correlation with manual counts, low variation coefficients, and high reproducibility. Thus, with the exception of clear CSF and peritoneal dialysate fluid specimens, the majority of samples can be analyzed on the LH 750, resulting in significant time and cost saving for most laboratories.

Evaluation n°8: LH750 vs Iris iQ200

Complete article in **Competitor evaluations** annex.

Article marketing points:

Coulter LH750

The specimens were analyzed using the manual aspiration mode. Before analysis, diluent was aspirated 3 times and the background count of the third aspiration was recorded. The CSF sample was then aspirated. Background counts were subtracted to determine the final TNC and RBC counts. Nucleated cells were counted using 2 decimal places and erythrocytes using 3 decimal places.

Glasser et al / CEREBROSPINAL FLUID CELL COUNTS

Our results for the LH750 showed poor reliability of automated nucleated cell counts less than 200/µL (200 × 10^6/L). In our laboratory, this would exclude about 94% of CSF specimens for analysis. This finding is similar to findings of other studies.[1-3] Barnes et al[3] reported a cutoff value of 300/µL, excluding more than 90% of the CSF samples and restricting the use of the LH750 to grossly bloody or cloudy fluids.

Results of the iQ200 automated digital imaging system were more encouraging. It is clear that differences between normal and abnormal results determine clinical decisions that, in some cases, would avoid medical misadventures. In the

Hematopathology / ORIGINAL ARTICLE

normal range, about 27% of the samples were misclassified. However, interpretation of results also differs among physicians. Whereas Fishman[5] considered values greater than 5/µL definitely abnormal, Merritt and Fremont-Smith[9] considered values of TNCs in the range of more than 5 to 10/µL (5-10 × 10^6/L) "suspicious." By this criterion, only 12% of the samples would be misclassified, a value that is also clinically unacceptable. It is less clear that misclassifications in other groups would alter clinical decisions. In only 1 sample was there a serious misclassification of the TNC count in the more than 50 to 200/µL group. Thus, 50 TNCs/µL (50 × 10^6/L) is our recommended lower limit of detection for the iQ200, a value similar to the lower limit of detection of TNCs of 30/µL (30 × 10^6/L) reported by Butch et al.[4]

There are conflicting data on the Advia 120 hematology analyzer (Bayer HealthCare, Tarrytown, NY). Aulesa et al[2] found the lower limit of detection for leukocytes to be 47/µL (47 × 10^6/L) and established their limit of reliability at 150/µL (150 × 10^6/L), which was equivalent to 3 SD. Aune et al[8] claim accurate results between leukocyte counts of 0 and 10/µL (0-10 × 10^6/L) in a study supported by Bayer Healthcare. It would be of interest to statistically evaluate data using the weighted κ statistic.

automation. As indicated previously, clinicians are held captive to the administrative and scientific decisions implemented by clinical laboratories. Our study demonstrates the importance of using measurements of reliability sensitive to clinical interpretation in addition to parametric analyses to evaluate the needs of clinicians and the impact on patient care. It also demonstrates the importance of evaluating demographic data to determine the actual cost savings of automation, if clinical reliability is to be maintained.

From the Departments of Pathology, [1]Rhode Island Hospital and [2]Brown University School of Medicine, Providence; and [3]Department of Biostatistics, Rhode Island Hospital and Lifespan.

Address reprint requests to Dr Glasser: Cbr, 6550 S Bay Colony Dr, Suite 160, Tucson, AZ 85756.

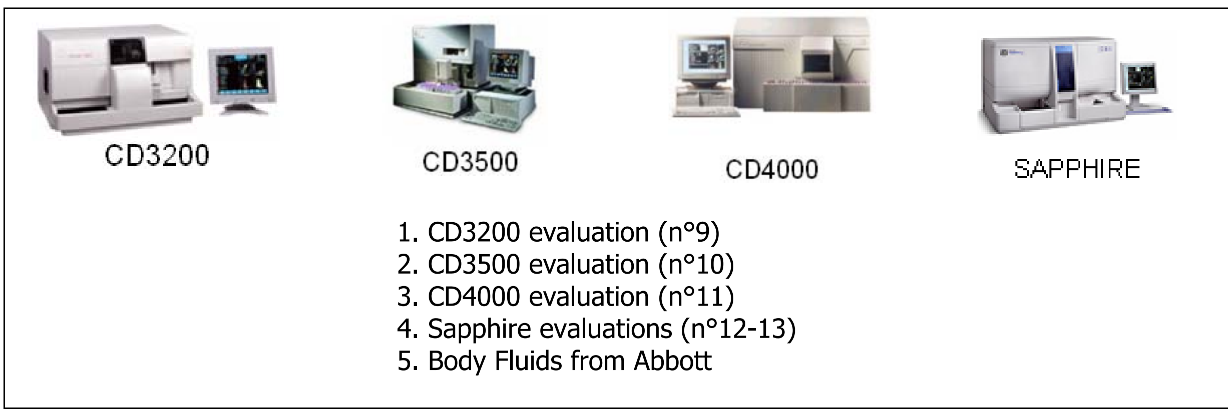
References

1. Brown W, Keeney M, Chin-Yee I, et al. Validation of body fluid analysis on the Coulter LH750. *Lab Hematol.* 2003;9:155-159.
2. Aulesa C, Mainar I, Prieto M, et al. Use of the Advia 120 hematology analyzer in differential cytologic analysis of biological fluids (cerebrospinal, peritoneal, pleural, pericardial

In their three-site evaluation of the Coulter LH 750 Body Fluid Application, the authors noted:

"Of the 372 samples, 106 had RBC counts greater than 0.01 X [10.sup.12]/L and were used for method comparison. Low-level sensitivity excluded the majority of cerebrospinal (119) and a small number of peritoneal dialysate fluid samples (8), which require accurate enumeration at clinical decision points between one to 100 cells/microliter. In the case of the synovial and serous fluids, however, most clinicians are interested in TNC counts above 0.2 X [10.sup.9]/L and RBC counts are relevant only if they are significantly increased ([greater than or equal to]0.05 X [10.sup.12]/L)." (1)

Content



1. CD3200 evaluation (n°9)
2. CD3500 evaluation (n°10)
3. CD4000 evaluation (n°11)
4. Sapphire evaluations (n°12-13)
5. Body Fluids from Abbott

1. CD3200 evaluation

Evaluation n°9: CD3200 vs Manual method

1: Lab Hematol. 2005;11(2):98-106.

An evaluation of the cell-dyn 3200 for counting cells in cerebrospinal and other body fluids.

Andrews J, Setran E, McDonnel L, Kussick S, Wood BL, Sabath DE.

Department of Laboratory Medicine, University of Washington, Seattle, Washington 98195-7110, USA. jandrews@u.washington.edu

This study compared the white blood cell (WBC) and red blood cell (RBC) counts obtained with the Cell-Dyn 3200 (CD 3200) with results obtained by hemocytometer, the reference method for counting cerebrospinal fluid (CSF) and other body fluid specimens. Ninety-six CSF and 65 body fluid specimens were evaluated. Background counts were maintained on the CD 3200 at 0.001 x 10(9)/L and 0.00 x 10(12)/L for WBC and RBC counts, respectively. Linearity and precision were acceptable for both the total nucleated cell (TNC) count and the RBC count. The CD 3200 WBC optical count was correlated with the TNC count obtained by the manual reference method for CSF specimens across the range of 0 x 10(9)/L to 7.863 x 10(9)/L (r2 = 0.9867) and for body fluid specimens across the range of 0 x 10(9)/L to 14.0 x 10(9)/L (r2 = 0.9955). An r2 value of 0.9016 was obtained for the 82 CSF specimens with manual TNC counts of <0.200 x 10(9)/L. Analysis of the CSF and body fluid specimens indicated that automated RBC counts could be reported at > or = 0.003 x 10(12)/L. In this study, 7 CSF and 30 body fluid specimens had RBC counts of >0.003 x 10(12)/L, and there was good agreement with manual RBC counts, with r2 values of 0.9893 and 0.9960 obtained for CSF and other body fluids, respectively. The CD 3200 in our experience has a lower reportable range than the ranges of most automated cell counters reported in the literature. In contrast to the only other instrument with comparable reportable ranges, the CD 3200 requires a smaller sample volume without any special sample preparation, reagents, or software. By using the CD 3200 with our laboratory-specific rules for agreement between duplicate counts, we would be able to reduce our manual CSF specimen counts from 192 TNC and 192 RBC counts to 2 TNC and 178 RBC counts. For body fluid specimens, our manual counts would be reduced from 130 TNC and 130 RBC counts to 10 TNC and 4 RBC counts.

PMID: 16024333 [PubMed - indexed for MEDLINE]

2. CD3500 evaluation

Evaluation n°10: CD3500 vs UF100

- Complete article in **Competitor evaluations** annex.
- Article marketing points:

Leukocyte Counts in Cerebrospinal Fluid with the Automated Hematology Analyzer CellDyn 3500 and the Urine Flow Cytometer UF-100

Reinhard Ziebig,* Andreas Lun, and Pranav Sinha

Background: The counting of leukocytes and erythrocytes in cerebrospinal fluid (CSF) is still performed microscopically, e.g., using a chamber in most laboratories. This requires sufficient practical experience, is time-consuming, and may constitute a problem in emergency diagnostics. Specific automated systems for CSF cell counting are not available at present.
Methods: We tested the hematology analyzer CellDyn 3500 (CD) and the urine flow cytometer UF-100 (UF), which are not designed for CSF analysis. We studied >104 samples with both analyzers, and the counts obtained were compared with the reference method (Fuchs-Rosenthal chamber).
Results: Good linearity in the medically relevant range of 15×10^6 to 1000×10^6 leukocytes/L and a high degree of within-run accuracy were seen for both analyzers. Cell counting on the UF was excellent, especially when low cell counts were encountered (CV, 4.9% compared with 28% observed for the CD). Method comparison showed that identical results could be detected for a majority of the count pairs. For a few samples, there was a discrepancy between the results from the analyzers and the counting chamber. In most cases, these were CSF samples containing a high proportion of lymphocytes. For these samples, the CD result led to a false-positive high leukocyte count, and on the UF these cells were not allocated to the leukocyte population, thus leading to false-negative counts.
Conclusions: Both analyzers should not be used for CSF cell counting in all cases at present. However, once the technical and software problems have been solved, routine use of the two analyzers for CSF analysis should be seriously contemplated.

© 2000 American Association for Clinical Chemistry

The counting of leukocytes and of erythrocytes in cerebrospinal fluid (CSF)[1] has not been automated. Cell counts are still performed microscopically, e.g., using the Fuchs-Rosenthal chamber in most laboratories. Fully automated analyzers meet time and quality requirements and are objective in material handling. On the other hand, laboratory technicians frequently must squeeze microscopic chamber counting into tight laboratory schedules (with increasing workloads), additionally needing to consider several other factors (e.g., number of samples and quality of the cells). Furthermore, insufficient practical experience in microscopic chamber counting and the subjectivity of individual laboratory workers adds to the unreliability of the results frequently observed.

The reason for the lack of more specific CSF diagnoses, including, e.g., a cell differentiation, often is insufficient sample volume and/or too few cells in a sample. Analyses performed with the modified sedimentation chamber technique according to Sayk *(1)* or with centrifugation *(2)* have contributed to the optimization of CSF cytology. They do not, however, solve the primary problem of the accurate determination of the number of cells.

Specific automated systems for CSF cell counting are not available at present. The following requirements, among others, would be necessary for such a system: (*a*) the ability to count small numbers of cells (e.g., 10×10^6 cells/L); (*b*) the ability to differentiate leukocytes into polymorphonuclear and mononuclear cell populations; and (*c*) the use of small sample volumes. On the basis of these requirements, we considered two analyzers appro-

ANALYZERS

The counting principles, specifications, and evaluations of the CD and the UF have been published previously *(3–8)*. For counting and differentiation of leukocytes, the CD uses a multiangle polarization scatter separation technology in the optical channel [white blood cell optical count (WOC)], combined with a second channel with impedance count (white blood cell impedance count). The erythrocyte counting is based on the impedance principle *(3–5)*. On the UF, cells in the urine are determined by light scatter (small-angle and wide-angle scattering) and the fluorescence of the cell membrane and the chromatin after staining with phenanthridine and carbocyanine as well as by impedance *(6–8)*.

In preparation for CSF cell counting, the CD was flushed three times with saline to obtain cell counts of $<3 \times 10^6$ cells/L. Only the leukocytes counted in the WOC channel were taken into consideration for the

the CD lead to falsely high values. In contrast, the UF leads to falsely low values. In both analyzers, the presence of the atypical fragile lymphocyte population can easily be suspected.

Regression analyses showed from the high degree of scattering of the data points around the line of identity that both analyzers do not offer a sufficient degree of safety for analyzing CSF as yet.

The high degree of accuracy and linearity that is offered by both analyzers should prompt us and the manufacturers to remedy the interfering factors as described by improving the algorithms these analyzers have to offer. Once this is done, these analyzers may be very useful for cell counts in CSF.

We thank the Sysmex Corporation for financial assistance and for helpful advice during the course of the study.

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3. CD4000 evaluation

Evaluation n°11: CD4000 vs Manual method

- Complete article in **Competitor evaluations** annex.
- Article marketing points:

Clin Chem Lab Med 2002; 40(11):1168–1173 © 2002 by Walter de Gruyter · Berlin · New York

Automated Counting of Cells in Cerebrospinal Fluid Using the CellDyn-4000 Haematology Analyser

Johannes J.M.L. Hoffmann* **and Willy C.M. Janssen**

Department of Clinical Laboratories, Catharina Hospital, Eindhoven, The Netherlands

Counting of cells in cerebrospinal fluid is currently performed manually. Because of the inherent analytical and economical disadvantages, we attempted to introduce a fully automated method. Therefore, we validated the Abbott CellDyn-4000 haematology analyser for counting cells in cerebrospinal fluid. The analyser was used in its standard configuration with the simple precaution of a preceding blank sample. As for leukocyte counting the analyser yielded high precision (CV ~5% above the upper reference limit), good linearity, low limit of detection (2/µl) and excellent correlation (r > 0.99) with the counting chamber method. The differential leukocyte count was equally accurate and precise, even in the low concentration range. Performance of the erythrocyte count was impaired by its high limit of detection (6/nl) and it appeared satisfactory only for detecting blood admixture due to traumatic puncture. The specificity of the analyser is excellent, since it correctly classified non-viable leukocytes and excluded yeast cells from the leukocyte count in a patient with cryptococcal meningitis. We conclude that the CellDyn-4000 is well suited for quickly and reliably counting leukocytes in cerebrospinal fluid. Developing some software modifications might make the analyser useful also for performing erythrocyte counting in cerebrospinal fluid. Clin Chem Lab Med 2002:40(11):1168–1173

(CSF). Yet, several attempts have been described of automated haematology analysers being used for this purpose. One of the very first reports on electronic CSF cell counting concluded that the then used haematology counters produced satisfactory results, but the lower limit of detection was rather high, varying from 100 to 200/µl, depending on the specific instrument (1). Lower detection limits could be obtained upon modification of the analysers and by increasing the sample volume, although this approach was considered not very practical (1). In addition, the early instruments were not specific enough; for example, yeast cells were incorrectly identified as leukocytes (2).

Recently, results of CSF cell counting applying more modern haematology analysers have been published. In a study using the Bayer H*2 analyser the authors concluded that this instrument yielded clinically acceptable results (3). The Abbott CellDyn-3500 haematology analyser was found to yield satisfactory precision and good linearity, but there were problems in accurately classifying fragile lymphocytes (4). These authors concluded that at present this analyser should not be used for CSF cell counting in all cases. They also concluded that a modification of algorithms might solve the problems and then make the instrument very useful for cell counting in CSF (4).

An entirely different approach for counting and differentiating CSF cells is the application of a urine flow cytometer, Sysmex UF-100 (4, 5). Although the results appear promising, the software in this instrument, too, needs to be improved before clinically reliable counts can be obtained.

One of the most advanced haematology analysers

Methods

CSF samples were analysed in the CellDyn-4000 (Abbott Diagnostics Division, Santa Clara, California, USA) in the manual mode with one background sample (normal saline) inserted immediately prior to it for reducing carry-over (see Results). The instrument was always switched into the so-called extended count mode for providing increased precision. In this mode the counting time is extended to 32 s, whereas in the standard mode it is only 9 s. The sample volume aspirated was 0.1 ml. The CellDyn-4000 was operated in accordance with the manufacturer's recommendations and checked daily following our standard operating procedures.

In the conventional method, erythrocytes (RBC) and leukocytes (WBC), the latter after staining with Türck's acidic methyl

nated as bl1 to bl6, in order to estimate the carry-over. Means ± SD of replicates (n=14) are shown.

after a normal blood sample. Figure 1 shows that in the second blank sample the WBC count drops to background level. Consequently, all further measurements were carried out with one blank sample immediately preceding the CSF sample to be analysed. As for RBC, the first blank still contained 22.9±8.9 RBC/nl (mean ± SD, n = 14), and only after four blanks the count became similar to the background (1.0±0.4 RBC/nl). So, at least four blank samples are necessary for eliminating erythrocyte carry-over.

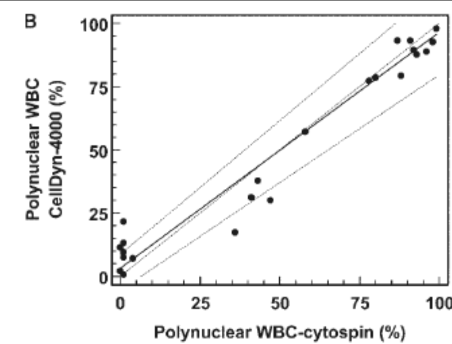


Figure 4 Regression analysis comparing the differential count by the CellDyn-4000 with the microscopic count in 24 samples with WBC > 10/µl. A: mononuclear cells with slope 0.93 (95% CI: 0.85 to 1.06), intercept 3.5 (95% CI: −0.2 to 6.1) and coefficient of correlation 0.977 (95% CI: 0.948 to 0.990). B: polynuclear cells with slope 0.93 (95% CI: 0.85 to 1.06), intercept 3.1 (95% CI: −5.5 to 8.6) and coefficient of correlation 0.978 (95% CI: 0.948 to 0.990). The utmost dotted lines indicate the 95% confidence limits of the regression (solid line); the

ran routine CSF samples in the CellDyn-4000 after a single blank sample. Using this procedure, the lower limit of detection was as low as 2 WBC/µl, which is comparable to the counting chamber and adequate for discriminating normal from pathological CSF. In contrast, the lower detection limit of RBC is insufficient for accurately measuring low RBC counts, even after as much as four blank samples. As a consequence, increased RBC counts resulting from cerebral bleeding will remain undetected as long as they are below the limit of 6.3/nl. Therefore, RBC counts in the CellDyn-4000 will only be increased in patients with severe bleeding. However, the RBC detection limit is sufficient for detecting admixture of WBC from peripheral blood caused by traumatic lumbar puncture. For example, 1 RBC/nl of normal blood is accompanied by approximately 1–2 WBC/µl. This implies that as long as the RBC count remains below the detection limit of the CellDyn-4000, any increased WBC count in CSF indicates true WBC pleocytosis. If the RBC are increased above their limit of detection, the WBC count may need to be corrected for admixture by comparing with RBC and WBC counts in blood (1).

4. Sapphire evaluations

Evaluation n°12: Sapphire vs Manual method

- Poster in **Competitor evaluations** annex.
- Poster marketing points:

For the CELL-DYN Sapphire's CBC mode, the background specification for WBC is less than 0.100 K/µL, or 100 cells /µL.

background cycles were processed until the reported WBC value was 5 cells / µL or less.

the CELL-DYN Sapphire has significant limitations (*figure 7c,d*), due in part to Poisson counting statistics, residual background, and white cell interference.

CSF ANALYSIS ON A ROUTINE HEMATOLOGY ANALYZER

STEPHEN GWILT [1] JOHN ROCHE[2],

[1]SUNY Upstate Medical University, Syracuse, New York, USA; [2]Abbott Diagnostics Division, Santa Clara, California, USA.

CELL-DYN Sapphire's CBC+C mode, which enables the system to perform an extended counting cycle for the WBC portion.

RBC counting of CSF has some significant limitations

The lowest reportable value that the CELL-DYN Sapphire can currently display is 0.001 x 1012 / L which is 1000 cells/ µL.

While the optical method may have several advantages over the impedance method, these advantages do not make the RBC values below 20,000 cells per microliter useful to the laboratory.

Evaluation n°13: Sapphire vs Manual method

- Poster in **Competitor evaluations** annex.
- Poster marketing points:

Figure 1. The CD-Sapphire distribution of a diluted normal blood for low-end linearity study. WBC and RBC concentrations were set at 1.000 x 10^3/µL and 1.000 x 10^6/µL, respectively. The CD-Sapphire WBC reagent is designed to lyse all RBCs, expose NRBC nuclei by stripping off their membrane and preserve all intact WBCs.[1] The reagent contains an intact membrane impermeant FL3 fluorescent dye (PI) to stain DNA of NRBC nuclei and damaged cells for cell viability test.[2] For WBC differential analysis, the CD-Sapphire utilizes axial light loss, multi-angle light scatter and FL3 fluorescence. The colors of each cluster represent: orange for neutrophils, blue for lymphocytes, purple for monocytes, green for eosinophils and black for basophils. Cell debris, if existing, are white on the screen but black on the print out. NRBC cluster is not shown here since this blood does not contain any NRBCs.

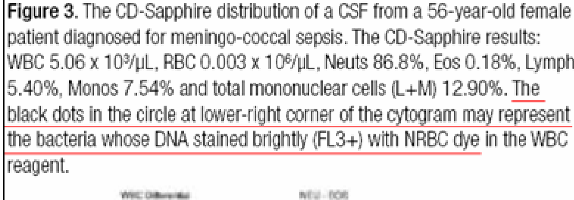
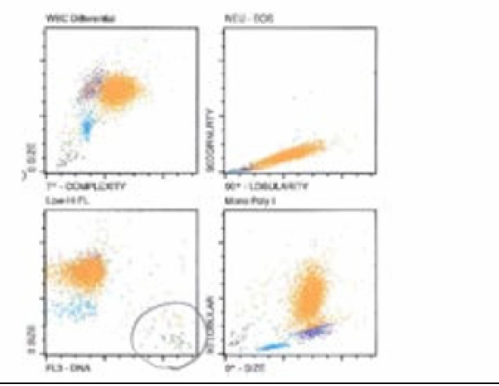


Figure 3. The CD-Sapphire distribution of a CSF from a 56-year-old female patient diagnosed for meningo-coccal sepsis. The CD-Sapphire results: WBC 5.06 x 10^3/µL, RBC 0.003 x 10^6/µL, Neuts 86.8%, Eos 0.18%, Lymph 5.40%, Monos 7.54% and total mononuclear cells (L+M) 12.90%. The black dots in the circle at lower-right corner of the cytogram may represent the bacteria whose DNA stained brightly (FL3+) with NRBC dye in the WBC reagent.



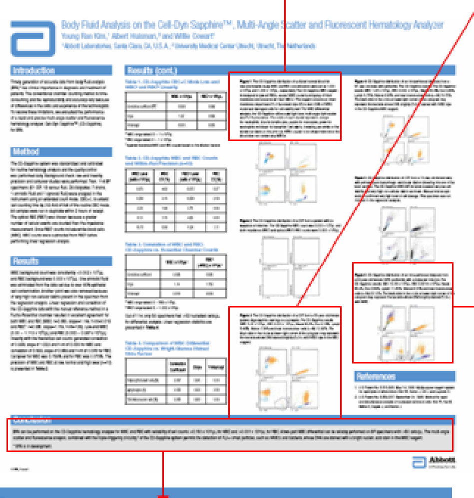
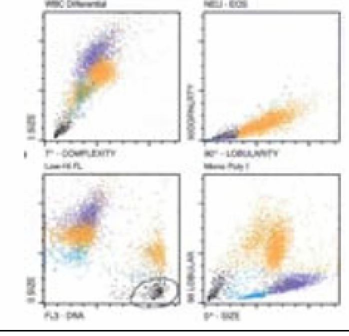


Figure 6. CD-Sapphire distribution of an intra-peritoneal dialysate from a 60-year-old female CAPD-peritonitis with acinobacter infection. The CD-Sapphire results: WBC 10.30 x 10^3/µL, RBC 0.0014 x 10^6/µL, Neuts 60.4%, Eos 0.69%, Lymph 11.80%, Monos 8.32% and total mononuclear cells (L+M) 20.12%. The black dots in the circle at lower-right corner of the cytogram may represent the bacteria whose DNA brightly stained (FL3+) with NRBC



Conclusion

BFA can be performed on the CD-Sapphire hematology analyzer for WBC and RBC with reliability of cell counts >0.150 x 10^3/µL for WBC and >0.001 x 10^6/µL for RBC. A two-part WBC differential can be reliably performed on BF specimens with >50 cells/µL. The multi-angle scatter and fluorescence analysis, combined with the triple-triggering circuitry,[2] of the CD-Sapphire system permits the detection of FL3+ small particles, such as NRBCs and bacteria, whose DNA are stained with a bright nucleic acid stain in the WBC reagent.

* BFA is in development.

5. Body Fluids from Abbott

🕮 Brochure in **Competitor flyers & brochures** annex.

CELL-DYN Sapphire Extended Immunofluorescent Applications

[…]

f) Monoclonal Antibody Analysis of Cerebrospinal Fluid (CSF)

Most of the previously discussed areas of extended CD-Sapphire analysis in this monograph have direct associations with routine haematology. However, it is also possible to consider applications where the role of the haematology laboratory is less obvious. One such example is the analysis of body fluids where automated analysers are widely used to obtain cell counts. While these numerical estimates have some utility, it is probably an understanding of the cellular components that are of greater diagnostic importance [Johnson *et al.,* 1987; Rathmell *et al.,* 1988]. For example, cytological preparations of CSF are a mandatory requirement for the differential diagnosis of diseases that impair the central and peripheral nervous system. Routine investigatory protocols therefore incorporate both classical and specialised staining procedures to assist the recognition of cellular components. This is relatively straightforward when the disease process is characterised by increased neutrophilic cells but may be more problematic when mononuclear cells are present. Cytochemical preparations can aid the identification of monocytes/macrophages but lymphoid cells are essentially uninformative unless plasmacytic forms are present. An ability to determine the lineage of lymphoid cells would be potentially useful for the differentiation of various CNS diseases and the feasibility of Mab analysis has been demonstrated in a number of reports [French *et al.,* 2000; Okuda *et al.,* 2005; Nuckel *et al.,* 2006] that have focussed on changes in specific cell populations, the detection of lymphomatous
CNS involvement and the assessment of activation markers. The CSF and Neuroimmunology Laboratory of Homolka Hospital, Prague examines a large number of CSF samples and is often faced with the need to diagnostically differentiate disorders such as neuroborreliosis (Lyme Disease), viral encephalitis, multiple sclerosis, and malignancy. In order to further evaluate the application of extended cellular investigations, a study exploring the utility of a restricted antibody panel is currently in progress with the aim being to devise Mab methods for CSF analysis. Initial methodological approaches are based on the CD-Sapphire CD3/4/8 procedure and although CSF cell numbers are often low, it has been possible to obtain some insights into cellular patterns by using a preliminary concentration step followed by Mab staining (Figure 17). Although these studies are ongoing, the initial indications are promising and it is hoped that further studies will define consistent patterns of change in defined neurological conditions (disease related patterns).

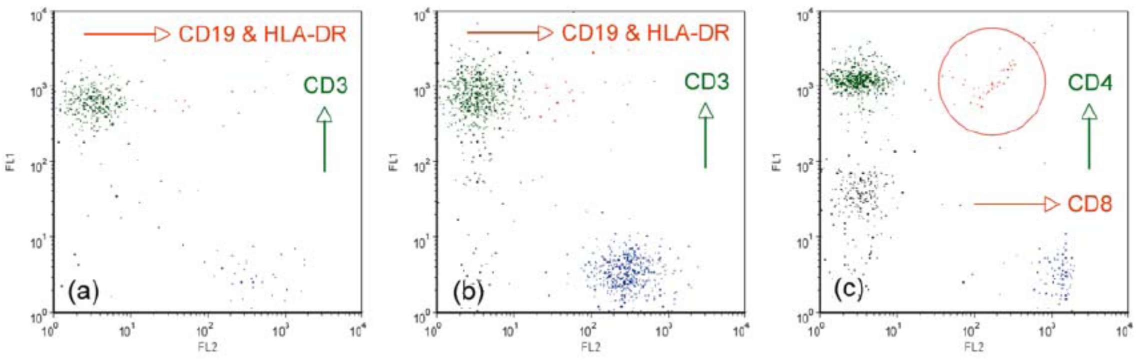
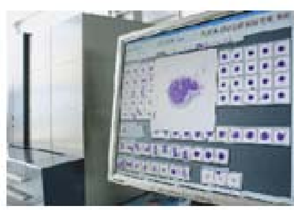


Figure 17: Representative Mab analyses of CSF cells. Plots (a) and (b) show the same combinations of reagents (CD3/FL1 and CD19/R-PE plus HLA-DR/R-PE). Both show the presence of distinct CD3+ T-cell fractions (green events) but plot (b) differs in that there is a more predominant CD19/HLA-DR+ fraction which, when taken into account with cytological examination, corresponds to B-cells. Plot (c) shows a CD4/FITC versus CD8/R-PE analysis. The CD4+ T-helper cell and CD8+ T-Suppressor cell components are seen as discrete green and blue-coded populations respectively but in addition there is a third CD4+CD8+ population. These almost certainly correspond to degenerate cellular particles with non-specific staining.

Content



DM96

1. Body Fluids application
2. FDA for DM96

1. Body Fluids application

📖 DM96 body fluids brochure in **Competitor flyers & brochures** annex.

📖 DM96 brochure in **Competitor flyers & brochures** annex.

Fluids: cerebrospinal, synovial, and pleural.

NEW CellaVision®
Body Fluid Application

Now body fluids and blood smears can be analyzed on the CellaVision DM96 at the same time, either batched in blue or orange magazines or in a random access mode when interfaced to an LIS.

Key features & benefits

- Designed to utilize standard cytocentrifuged slides.
- Pre-classification of seven classes.
- View cell classes side by side or all cells in a full screen view.
  Digital scan of entire sample area:
  - navigation in a digital sample overview
  - available in 10x or 50x magnification
- Regions Of Interest (ROI):
  - Tag interesting areas for Pathology review and for educational purposes
  - Export your ROI into presentations and educational material.
- Add pre-coded or free text comments to any slide, cell class or specific cell.
- Permanently store images —access a patient's image history.

Body Fluids

Slide preparation methods
- Standard cytocentrifuge preparation
- Default settings for Shandon™/Wescor™/Statspin™

Stains
Romanowsky stains (May Grünwald/Wright/Giemsa)

Number of cells counted User definable

Quality control
- Built-in QC module for verification of the cell location accuracy

Throughput
Based on 6 mm sample area
- Up to 25 slides/h for differential (100 WBCs + 10x)
- Up to 7 slides/h for differential (100 WBCs + 10x + 50x)

Slide image size
Based on 6 mm sample area

100 WBCs:	~5 MB
100 WBCs + 10x:	~10 MB
100 WBCs + 10x + 50x:	~150 MB

Result parameters
- ***WBC pre-classification:*** Neutrophils, Eosinophils, Lymphocytes, Macrophages (including Monocytes), Other (Basophils, Lymphoma cells, Atypical lymphocytes, Blasts and Tumor cells) and Unidentified.
- ***Non WBC pre-classification:*** Smudge cells and Artefacts

Optional Software

CellaVision® Body Fluid Application

CellaVision® Remote Review Software
Recommended PC specifications
- 64 MB graphics RAM with Open GL 1.2 support
- Ethernet adapter 10/100 Mbps • 512 MB RAM
- 100 MB free disk space • CPU Pentium IV

CellaVision® Competency Software

2. FDA for DM96

- 510K in **Competitor FDA** annex.
- 510K marketing points:

1. Analytical Performance:

   a. *Accuracy*:

A mixture of 156 samples (89 CSF and 69 BF) was identified according to EP9A-2 from two sites. All samples were initially analyzed on a cell counter or counted in a hemocytometer to get the leukocyte concentration. From each sample two cytocentrifuged smears were prepared. 200-cell differential counts were performed (400 cells/sample) with both methods and analyzed. The same examiners analyzed the same slides. The accuracy was tested through scatter-plots for each cell class.

Samples included in the study.

Defined as in study	Type	number
CSF	CSF	89
Serous	Peritoneal fluid	24
Serous	Pleural fluid	46

Results for all samples included are as follows:

Cell Class	Accuracy	95% CI Slope	n
Neutrophils	y= 1.0166x - 0.0030 r^2= 0.9903	1.0006 - 1.0326	156
Lymphocytes	y= 1.9840x - 0.0011 r^2= 0.9788	0.9609 - 1.0070	156
Macrophages	y= 0.9554x - 0.0113 r^2= 0.9648	0.9264 - 0.9845	156
Eosinophils	y= 1.1352x - 0.0018 r^2= 0.9737	1.1055 - 1.1649	156
Other cells	y= 1.0808x - 0.0019 r^2= 0.9566	1.0442 - 1.1174	156

Cells pre-classified as Basophils, Lymphoma cells, Atypical lymphocytes, Blasts and Tumor cells are automatically are forwarded to the cell class *Other*.

Body fluids controls

Home > Diagnostics > Hematology Controls > Cell-Chex Auto Automated Body Fluid Cell Count Control

Cell-Chex Auto Automated Body Fluid Cell Count Control

request information

General Information

Vendor	**Streck**
Item	**Cell-Chex Auto Automated Body Fluid Cell Count Control**
Product Number(s)	200067 200068 200069
Analyte(s) / Parameter(s)	Total RBC and WBC values
Levels	Level 1, 2 and 3
Quantity/Volume (mL)	3x3.0ml (Level 1) 3x3.0ml (Level 2) 3x3.0ml (Level 3)
Instrument Compatibility	Abbott CELL-DYN® 3200, 4000 and Sapphire™ Beckman Coulter® LH750/LH 755 (levels 2 and 3 only) Sysmex XE-2100™
Pricing	REQUEST QUOTE

Product Description

Cell-Chex Auto is a whole blood control for evaluating the accuracy and precision of hematology instruments that measure automated blood cell counts in patient body fluid samples. The assay provides total RBC and WBC values for the Abbott CELL-DYN® 3200, 4000 and Sapphire™, Beckman Coulter® LH750/LH 755 (levels 2 and 3 only) and Sysmex XE-2100™ instruments. Cell-Chex Auto is a three-level control packaged in a plastic 76mm vial with a pierceable cap. Cell-Chex Auto has 75-day closed-vial stability and 30-day open-vial stability when stored at 2°-10°C.

Go to Vendor Product Page

Go to Vendor Website

PRODUCTS SERVICES CONTACT US CBC-MONITOR ONLINE RESEARCH PRODUCTS

Product Information

R&D CSF Automated Control

R&D CSF Automated Control is an assayed whole blood control designed to monitor values obtained using hematology instruments that measure CSF samples. Assays are provided for the Advia 120 and 2120. CSF Automated Control has 105-day closed vial stability with 14-day open vial stability.

Features:

- 105-day closed vial stability; 3 QC months
- 14-day open vial stability
- Packaged in 3mL pierceable tubes
- Assayed for Bayer Advia 120, 2120

PRODUCTS SERVICES CONTACT US CBC-MONITOR ONLINE RESEARCH PRODUCTS

Product Information

R&D Body Fluid Manual Control

Body Fluid is a bi-level control used to monitor total cell counts performed manually using a hemocytometer. The two levels of this control are designed to monitor values in the normal and abnormal ranges. This product contains mammalian erythrocytes and leukocytes in a plasma like fluid. Body Fluid Control has 105-day closed vial stability with open vial stability of 90 days (31 thermal cycles).

Features:

- 105-day closed vial stability; 3 QC months
- 90-day (31 thermal cycles) open vial stability
- Packaged in 2mL vials
- Assayed for Manual Methods

Ordering Information:

Catalog No.	Description
BF001	R&D Body Fluid: 2 x 2mL (1 Each Levels 1,2)
BF002	R&D Body Fluid: 4 x 2mL (2 Each Levels 1,2)

3. Competition arguments

System	Weaknesses	Page	Strengths	Page
Advia series	• 4 minutes pretreatment for CSF (fix and spherize the cells) • pleural no good correlation • Advia 120 not good for patients with tumors	29 32 32	• Pre-treatment kit for CSF • FDA • 20 WBC /mm^3 • synovial + hyaluronidase • test price of hyaluronidase (≈ 6 €) ?	28 30 27 32
XT series	• No Diff parameters • Linearity : WBC > 50 cells/mm^3	33 33	• Volume 85 µl • FDA	33 34
XE series	• Linearity : WBC > 50 cells/mm^3 • Re-switch in whole blood = 2min	34 36	• Volume 130 µl • FDA • Extended count volume • Algorithm to exclude mesothelial & macrophage cells • Cost of test	35 37 36 36 37
UF 100	• False negative for lympho • Don't use for body fluids	53		
LH series	• Hyaluronidase • Recall in 2006 • 3 blank cycles • Not good for CSF	44 47 49 49	• FDA	47
CD 3500	• Don't use for CSF • 3 blank cycles • False positives	52 53 52		
CD 4000	• RBC contamination • 4 blank cycles	55 54	• Extended counting for WBC (+ 32 sec)	54
Sapphire	• Linearity : WBC > 100 cells/mm^3 • No FDA • Limitation interferences	56 56	• Extended counting for WBC (+ 32 sec) • CD markers CD3,4,8 for CSF • Plus bacteria cells	56 57 57
Pentra DX	• Sample volume • No FDA	23	• Linearity • Blank cycle included	16 12

Frequently asked questions

1. Why don't we use the channel WBC/HGB for the WBC counting?

The dilution is lower on the LMNE channel, and the precision is better for few cells.

Dilution ratios

WBC/HGB	1/234
LMNE	1/80
RBC/PLT	1/10000
BASO	1/200
RETIC	1/3125

2. We use the current channel and cycles. Why did we create the CBF mode?

On the current results, the minimum value that we can display is 0.1 $10^3/mm^3$.
This value is obtained from the raw data and after several rounded calculation.
To obtain the best linearity and precision for the CBF, the algorithm uses the raw data **without rounded calculation** and with **more decimals** to give the most right number of cells.

3. Why aren't the graphs displayed on the ML?

Technically, on the ML, the graphs are associated with parameters.
Eg: Double Matrix associated with Diff parameters.
On the CBF mode, we created new parameters (RUO) and we have no corresponding graphs.

4. Why is the volume so large?

Without optical detection, to be sure the shear valve is completely full, the aspiration duration is 4 seconds. This duration was evaluated during the first study, according to the viscosity of the liquids, and confirmed by the other evaluations.

5. Can the mesothelial cells, the endothelium cells or the synovial cells cause an inference with leukocytes when a fluid (pleural, synovial) is run?

No, in general, the cells are too larges.
In some cases, the mesothelial cells could be small but we never noted these kinds of interferences in the Barcelona study.

6. If the other cells are counted in the LMNE then where do they appear in the LMNE matrix?

We are studying the eventual counting of these cells but the conclusion is that the "other" cells are not in the matrix.

Tools

Movie

"CBF mode" movie available in French and English.
Translation in all languages possible.

Brochures

- Flyer available in PDF format (English, French languages) in the Communication Database.
- CBF Mini procedure available in Word format, English language.

Competitors evaluations

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ISLH

Official Publication

The ADVIA 2120 Hematology System: Flow Cytometry–Based Analysis of Blood and Body Fluids in the Routine Hematology Laboratory

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ABSTRACT

The ADVIA 2120 Hematology System was recently released by Bayer HealthCare, Diagnostics Division, as a bench-top analyzer designed for medium- to large-volume laboratories. This flow cytometry–based system uses light scatter, differential white blood cell (WBC) lysis, and myeloperoxidase and oxazine 750 staining to provide a complete blood cell count, a WBC differential, and a reticulocyte count. A cyanide-free method is used to measure hemoglobin colorimetrically. The system is automation ready; in addition to its capability for analyzing peripheral blood specimens, the analyzer is also equipped to analyze cerebrospinal fluid samples. In this article we explain the underlying technology of the ADVIA 2120, provide linearity ranges, method-specific reference ranges, and stability data, and describe novel parameters and applications that are unique to the methodology used by this instrument. Finally, we discuss research applications and future directions, such as the use of this hematology analyzer in the determination of fetal lung maturity. *Lab Hematol.* 2005;11:47-61.

KEY WORDS: Cell counters · Hematology analyzers · ADVIA 2120 · ADVIA 120 · Automated cell analysis · Flow cytometry

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INTRODUCTION

The hematologic evaluation of peripheral blood and body fluids has an important role in the laboratory workup of most patients. Historically, manual, nonautomated, and labor-intensive methods were central in this analysis, which was often beset by high costs and difficulties in standardization. More recently, technological advances have made it possible to automate many aspects of this analysis, leading to decreased turnaround times, more reproducible results, and lower costs. The first automated hematology systems introduced in the 1950s were based on the electrical impedance principle and were able to provide data for only the most basic hematologic parameters. These instruments were restricted to the analysis of peripheral blood samples [1]. Such first-generation instruments have increasingly been replaced by more advanced systems that can analyze body fluids in addition to blood and that distinguish themselves by providing the ability to measure novel parameters that allow new clinical applications. Many of these newer systems also use methodologies other than impedance measurements, such as flow cytometry and radio frequency determinations.

The Technicon H*1 (Technicon Instruments Corporation, Tarrytown, NY, USA) was introduced in the 1980s as a flow cytometry–based single-sample analyzer capable of providing a complete blood count (CBC) and a full white blood cell (WBC) differential [2]. This instrument was improved in the H*2 model, and a reticulocyte counting ability was added with the H*3 (Miles Diagnostics Division, Tarrytown, NY, USA) [3,4]. The H*3 series of instruments was replaced by the ADVIA 120 (Bayer HealthCare, Diagnostics Division, Tarrytown, NY, USA). Most recently, Bayer has released the ADVIA 2120, which includes in addition to the established features of the ADVIA 120 a cyanide-free hemoglobin method, automa-

tion readiness, and an improved user interface. We provide an overview of the technical aspects and unique clinical applications of the ADVIA 2120 Hematology System, supply linearity and reference ranges as well as sample-stability data, and review future directions of this methodology.

TECHNOLOGY

Overview

The ADVIA 2120 Hematology System is a bench-top analyzer designed for medium- to large-volume laboratories. It has 5 sample-acquisition selectivities: CBC (for CBC alone), CBC/Diff (for CBC with automated differential), CBC/Diff/Retic (for the simultaneous determination of CBC, differential, and reticulocyte count), CBC/Retic, and Retic only. The rack-based system has a throughput of 120 samples/hour in the CBC/Diff mode and 74 samples/hour when reticulocyte analysis is performed. Three aspiration modes are available to run different samples: a rack-based autosampling provides a walk-away feature, manual closed-tube sampling is ideal for high-risk/urgent samples, and manual open-tube sampling accommodates small samples.

Methods of Sample Analysis

The ADVIA 2120 Hematology System is a flow cytometer. The analyzer uses light scatter, differential WBC lysis, and myeloperoxidase staining to determine WBC parameters. Unique red blood cell (RBC) indices are directly measured by means of a mathematical principle termed *Mie* (or *Lorenz-Mie*) *theory*, which provides an equation for the analysis of light scattering from a homogeneous spherical particle [5]. The instrument uses 5 channels to analyze blood samples: a hemoglobin channel for the colorimetric measurement of hemoglobin concentration, a combined RBC and platelet channel, 2 channels (the peroxidase and lobularity/nuclear density channels) for WBC counts and differentials, and a reticulocyte channel [3,6]. In the 2 WBC analysis channels, for example, the blood sample is diluted approximately 50-fold so that 1 μL of diluted suspension corresponds to 0.02 μL of the undiluted blood sample. Forty microliters of this suspension are passed through the flow cell for analysis; therefore, 0.8 μL of whole blood is analyzed in each WBC analysis channel.

Hemoglobin Channel. A cyanide-free, environment-sensitive colorimetric method of hemoglobin measurement is used on the ADVIA 2120. In this method, hemoglobin is converted to a sulfated derivative by the addition of sodium lauryl sulfate, and light absorption is measured at 564 nm [7,8]. On the ADVIA 120, the hemoglobin concentration in the sample was determined by a modification of the manual cyanmethemoglobin method, which used potassium ferricyanide to convert hemoglobin iron from the ferrous to the ferric state and subsequently measured cyanmethemoglobin colorimetrically at 546 nm. The cyanide-free method is available as an option on the ADVIA 120.

RBC/Platelet Channel. RBCs are converted to spheres with sodium dodecyl sulfate and glutaraldehyde. Platelets suspended in K_3EDTA are converted into pseudospheres. This treatment permits a low-angle (2°-3°) and high-angle (5°-15°) light-scatter analysis that is independent of the original shape of the cell. The 2 light-scatter signals are detected, electronically amplified, and split into 4 signals, with a pair of low-angle and high-angle light-scatter signals used to analyze RBCs. For platelet analysis, a low-angle light-scatter signal is amplified 30 times, and a high-angle light-scatter signal is amplified 12 times to achieve higher resolution in the discrimination of collected events. Thus, 4 sets of scatter signals resulting from the 2 scatter measurements are digitized and analyzed by the instrument. These scatter measurements are converted into volume and refractive index values by means of the Mie theory of light scattering for homogeneous spheres [5,9]. The RBC cytogram map resolves volumes between 1 fL and 180 fL and refractive index values between 1.38 and 1.44 (Figure 1A). The platelet cytogram maps resolve volumes between 1 fL and 60 fL and refractive index values between 1.35 and 1.40 (Figure 1B).

For RBC analysis, the pair of signals consisting of the low-angle light-scatter measurement and the high-angle light-scatter measurement is converted into cell volume (in femtoliters) and hemoglobin concentration (in grams per deciliter) by means of a lookup table based on Mie scattering theory. The mean hemoglobin concentration defines the corpuscular hemoglobin concentration mean (CHCM) for RBCs. This measurement is analogous to the classic mean corpuscular hemoglobin concentration (MCHC), which is derived from the hemoglobin concentration and the hematocrit.

For platelet analysis, the light-scatter signals are volume (in femtoliters) and platelet component concentration values (in grams per deciliter; Figure 1B).

Integrated RBC/platelet analysis is used to distinguish RBCs, platelets, large platelets, RBC fragments, RBC ghosts, and debris. The refractive index measurement makes it possible to distinguish platelets from particles of similar size, thus providing an accurate platelet count. The reportable platelet count includes large platelets with volumes of up to 60 fL. Microcytes, RBC fragments, and RBC ghosts are excluded from the platelet count.

Peroxidase Channel. Two separate methods, the peroxidase and lobularity/nuclear density methods, are used on the ADVIA 2120 system to analyze WBCs. In the peroxidase method, the RBCs are lysed, and peroxidase reagents are used to distinguish between peroxidase-positive cells, such as neutrophils, eosinophils, and monocytes, and peroxidase-negative cells, which include lymphocytes, basophils, and "large unstained cells" (LUCs). Platelets are distinguished from WBCs on the basis of size. A tungsten-based optical system is used to count all WBCs and to determine the absorbance (stain intensity) and cell size (by forward light scatter) for each

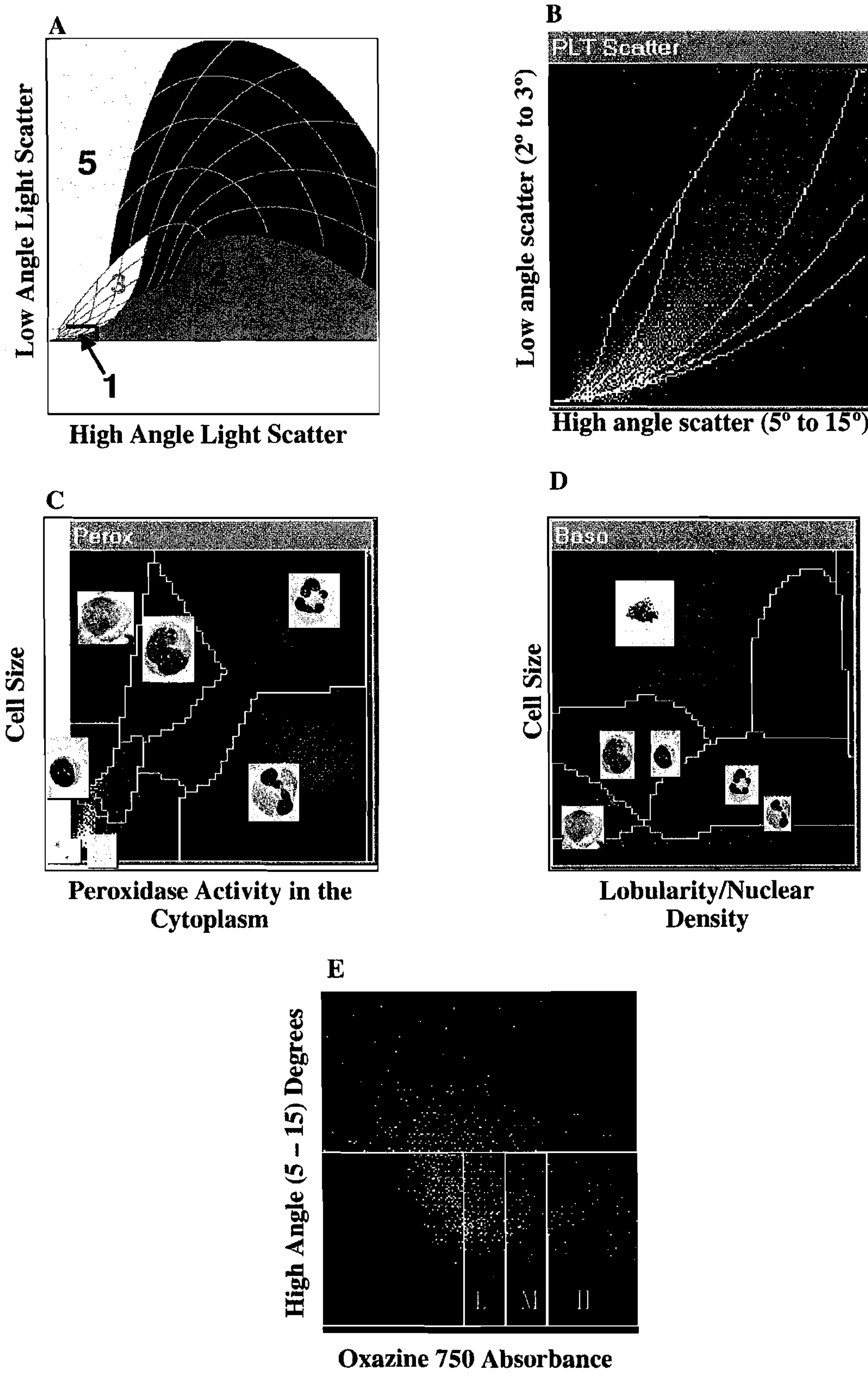


FIGURE 1. Cytograms derived from the various channels of the ADVIA 2120 Hematology System. A, Combined red blood cell (RBC)/platelet cytogram. Indicated are the area covered by the platelet cytogram (1), RBC fragments (2), large platelets (3), RBCs (4), and RBC ghosts (5). B, Platelet cytogram. C, Peroxidase cytogram. D, Lobularity/nuclear density (basophil) channel cytogram. E, Reticulocyte cytogram.

TABLE 1. Linear Ranges of the ADVIA 2120 Hematology System

White blood cells, $\times 10^3/\mu L$	0.02-400
Red blood cells, $\times 10^6/\mu L$	0.0-7.0
Platelets, $\times 10^3/\mu L$	5.00-3500
Hemoglobin, g/dL	0.0-22.5
Reticulocytes, %	0.2-24.5

cell. The cells absorb light in proportion to the amount of peroxidase stain present, and this peroxidase activity parameter is represented on the x-axis of the peroxidase cytogram. Cells scatter light in proportion to their size, and this cell size parameter is represented on the y-axis of the cytogram (Figure 1C). When the light-scatter and absorption data are plotted, distinct populations or clusters are formed, and cluster analysis is applied to identify different cell populations.

Lobularity/Nuclear Density Channel. The lobularity/nuclear density channel, also called the basophil channel, is the primary channel used to report the WBC count. In this channel, surfactant and phthalic acid are used to lyse RBCs and platelets and to strip away the cytoplasmic membrane from all leukocytes except basophils. Cells are then counted and classified according to size (with basophils being the "largest" cells because they still possess a cytoplasmic membrane), lobularity, and nuclear density (Figure 1D). In addition to providing the reported WBC count and the basophil count, this channel also provides valuable information about the degree of maturity of each WBC's nucleus by measuring its lobularity and density [10].

Reticulocyte Channel. Reticulocyte analysis is performed with oxazine 750, a nucleic acid dye that selectively stains reticulocytes and distinguishes them from mature RBCs (Figure 1E) [4,11]. Reticulocyte analysis includes the measurement via light scatter of the volume and hemoglobin content of each individual cell. Mature RBCs and immature RBCs (reticulocytes) are analyzed by the same method at the same time. This measurement provides additional clinical information on the interrelationship of these 2 RBC populations.

Use of the Information from the Various Channels. Comparison of the data obtained from the various channels of the ADVIA 2120 Hematology System is used for internal cross-checks and for the generation of additional information. For example, although the primary reported WBC count is derived from the lobularity/nuclear density channel, the WBC count is also determined from the peroxidase channel, and a flag is triggered if the 2 parameters differ to a significant degree. Similarly, the hemoglobin concentration measured colorimetrically is compared with the value derived from the light-scatter analysis, which is the product of the RBC count, the mean corpuscular volume, and the CHCM, and a significant discrepancy will lead to flagging of the specimen. Such discrepancies may be caused by significant hemolysis, icterus, or lipemia. LUCs from the peroxidase channel are used in the algorithm together with the information from the lobularity/nuclear density channel to trigger a blast flag. If no blasts are seen in the basophil channel, an "atypical lymphocyte" flag will be seen by the technologist.

Other Technical Information

Consolidated Data Manager. An optional consolidated data manager called ADVIA CentraLink is available to expedite result reporting and to allow data editing. This feature is of importance when multiple ADVIA 2120 instruments are used in the laboratory. It provides the ability to review and edit results at any workstation, to easily monitor real-time quality control performance across instruments, and to review patient results and cytograms at microscope stations.

Automation Readiness. The ADVIA Autoslide, an automated slide maker/stainer (in development), is an integrated option for the ADVIA 2120 Hematology System. The Autoslide automatically prepares blood smears and stains the slides with user-definable smearing and staining protocols. Blood sampling for the Autoslide can be automatically performed on the ADVIA 2120 analyzer on the basis of reflective slide-making criteria.

Multispecies Capabilities. Software applications for the analysis of blood cells from 15 animal species are available on the ADVIA 120/2120 systems. Adaptation of the instrument for different species is accomplished via dedicated software packages that automatically adjust instrument settings [12].

LINEARITY, REFERENCE RANGES, MEAN VALUES, AND SAMPLE-STABILITY DATA

Linearity

Reproducible results at very low and very high levels of hematologic parameters are crucial for (1) an efficient workflow in the clinical laboratory to avoiding time-consuming manual dilutions and for (2) patient safety, by providing reliable results at clinical-decision points, such as extremely low platelet and WBC counts. Table 1 shows the linearity ranges of the ADVIA 2120 Hematology System. Platelet counts as low as 5000/µL can be reported as reproducibly as counts of 3,500,000/µL. Similarly, WBC counts can be reported reliably at levels as low as 20/µL.

Reference Ranges for Conventional Parameters

Laboratory reference ranges are affected by many variables, including age, sex, diet, patient population, and laboratory methods [13]. We recommend that each laboratory establish its own range of expected values. Table 2 shows ranges of expected values from the ADVIA 2120 system that are based on duplicate assays of 60 specimens of whole blood obtained from an adult population of presumed healthy individuals [14]. Similar results have been published by others [15,16].

TABLE 2. Ranges of Expected Values for ADVIA 2120 Reportable Parameters

Parameter	Name	Unit	Range
WBC	White blood cell count	$10^3/\mu L$	3.8-8.6
RBC	Red blood cell count	$10^6/\mu L$	4.1-6.0
Hgb	Hemoglobin	g/dL	11.1-17.1
HCT	Hematocrit	%	33.0-57.0
MCV	Mean corpuscular volume	fL	76.0-100.0
MCH	Mean corpuscular hemoglobin	pg	24.0-31.0
MCHC	Mean corpuscular hemoglobin concentration	g/dL	28.0-34.0
CH	Mean hemoglobin content	pg	24.0-35.0
CHCM	Hemoglobin concentration mean	g/dL	29.0-34.0
RDW	Red cell volume distribution width	%	12.0-15.0
HDW	Hemoglobin concentration distribution width	g/dL	1.9-3.0
PLT	Platelet count	$10^3/\mu L$	140-360
MPV	Mean platelet volume	fL	7.0-9.0
%NEUT	Neutrophil proportional count	%	40-77
%LYMPH	Lymphocyte proportional count	%	16-44
%MONO	Monocyte proportional count	%	4-9
%EOS	Eosinophil proportional count	%	1-7
%BASO	Basophil proportional count	%	0-1
%LUC	Large unstained cell proportional count	%	1-4
#RETIC	Reticulocyte absolute count	$10^9/L$	40-79
%RETIC	Reticulocyte proportional count	%	0.8-2.1
MCVg*	Mean corpuscular volume of all gated red cells	fL	76-101
MCVr	Mean corpuscular volume of reticulocytes	fL	93-121
CHCMg*	Hemoglobin concentration mean of all gated red cells	g/dL	29-35
CHCMr	Hemoglobin concentration mean of reticulocytes	g/dL	27-34
CHg*	Mean hemoglobin content of all gated red cells	pg	24-35
CHr	Mean hemoglobin content of reticulocytes	pg	24-36

*Gated cells comprise both reticulocytes and mature red cells.

Sample Stability

A study on the effects of the prolonged storage of blood samples was performed over a 72-hour period. Specimens of whole blood drawn from 15 apparently healthy donors were assayed shortly after phlebotomy and then again at intervals of 8, 24, 36, 48, 56, and 72 hours. The specimens of whole blood were stored at room temperature (approximately 25°C) and refrigerated at a temperature between 2°C and 8°C in capped blood-collection tubes that contained K_3EDTA as the anticoagulant. The results from the study indicate that each parameter is stable within 2 standard deviations of the initial recovery value for the time intervals listed in Table 3 [14].

Mean Values and Stability Data for the Novel Platelet Parameters Available on the ADVIA 120/2120

Available from the ADVIA 120/2120 systems is the capability to measure novel platelet parameters, which have been shown to be useful for a variety of research applications and are used in clinical trials. It has therefore been necessary to establish reference ranges for these parameters. Table 4 shows mean values for these research use–only parameters obtained 60 minutes postphlebotomy from EDTA-containing samples from 24 healthy donors (samples courtesy of Dr. S. Chapman-Montgomery). Our data are very similar to those reported by others [17-19].

Many of the novel platelet parameters available on the ADVIA 2120 reflect the activation status of platelets, and their values can therefore be subject to change if the parameters are not measured shortly after sample collection. Several reports have shown that the mean platelet volume will significantly increase during a 24-hour period of blood storage, whereas the mean platelet component concentration (MPC) will decrease [20-22]. This finding is consistent with the microscopical observation that platelets swell and loose refractility with prolonged storage.

CLINICAL APPLICATIONS UNIQUE TO THE ADVIA 120/2120 HEMATOLOGY SYSTEMS

Red Cells

The unique methodology of RBC analysis of the ADVIA 120/2120 Hematology Systems allows for a number of novel

TABLE 3. Expected Sample Stability*

Parameter (Unit)	Room Temperature Stability, h	Refrigerated Stability, h
WBC ($10^3/\mu L$)	36	56
RBC ($10^6/\mu L$)	48	72
Hgb (g/dL)	72	72
MCV (fL)	8	24
CHCM (g/dL)	8	24
RDW (%)	72	72
HDW (g/dL)	72	72
PLT ($10^3/\mu L$)	48	48
MPV (fL)	8	8
NEUT (%)	36	72
LYMPH (%)	36	72
MONO (%)	72	72
EOS (%)	8	72
BASO (%)	72	56
LUC (%)	72	72
RETIC (%)	24	72
MCVg (fL)	8	24
MCVr (fL)	8	24
CHCMg (g/dL)	8	24
CHCMr (g/dL)	8	24
CHg (pg)	36	56
CHr (pg)	24	72

*Parameter abbreviations are expanded in Table 2.

applications that are not available on other platforms. These applications include the generation of an RBC cytogram, the reporting of RBC parameters available only on the ADVIA 120/2120, the analysis of samples from patients who have received blood substitutes, and the generation of reticulocyte-specific parameters.

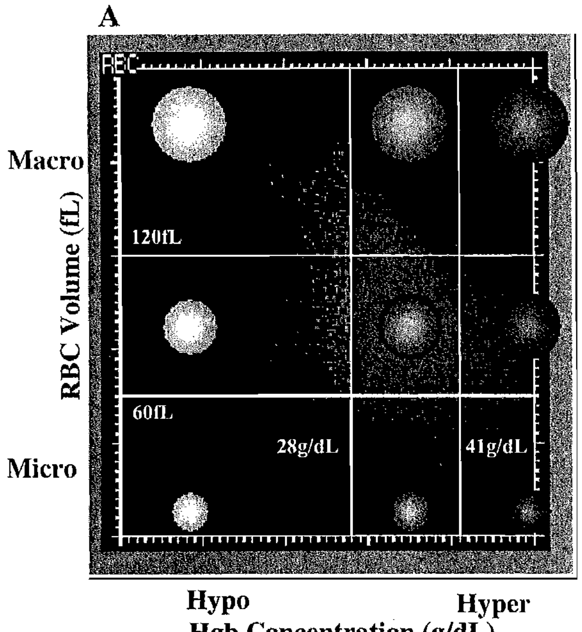
The RBC Cytogram. The ADVIA 2120 Hematology System directly measures the volumes and hemoglobin concentrations of individual RBCs and therefore allows the determination of chromasia and erythrocyte size on a cell-by-cell basis. This information is presented in a RBC cytogram. Visual inspection of this graphic representation of the size and hemoglobin content of individual RBCs can be used to supplement and sometimes even to replace conventional slide review. After minimal training, one can very quickly determine chromasia and the size of the patient's RBCs, as well as the presence or absence of multiple populations of RBCs, by reviewing a RBC cytogram and drawing conclusions about the underlying pathophysiology (Figure 2). For example, most of the RBCs in a patient with iron deficiency will be both hypochromic and microcytic, whereas microcytosis will be more pronounced than hypochromasia in patients with a thalassemic trait. The presence of more than 1 population of RBCs may indicate myelodysplastic syndrome, correcting iron deficiency, or the transfusion of RBC concentrates (Figure 3).

Novel RBC Parameters Unique to the ADVIA 120/2120 System. In addition to the graphic presentation of RBC data in the form of the RBC cytogram, the ADVIA 2120 Hematology System also provides for measurements of a number of novel numeric RBC parameters that can be obtained only with flow cytometry. These parameters include the percentages of microcytic, macrocytic, hypochromic, and hyperchromic cells. Clinical applications for some of these parameters have already been described; applications for others are presently undergoing intensive studies. For example, the percentage of hypochromic cells has been shown to be a useful predictor of iron deficiency [23-25]. Determination of the percentage of hypochromic cells has been included in the European guidelines for the management of anemia in hemodialysis patients [26]. The ratio of microcytic to hypochromic cells (the "M/H ratio") has also been reported to be useful in distinguishing β-thalassemia and iron deficiency [27,28]. Pati and colleagues and Kutter and coworkers have reported that the percentage of hyperchromic cells can be used as a screening tool for the presence of spherocytosis (Figure 4) [29,30].

Analysis of Samples from Patients Who Have Received Blood Substitutes. Hemoglobin-based blood substitutes are presently used in the setting of large clinical trials, and laboratories are often faced with urgent requests from clinicians for the measurement of CBC parameters for patients who have received such products. Because the ADVIA 2120 Hematology System routinely measures both the total hemoglobin present in the sample (by a colorimetric method) and the cellular hemoglobin (by light scatter), the instrument can be used to monitor patients who have received such hemoglobin-based blood substitutes [31]. The

TABLE 4. ADVIA 120/2120 Platelet Parameter Values Derived from a Healthy Population

Parameter	Name	Unit	Mean ± SD
PDW	Platelet volume distribution width	%	53.5 ± 5.8
PCT	Platelet crit	%	0.19 ± 0.03
MPC	Mean platelet (component) concentration	g/dL	27.6 ± 0.6
PCDW	Platelet component concentration distribution width	g/dL	5.1 ± 0.4
MPM	Mean platelet (dry) mass	pg	2.14 ± 0.19
PMDW	Platelet dry mass distribution width	pg	0.91 ± 0.11



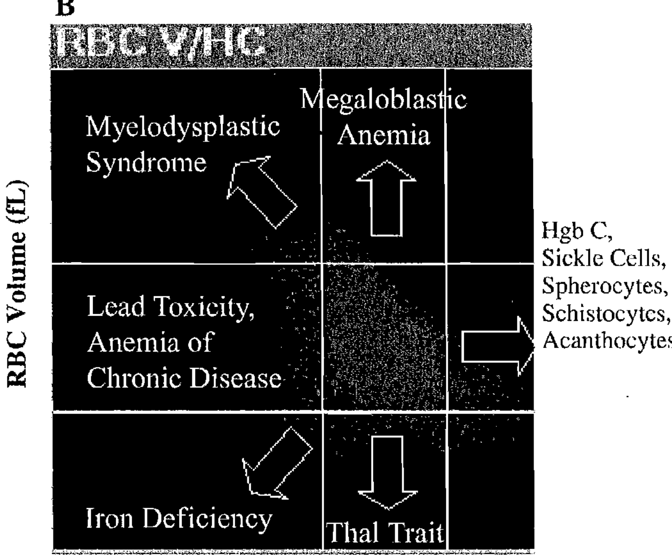


FIGURE 2. Red cell cytograms. A, A red blood cell (RBC) cytogram and the positions of hypochromic, normochromic, hyperchromic, microcytic, normocytic, and macrocytic cells. B, Effects of various disease states on the locations of RBCs in the RBC cytogram. Hgb indicates hemoglobin; V, volume; HC, hemoglobin concentration; Thal, thalassemia. Figure courtesy of Dr. Timothy Skelton, Lahey Clinic, Burlington, MA, USA.

blood substitute level (extracellular hemoglobin) can be calculated with the following formula:

Extracellular Hemoglobin = Total Hemoglobin – Cellular Hemoglobin.

In addition, the MCH and MCHC parameters of the patient's own RBCs can be accurately determined in this setting with the ADVIA 2120 technology.

Novel Reticulocyte Parameters. By combining the ADVIA 2120's cell-by-cell analysis of RBCs for volume and hemoglobin concentration with the determination of the RNA content of individual cells, this system can directly measure reticulocyte volume, reticulocyte hemoglobin concentration, and reticulocyte hemoglobin content (CHr). In addition, a large number of other reticulocyte-specific values can be derived from these directly measured parameters. Measurement of these parameters has been demonstrated to enable a real-time assessment of the functional state of the erythroid marrow (Figure 5) [6]. In particular, a series of articles has shown that the CHr can be used to determine iron deficiency in dialysis patients [32-35]. This application has gained widespread use, and the CHr parameter has been cleared by the US Food and Drug Administration for this purpose. The CHr has also been used, frequently in combination with other measurements such as the percentage of hypochromic RBCs, in the evaluation of other disease states in which the determination of iron deficiency by conventional parameters alone can be difficult, as in patients with acute-phase reaction [36]. Most exciting are recent reports that the CHr appears to be the strongest predictor of iron deficiency in pediatric populations and that CHr measurement may be an acceptable alternative to biochemical iron studies [37,38].

Platelets

Excellent Accuracy of the ADVIA 2120 Platelet Count due to 2-Dimensional Analysis. By using a 2-dimensional method to identify platelets, the ADVIA 2120 Hematology System is able to provide improved platelet discrimination, thereby eliminating platelet-counting errors due to the inclusion of interfering substances and the exclusion of large platelets [39]. This excellent accuracy is especially important at low platelet counts, because fear of the transmission of infectious agents, limitations of supply, and cost pressures have led to a lowering of the platelet count threshold at which a platelet transfusion will be given. The results obtained with this dual-angle optical method compared well with those obtained with the reference immunoplatelet-counting method, and the ADVIA 2120 method was more accurate at determining platelet counts in thrombocytopenic samples [40]. The ability of the ADVIA 2120 to accurately enumerate platelets, especially at such low counts, is therefore of high clinical importance.

Novel Platelet Parameters. In addition to the established parameters such as platelet count and platelet size, the

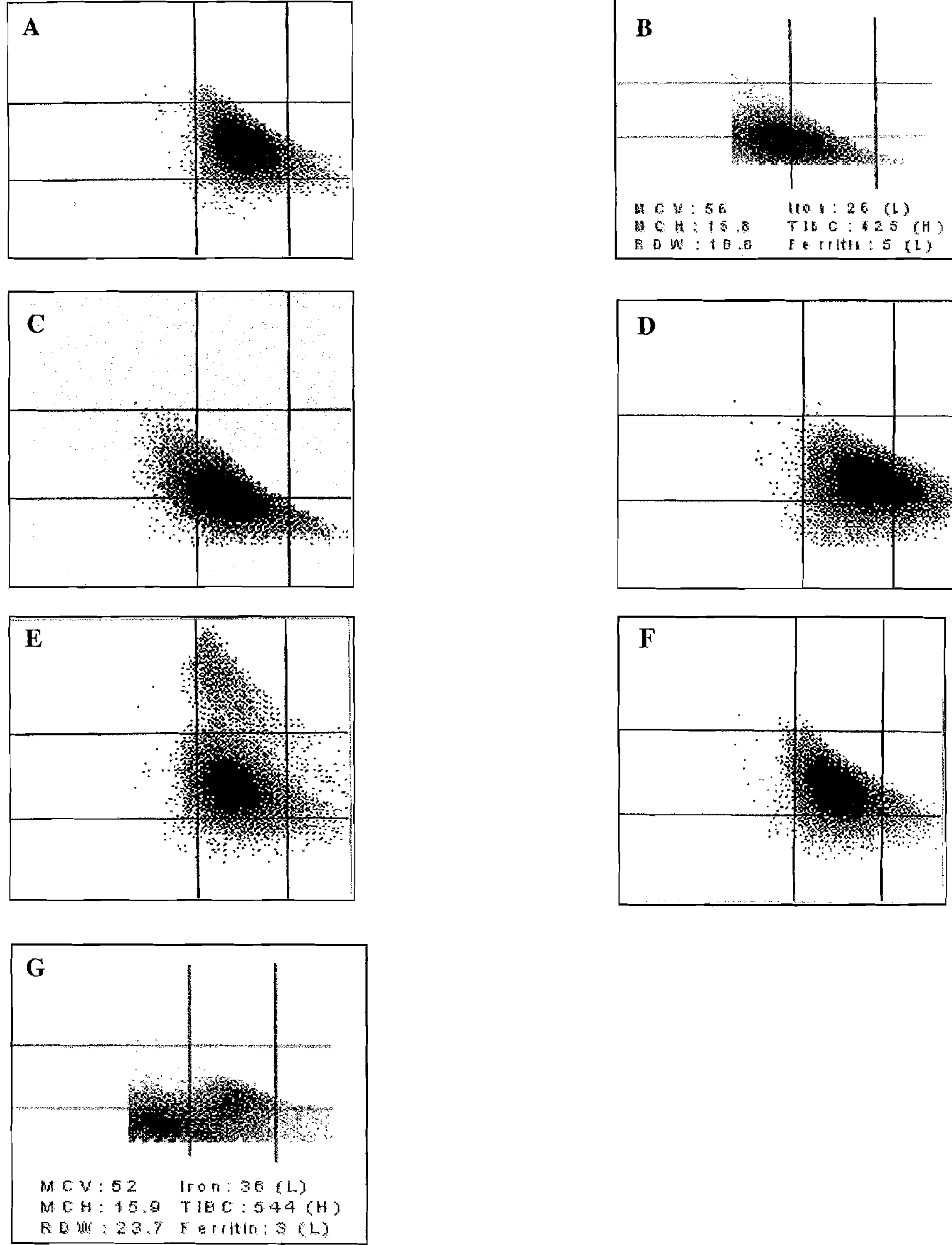
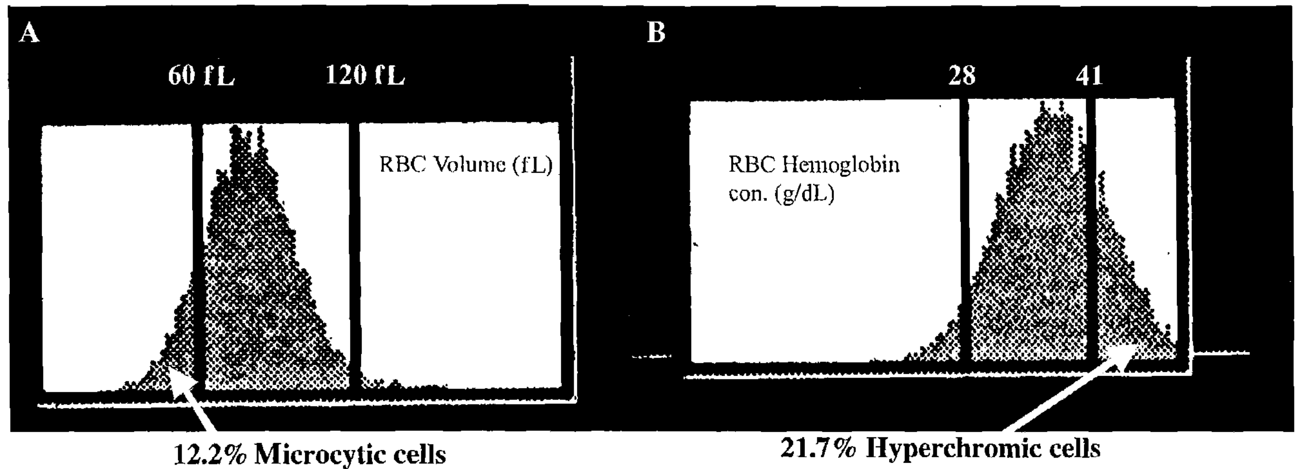


FIGURE 3. Red cell cytograms. A, Normal red cell cytogram. B, Patient with iron deficiency. Red cells are hypochromic and microcytic. C, Patient with β-thalassemia trait. Red cells are microcytic but less severely hypochromic than in iron deficiency. D, Patient with spherocytosis. Red cells are microcytic and hyperchromic. E, Patient with cold agglutinin. A second population of macrocytic and normochromic red cells is present in addition to normocytic and normochromic red cells. This second population represents 2 red cells sticking to each other. F, Same patient as in E after the sample had been warmed for 30 minutes at 37°C and reanalyzed. The second population of large cells has disappeared. G, Patient with iron deficiency who has received a blood transfusion. The patient's own red cells are hypochromic and microcytic; the transfused red cells are normocytic and normochromic.



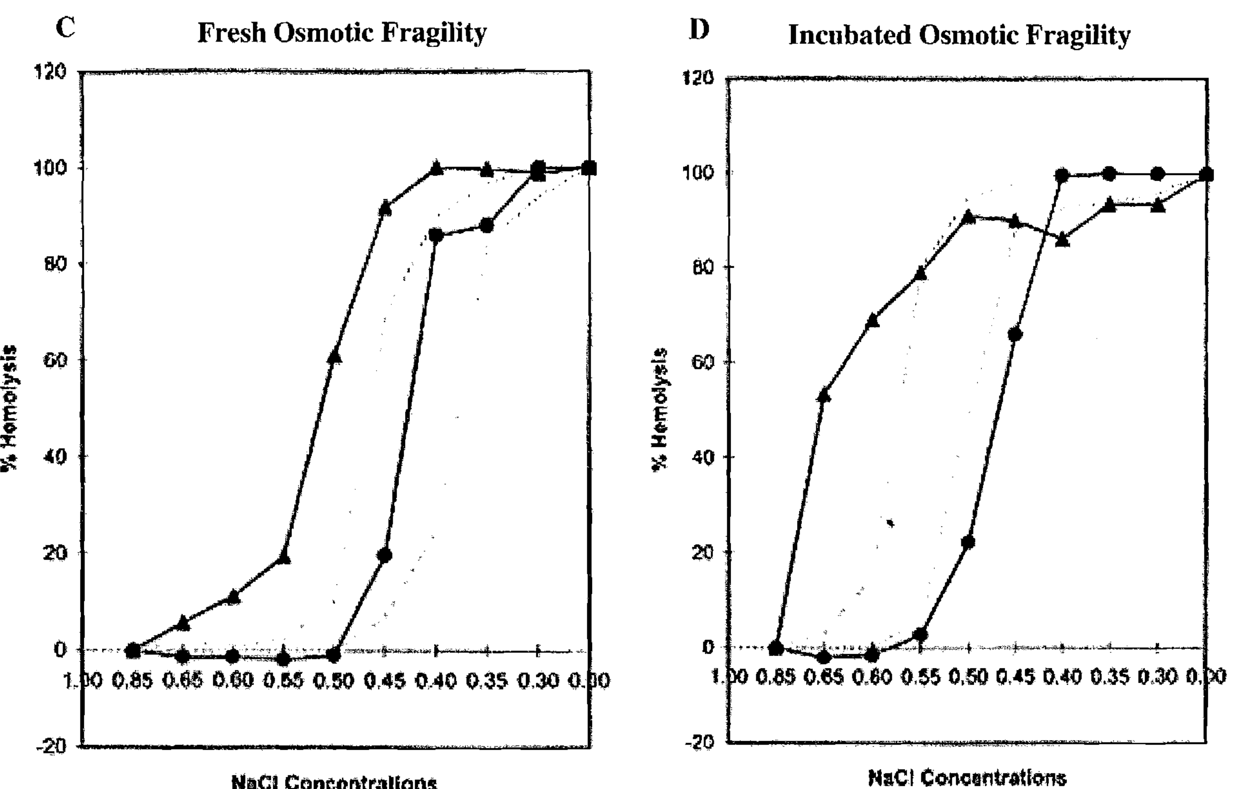
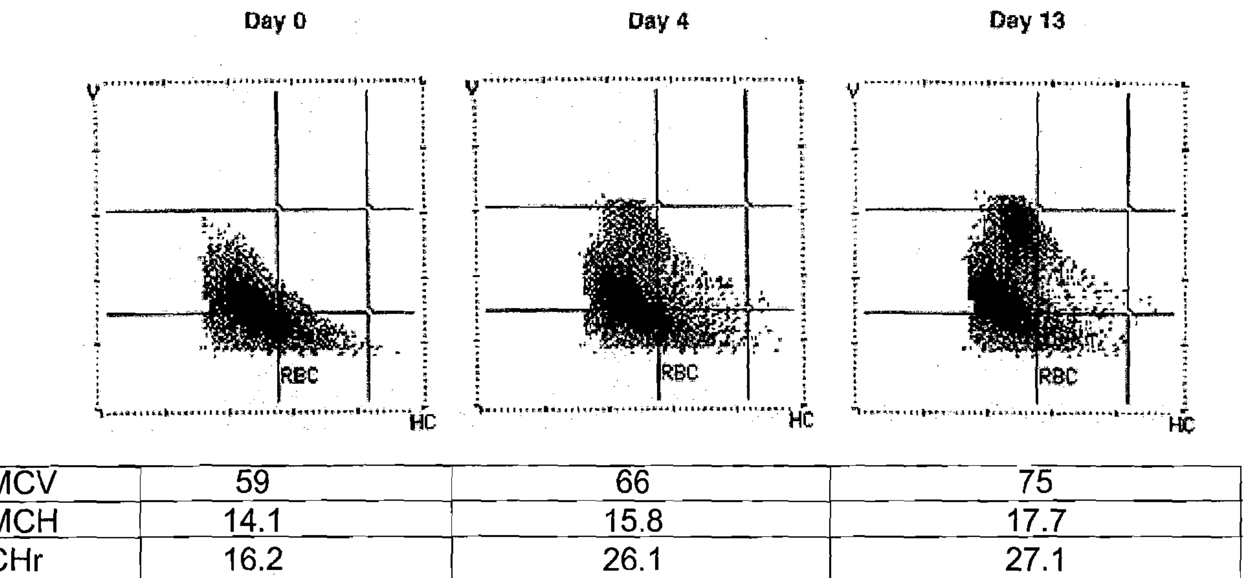


FIGURE 4. Hereditary spherocytosis. An increased percentage of microcytic cells (A) and hyperchromic cells (B) cells are present on red cell (RBC) analysis on the ADVIA 2120. The diagnosis of spherocytosis is confirmed by the increased osmotic fragility of the fresh (C) and the incubated (D) sample. ● indicates control sample; ▲, patient sample.

2-dimensional flow cytometric method used by the ADVIA 2120 makes it possible to evaluate a number of novel parameters [41,42]. The low- and high-angle light-scatter platelet cytogram (marked as area 1 in Figure 1A; Figure 1B) can be transformed by Mie light-scatter theory into a plot of platelet refractive index (x-axis) versus platelet volume (y-axis). The platelet refractive index is a measure of platelet density and is expressed in grams per deciliter [42,43]. Other parameters measured on the analyzer include the platelet volume distribution width, which is a measure of platelet size variation; the MPC; the platelet component distribution width (PCDW), which is a measure of the variation in platelet



MCV	59	66	75
MCH	14.1	15.8	17.7
CHr	16.2	26.1	27.1

FIGURE 5. Correcting iron deficiency: sequential red blood cell (RBC) cytograms and selected numeric parameters. On day 0, all of the patient's RBCs are hypochromic and microcytic. Mean corpuscular hemoglobin (MCH) and mean hemoglobin content of reticulocytes (CHr) differ by only 2 pg. On day 4 after the start of iron replacement, a second, small population of normocytic RBCs is present in addition to the hypochromic and microcytic RBCs. Although the MCH has increased by less than 3 pg, the CHr has already increased by almost 10 pg. On day 13 after the start of iron replacement, the 2 RBC populations are clearly discernible; the difference between the MCH and the CHr is still substantial. V indicates volume; HC, hemoglobin content; MCV, mean corpuscular volume. Reprinted with permission from [62]. Copyright 1996, Dr. Carlo Brugnara.

shape; mean platelet mass; and platelet mass distribution width [42]. Reference ranges for many of these parameters have been published [19,42,44], and reductions in the MPC (reflecting a decrease in platelet density) have been shown to be indicative of platelet activation [18,20,42,43]. The decrease in MPC was found to be associated with an increase in CD62P expression [20,42,43]. The MPC parameter has been used to investigate the role of platelet activation in coronary disease and in diabetic retinopathy [45,46], as well as in monitoring the efficacy of antiplatelet therapies [47]. MPC measurement has also been suggested as an inexpensive and rapid test to screen for platelet dysfunction caused by myelodysplasia [48]. The PCDW may constitute a convenient quality indicator for determining the viability of stored platelets [49].

White Cells

Peroxidase and Nuclear Density Analysis. The 2 WBC channel cytograms of the ADVIA 2120 System provide a simultaneous analysis of leukocyte myeloperoxidase content together with nuclear shape and density as well as total cell volume. This capability has led to the development of a diagnostic principle termed peroxidase and nuclear density analysis (PANDA) [50,51]. The PANDA method has the potential to become a new diagnostic tool, along with established methods such as immunophenotyping and genetic analysis, in the workup of leukemia.

Seven distinct peroxidase patterns (P0-P6) and 3 nuclear density patterns (D0-D2) have been identified (Figure 6). The 7 peroxidase patterns describe a gradient of increasing cellular myeloperoxidase content, with P0 reflecting the total absence of myeloperoxidase and P6 indicating an unusually high peroxidase content. With respect to nuclear density, the D0 pattern shows a normal rounded mononuclear cell cluster, and a D1 pattern indicates a mononuclear cell cluster that is shifted down and left to the blast area. In the D2 pattern, the main mononuclear cluster is shifted upwards. In the final analysis, a diagnostic grid is constructed with 7 columns representing the peroxidase content (P0-P6) and 3 rows of nuclear density (D0-D2). The various PANDA patterns can then be correlated with distinct diagnostic categories (Table 5) [51]. For example, acute myelogenous leukemia (AML) type M0 gives a P0 pattern, whereas AML M1, M2, M4, and M5 yield peroxidase patterns ranging from P0 to P4. AML M3 (acute promyelocytic leukemia) gives a characteristic P6 cytogram. We have noted that although AML is typically associated with a D1 density pattern, most acute lymphoblastic leukemias of the L1 type give a D0 or a weak D1 pattern.

Diagnosis of Myeloperoxidase Deficiency. Peroxidase analysis is a routine component of all automatic differentials performed on the ADVIA 2120. This capability has highlighted

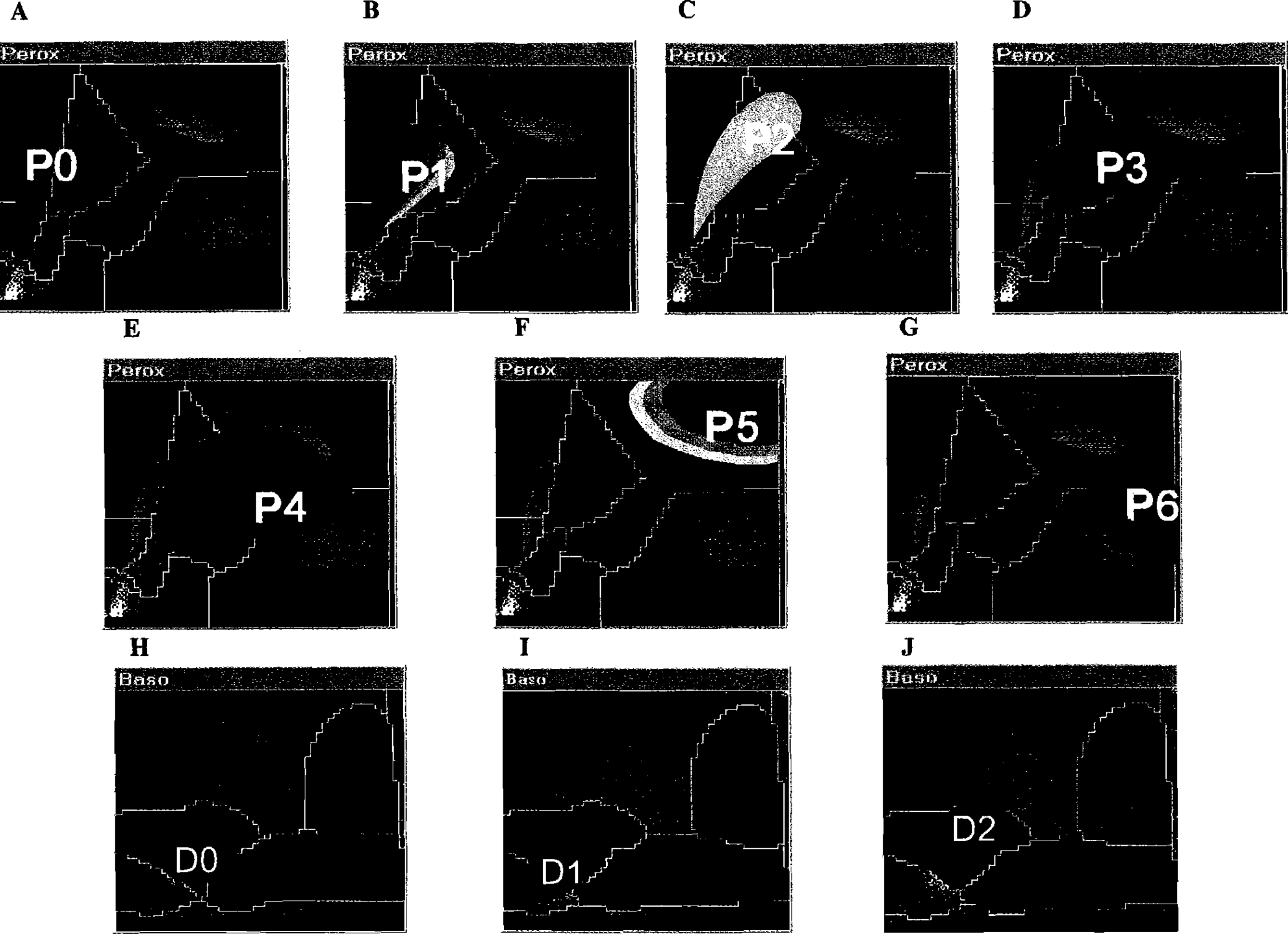


FIGURE 6. Peroxidase and nuclear density analysis (PANDA). The peroxidase patterns (A-G) represent different levels of cellular myeloperoxidase (P). In combination with the 3 nuclear density patterns (H-J), the peroxidase patterns can be used as a diagnostic tool in the workup of hematologic diseases.

the importance of myeloperoxidase deficiency, a condition with an approximate prevalence in Caucasians of 1 in 2500 [52]. Severe myeloperoxidase deficiency is associated with the P0 pattern in the PANDA system. Although the condition is usually clinically benign, it is occasionally associated with an increased susceptibility to certain infections. An association between myeloperoxidase deficiency and a vulnerability to certain malignancies has also been proposed [53].

Activation Status of Neutrophils. The ADVIA 2120 Hematology System compares the location of the neutrophil cluster in the peroxidase cytogram to a standard cluster and uses this information to calculate the myeloperoxidase intracellular index (MPXI). This parameter can be used to diagnose partial and total myeloperoxidase deficiency [10]. The MPXI has also been shown to be a measure of systemic neutrophil activation. Several reports have shown its potential usefulness in determining neutrophil activation in patients with myocardial ischemia [54,55], in determining the therapeutic efficacy of recombinant granulocyte colony-stimulating factor [56], and in studying neutrophil degranulation patterns in lymphoma transplantation patients with bacteremia [57].

Cerebrospinal Fluid Analysis

The ADVIA 2120 CSF Assay is an automated method that uses direct cytometry to enumerate RBCs and WBCs and provide a WBC differential for cerebrospinal fluid (CSF) patient samples [58]. Before the CSF specimen is loaded on the analyzer, a 4-minute pretreatment with CSF reagent is required to fix and spherize the cells. The cells are differentiated and enumerated via 3 optical measurements: (1) high-angle scatter, (2) low-angle scatter, and (3) absorbance. The signals are digitized and used to construct the CSF cytogram (Figure 7). The automated differential parameters include the percentages of mononuclear cells, polymorphonuclear cells, neutrophils, lympho-

TABLE 5. Peroxidase and Nuclear Density Analysis (PANDA)*

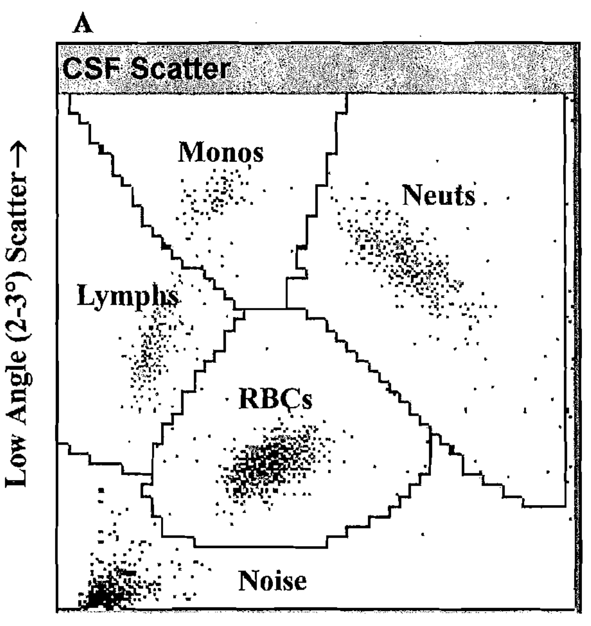
	P0	P1	P2	P3	P4	P5	P6
D0	CLL, PLL, HCL, ALL-L1, total MPO deficiency	Severe MPO deficiency	Partial MPO deficiency	CML	CML	AIDS, MDS	
D1	ALL (L1-L3), NHL, M0, M5a, M6, M7	M1, M5a, M2, M4	M2, M4, M5a, M5b, M1	M2, M4, CML-BC	M2, M3v, M4	M3v, atypical CML	M3
D2	Infectious mononucleosis, viral diseases						

*Adapted from [51] with permission from *Bloodline Reviews*. Copyright 2001, Carden Jennings Publishing Company, Ltd. CLL indicates chronic lymphocytic leukemia; PLL, prolymphocytic leukemia; HCL, hairy cell leukemia; ALL, acute lymphoblastic leukemia; MPO, myeloperoxidase; CML, chronic myelocytic leukemia; AIDS, acquired immunodeficiency syndrome; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; CML-BC, CML in blast crisis.

cytes, and monocytes. The cell counting is accurate up to 5100 WBCs/μL and 2100 RBCs/μL. If the RBC count exceeds 1500/μL, a specimen dilution to a maximum of 1:10 is recommended.

Studies by Aune et al and others have demonstrated excellent correlation between results with the standard manual CSF analysis and those with the automated method [58,59]. The slope and intercept were 0.87 and 8, respectively, for WBCs and 0.93 and 5 for RBCs. Precision was also very good. For low WBC counts (mean, 9/μL) and low RBC counts (mean, 22/μL), the between-run precision values were 20% and 19.5%. For high counts (WBC mean, 88/μL; RBC mean, 203/μL), the between-run precision values were 9.3% and 10.1%. Carryover was minimal. Hypochromic and microcytic specimens require a longer pretreatment than normochromic, normocytic CSF specimens.

The advantages of the automated CSF analysis over manual methods include a more reliable analysis, because more cells are counted than with the traditional chamber method,



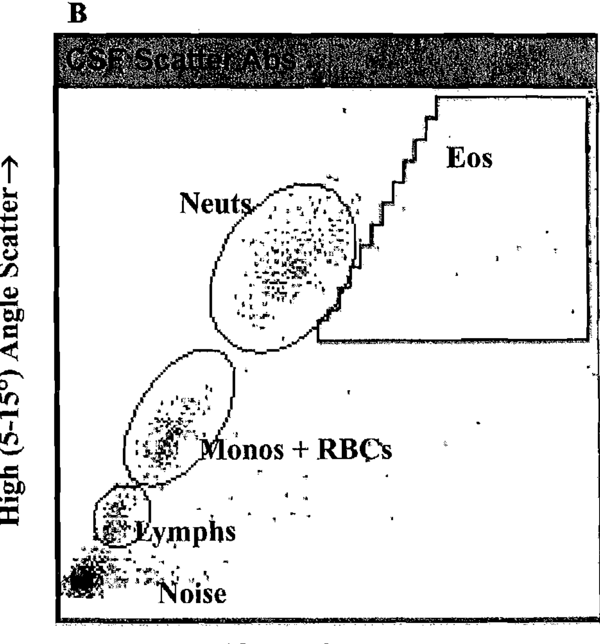


FIGURE 7. ADVIA 2120 CSF Assay cytograms. Three optical signals are measured for cerebrospinal fluid (CSF) analysis. Two signals represent low-angle (2°-3°) and high-angle (5°-15°) scatter signals. The third signal is an absorption measurement. After conversion to digital form, scatter and absorption signals are paired to generate CSF cytograms. High-angle scatter signals are paired with low-angle signals to form the CSF scatter/scatter cytogram (A), and low-angle signals are paired with absorption signals to form the CSF scatter/absorption cytogram (B). Both CSF cytograms have a resolution of 100 × 100 channels. Monos indicates monocytes; lymphs, lymphocytes; neuts, neutrophils; RBCs, red blood cells; Eos, eosinophils.

therefore achieving increased accuracy and precision. In addition, turnaround times can be decreased significantly, and analysis can be performed at all hours by personnel who are not experts at reading microscopy slides.

FUTURE DIRECTIONS

Fetal Lung Maturity

The determination of fetal lung maturity is an important diagnostic component in the prevention of respiratory distress syndrome (RDS) in the newborn in instances of premature delivery. Lung maturity analysis is aimed at evaluating (1) the risk for RDS and (2) whether delivery should be delayed. Current methods of determining lung maturity include measuring the "FLM" (surfactant-to-albumin ratio), which is an automated assay, and determining the lecithin-sphingomyelin ratio. The latter is very labor intensive, requiring several hours of analysis. In many academic centers, clinicians demand 2 or more independent methods of determining lung maturity.

Lamellar bodies are small, surfactant-containing structures produced by type II pneumocytes in the fetal lung. They appear in amniotic fluid in increasing numbers as the fetal lungs mature. Counts of lamellar bodies are therefore an index of fetal lung maturity. Counts of lamellar bodies in the amniotic fluid can be rapidly determined in the clinical laboratory by using the platelet channel of several hematology analyzers. The ADVIA 2120 also can be used for this purpose. Lamellar bodies can be counted in the platelet channel by using high- and low-angle laser light scatter [60]. Unlike platelet counting, all of the data points in this area of the scattergram are collected and counted (in platelet counting, certain light-scatter thresholds are set to exclude RBC fragments and RBC ghosts). A detailed study of 88 amniotic fluid samples by Chapman et al demonstrated that the counting of amniotic fluid lamellar bodies with the ADVIA 120 has a very good diagnostic performance [60]. The prevalence of RDS in this study was 15.9%. A cutoff lamellar body count of 35,400/µL resulted in a predictive value of 100% for lung maturity. The area under the receiver operating characteristic curve was 0.935. The predictive value for lung immaturity was 36.8%. This study indicates that counting lamellar bodies on the ADVIA instrument is a rapid, automated diagnostic test that is relatively easy to perform and that provides useful information regarding fetal lung maturity.

Bone Marrow

Microscopical review of bone marrow aspirates is often performed for diagnostic evaluation and monitoring patients with hematologic malignancies. Pedemonte et al reported that the ADVIA 120 system can be used as an alternative method for evaluating bone marrow aspirates [61]. Combined use of light-scatter measurements and peroxidase staining intensity enables the determination of marrow cellularity, the presence of different cell clusters, the presence of blasts, and the status of dyspoiesis. The automated analysis could be used as a rapid and objective screening method to evaluate bone marrow aspirates and could serve as an aid in assessing most hematologic malignancies [61].

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Automated Flow Cytometric Analysis of Blood Cells in Cerebrospinal Fluid

Analytic Performance

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Key Words: Cerebrospinal fluid; ADVIA 120; CSF WBC count; CSF RBC count; CSF WBC differential; Flow cytometry; Automated cell analysis

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Abstract

We compared the performance of an automated method for obtaining RBC and WBC counts and WBC differential counts in cerebrospinal fluid (CSF) samples with the reference manual method. Results from 325 samples from 10 worldwide clinical sites were used to demonstrate the accuracy, precision, and linearity of the method.

Accuracy statistics for absolute cell counts showed a high correlation between methods, with correlation coefficients for all reportable absolute counts greater than 0.9. Linearity results demonstrated that the method provides accurate results throughout the reportable ranges, including clinical decision points for WBCs of 0 to 10/µL. Interassay precision and intra-assay precision for the ADVIA 120 (Bayer HealthCare, Tarrytown, NY) method were acceptable at all levels.

The ADVIA 120 CSF Assay enumerates and differentiates cells via flow cytometry in a minimally diluted sample, improving the analysis of typically hypocellular CSF samples. Study results demonstrate that the automated ADVIA 120 CSF Assay is an acceptable alternative to the labor-intensive manual method.

Hematologic analysis of cerebrospinal fluid (CSF) specimens can provide clinicians with valuable diagnostic information, because abnormal numbers of WBCs in a CSF sample (a mononuclear cell count of >5/µL in adults or >30/µL in newborns)[1] can indicate one of several serious medical conditions, such as meningitis, encephalitis, neurologic disorders, and leukemic CSF infiltrations. CSF WBC counts also can be used to monitor the effectiveness of therapy for patients with leukemia or lymphoma. Unusually elevated RBC counts can indicate cerebral hemorrhage or a traumatic spinal tap because normal specimens contain no RBCs.[1]

Conventional methods for the hematologic analysis of CSF specimens require subjective, visual cell counting and cell differentiation using a small specimen. These time-consuming, technique-dependent procedures are prone to interoperator variability and low precision.[1-3]

The introduction of semiautomated and automated methods of analysis has reduced interoperator variability and improved turnaround time and precision[4]; however, these methods often are hampered by electronic background noise, which might falsely elevate cell counts, most notably in cytopenic specimens. In addition, the imprecision of the ratio-derived WBC differential also is a concern because the number of certain cell types might be of substantial value in diagnosing and monitoring treatment in conditions such as meningitis and leukemia.[1]

This study was designed to evaluate the performance of the automated ADVIA 120 CSF Assay, with respect to accuracy, precision, linearity, and ease-of-use. The assay is available on the ADVIA 120 hematology analyzer (Bayer HealthCare, Tarrytown, NY).

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Materials and Methods

Patient CSF Samples

We tested 325 clinical samples at 10 worldwide clinical trial sites. The CSF samples represented a cross-section of patient populations, including pediatric, oncology, and neurology patients. Cytograms from specimens run on the ADVIA 120 hematology analyzer were reviewed for interfering substances and analysis artifacts.

Results were excluded from the analysis only if a sample displayed interference or cell counts outside the range limits of the method. *Interference* included conditions in which cell populations crossed predefined cell counting thresholds, ie, specimens that could not be diluted to an RBC level of less than 1,500/µL without exceeding the 1:10 dilution limit of the assay. When the RBC count exceeds 1,500/µL, the WBC and neutrophil counts might be falsely elevated. Hypochromic RBCs might interfere with the WBC and lymphocyte counts. As shown in studies performed at Haukeland University Hospital, Bergen, Norway, increasing the prepared sample incubation time for CSF samples containing hypochromic and/or microcytic RBCs might increase the number of samples that can be analyzed on the ADVIA 120. We excluded 62 samples (19.1%) from the analysis based on these criteria. The exclusion rate varied among the sites, averaging 6% at 9 sites. One clinical site had an exclusion rate of 49% because almost half the samples submitted by this teaching hospital demonstrated markedly elevated RBC counts, probably caused by contamination of CSF specimens with peripheral blood.

Reference Manual Method

Reference manual RBC and WBC counts and WBC proportional counts were performed according to the standard protocol used at each of the 10 clinical sites.[5] For the absolute RBC counts, this involved visual enumeration of cells in a counting chamber after dilution with 0.9% sodium chloride, if needed. Absolute WBC counts also were obtained visually after a counting chamber was charged with cells stained by methyl violet acetic acid. The volume of sample typically analyzed in a Neubauer counting chamber is 1 µL. The 100-cell WBC differential was performed after cytocentrifugation of the samples, followed by Wright staining. The ratio-derived differential was calculated by multiplying the number of lymphocytes, monocytes, and granulocytes found by the absolute WBC count for that specimen. For the present study, manual counts were performed by 2 independent technologists. A third cell count was performed if the WBC counts were discrepant by 30% or more.

ADVIA 120 CSF Assay

The ADVIA 120 CSF Assay, available on the ADVIA 120 hematology analyzer, is an automated method that uses direct cytometry to enumerate RBCs and WBCs and provides a WBC differential on CSF patient samples. Reportable parameters for the WBC differential include absolute and proportional counts for neutrophils, lymphocytes, and monocytes and for a research-use-only eosinophil count.

The ADVIA 120 CSF Assay procedure consists of mixing 300 µL of the CSF specimen with ADVIA 120 CSF Reagent (Bayer HealthCare) in a 1:1 ratio to sphere and fix the cells. After a minimum 4-minute to a maximum 4-hour incubation period, the prepared sample is aspirated directly into the ADVIA 120 system set to CSF Analysis mode, in which the cells are differentiated and enumerated via 3 optical signals (2 different light scattering angles and 1 absorption measurement) that are detected and digitized and used to generate CSF cytograms with 100 × 100-channel resolution. Results are calculated automatically **Figure 1**. Automated results are based on the analysis of approximately 4 µL of patient sample, or 4 times that of the manual method.

Owing to the low cell numbers typically seen in CSF samples, it is extremely important to eliminate potential background interference. To accomplish this, the assay procedure requires the operator to verify that the total number of events displayed in the CSF scatter/scatter cytogram, including the noise region, is less than 10 and that the background count for all cell counting areas is zero before analysis. If counts fall outside these parameters, the operator must perform a manufacturer-defined cleaning procedure.

Reportable parameter ranges for the ADVIA 120 CSF Assay are as follows: CSF WBC, 0 to 5,000 cells per microliter; and proportional CSF WBC differentials for samples with CSF WBC counts greater than 20 cells per microliter. The reportable range for CSF RBC count is 0 to 2,880 cells per microliter; however, if the RBC count is greater than 1,500 cells per microliter, RBC coincidence events might overlay WBC counting areas, interfering with the WBC count and differential. Samples with more than 1,500 RBCs per microliter can be diluted up to 1:10 with phosphate-buffered saline or normal saline before preparing the sample for ADVIA 120 CSF analysis.

Performance Characteristics

Accuracy

Accuracy of the ADVIA 120 CSF method was evaluated by regression analysis, comparing 263 automated results for absolute RBC and WBC counts and absolute and proportional WBC differential values with the reference manual counts.

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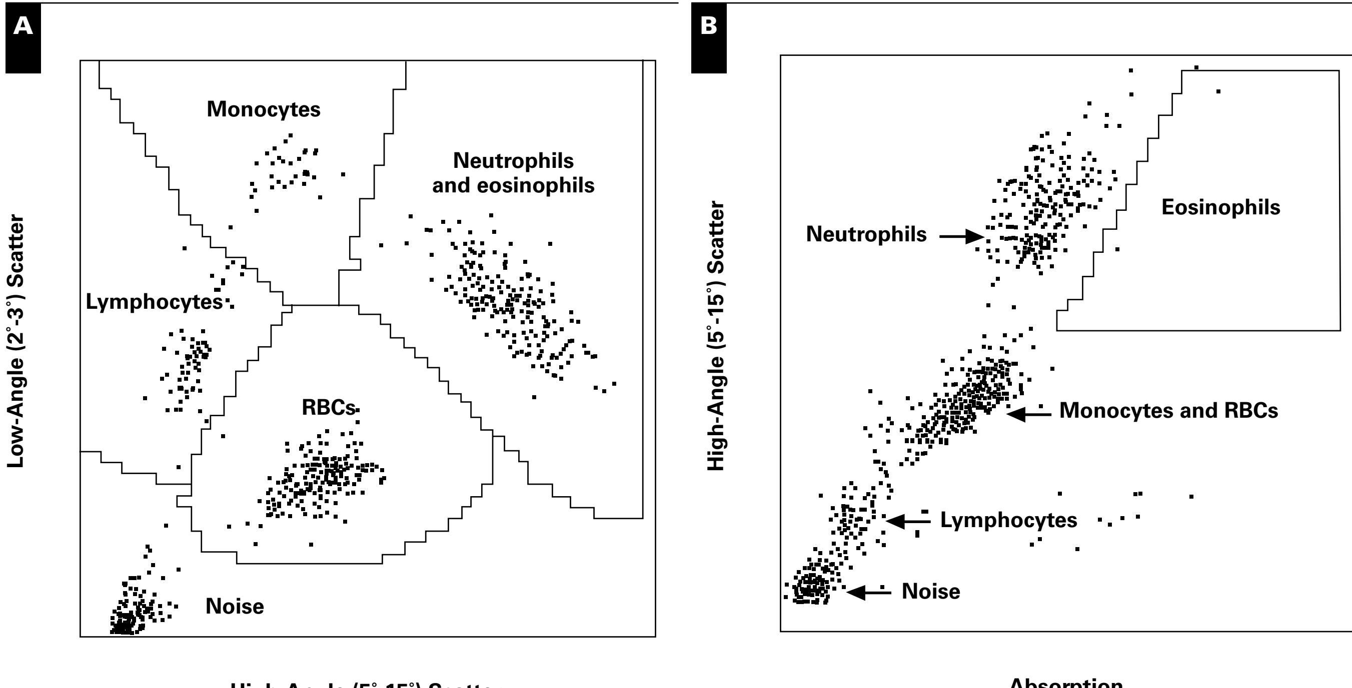

Figure 1 ADVIA 120 CSF [cerebrospinal fluid] Assay cytograms. Three optical signals are measured for CSF analysis. Two of these represent low-angle (2°-3°) and high-angle (5°-15°) scatter signals. The third signal is an absorption measurement. After conversion to digital form, scatter and absorption signals are paired to generate CSF cytograms. **A**, High-angle scatter signals are paired with low-angle signals to form the CSF scatter/scatter cytogram. **B**, Low-angle signals are paired with absorption signals to form the CSF scatter/absorption cytogram. Both CSF cytograms have 100 × 100-channel resolution. For proprietary information, see the text.

Precision

Interassay precision for the absolute counts was obtained by analyzing 38 samples from the accuracy study that were assayed more than once. The samples were run 2 to 10 times each on the ADVIA 120 hematology analyzer, depending on available sample volume. Fifteen samples from the accuracy study with WBC counts of more than 20/μL were used to determine interassay precision for the proportional WBC differential. These samples also were assayed 2 to 10 times each on the hematology analyzer, depending on available sample volume. Intra-assay precision was calculated by using values obtained from 2-level ADVIA TESTpoint CSF control materials (Bayer HealthCare), which were run daily at the clinical trial sites.

Linearity and Carryover

Linearity was calculated by using separate WBC and RBC serial sample dilutions prepared at the high extreme of the proposed linearity ranges. A concentrated WBC cell suspension was diluted serially to produce 7 concentration levels containing 0 to 5,000 WBCs per microliter. Similarly, a concentrated RBC cell suspension was diluted serially to produce 7 concentration levels containing 0 to 2,880 RBCs per microliter. Carryover was evaluated with the linearity pools by assaying the high-level pool followed by 3 assays of the cell-free pool.

Prepared Sample Stability

In studies conducted at Haukeland University Hospital, 10 CSF patient samples were prepared with ADVIA 120 CSF reagent and tested at 4 minutes and 4 hours. Automated values for the number of WBCs and RBCs and the percentage of mononuclear cells and polymorphonuclear cells at the 2 time points were compared.

Effect of Increased Incubation Time on Samples Containing Hypochromic and/or Microcytic RBCs

It was observed that hypochromic and/or microcytic RBCs in some CSF samples failed to sphere completely within the standard 4 minutes after preparation with CSF reagent. An experiment was performed at Haukeland University Hospital to address this observation. A volume of homologous K_2EDTA-anticoagulated whole blood with the desired RBC parameter profiles was added to filtered CSF to create surrogate CSF samples. Two such samples were made to contain hypochromic and/or microcytic RBCs, and 1 was

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made to contain normochromic and normocytic RBCs as a control sample. These 3 samples were tested on the ADVIA 120 for a period of 4 to 30 minutes. Automated RBC and WBC counts at 8 time points were compared with the initial manual RBC and WBC counts.

Results

Accuracy

The agreement between the ADVIA 120 CSF Assay and reference manual cell counts for RBC and WBC counts and proportional WBC differential values was determined. Accuracy statistics comparing 263 absolute counts on the ADVIA 120 CSF Assay with the manual method demonstrated excellent correlation between the methods. For WBC counts in the range of 0 to 3,000/μL, the correlation coefficient was 0.988, with a mean ADVIA 120 CSF WBC value of 56/μL and a mean manual value of 55/μL. The correlation coefficient for the absolute RBC counts in the range of 0 to 2,700/μL was 0.988 with the mean ADVIA 120 CSF RBC value of 135/μL and a mean manual value of 139/μL. Regression analysis results are shown in **Table 1**. The x, y regression plots are displayed in **Figure 2A** and **Figure 2B**.

Only samples with WBC counts greater than 20/μL were used for differential WBC count comparisons. As shown in Table 1, these samples demonstrated excellent correlation, with correlations ranging from 0.967 to 0.928 with the respective mean values for the manual and automated methods equivalent for lymphocytes (10 and 10/μL) and differing by 1 cell for neutrophils (3 and 4/μL) and monocytes (4 and 5/μL). The x, y regression plots are shown in **Figure 2C**, **Figure 2D**, **Figure 2E**, **Figure 2F**, and **Figure 2G**.

Proportional WBC differential results also were analyzed according to the National Committee for Clinical Laboratory Standards H20-A guidelines,[5] using 15 samples with WBC counts greater than 20/μL. As shown in **Table 2**, the paired *t* test demonstrated statistically insignificant differences; 98% of the mononuclear and polymorphonuclear counts fell within the binomial envelope surrounding the manual counts.[2,3]

Typical cell distribution patterns were clearly demonstrated on the ADVIA 120 cytograms from representative clinical meningitis cases **Image 1** and **Image 2**. In the viral meningitis case (Image 1), the automated WBC population was predominantly mononuclear, consisting of 76% monocytes and 3% lymphocytes, which is similar to the manual proportional count and reflects the cell distribution seen on the stained blood smear. In the bacterial meningitis case (Image 2), the automated WBC population was predominantly neutrophilic, similar to the manual proportional count, again reflecting the cell distribution seen on the stained blood smear.

Precision

ADVIA 120 Within-Run Precision

A total of 121 ADVIA 120 CSF Assay tests were performed on 38 samples from the accuracy study to determine the within-run precision for the absolute counts, giving 121 – 38 = 83 degrees of freedom for the resulting pooled variance. Only samples with WBC counts of more than 20/μL were used for analysis of the proportional WBC differential. A total of 40 ADVIA 120 CSF differential counts were performed on 15 samples from the accuracy study, giving 40 – 15 = 25 degrees of freedom for the resulting pooled variance. The assay demonstrated excellent precision with coefficients of variation (CVs) of 8.2% and 3.8% for WBC and RBC absolute counts, respectively **Table 3**.

A separate analysis was performed to determine the within-run precision for samples with WBC counts in the range of 0 to 5/μL. A total of 55 assays were performed on 16 samples giving 55 – 16 = 39 degrees of freedom for the resulting pooled variance. Observed CVs were 30.0% and 3.4% for the WBC and RBC counts at levels of 2 and 253 cells per microliter, respectively **Table 4**.

Table 1
Accuracy of Absolute Counts in Cerebrospinal Fluid Samples*

Parameter (/μL)	*r*	Slope	Intercept	S_{yx}	Manual Mean	ADVIA 120 Mean	Bias
WBC	0.988	0.87	8	29	55	56	1
RBC	0.988	0.93	5	62	139	135	–4
Mononuclear cells	0.953	1.02	3	16	18	21	3
Polymorphonuclear cells	0.981	0.81	2	14	18	17	–1
Neutrophils	0.967	1.23	0	6	3	4	1
Lymphocytes	0.964	0.86	1	9	10	10	0
Monocytes	0.928	1.24	0	8	4	5	1

S_{yx}, standard error of the regression line.
* Results from the ADVIA 120 CSF [cerebrospinal fluid] Assay were compared with the results obtained with the manual methods performed at 10 sites.

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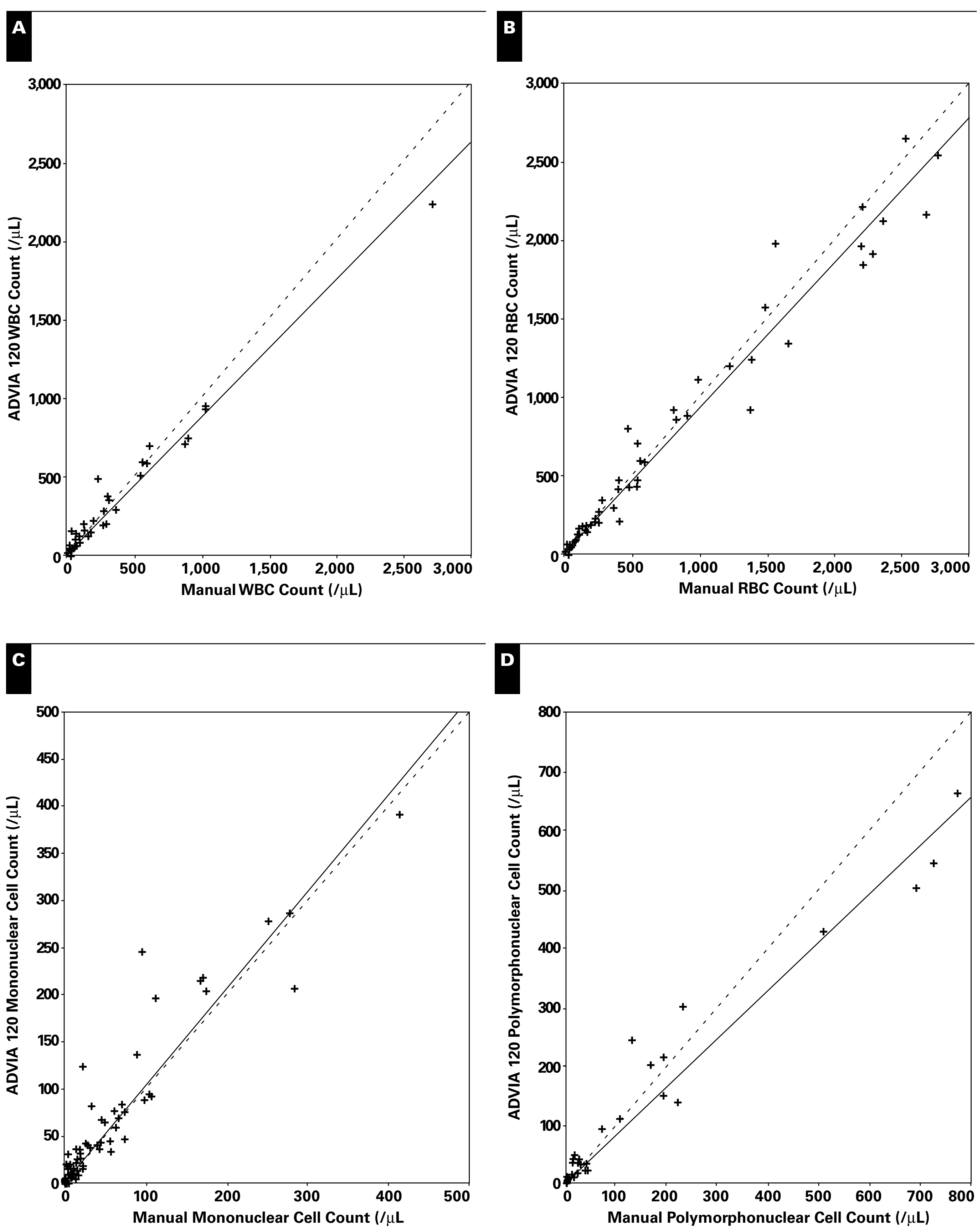

Figure 2 Graphic representations of the regression comparing the automated and manual results for absolute counts. **A**, WBC count. **B**, RBC count. **C**, Mononuclear cell count. **D**, Polymorphonuclear cell count.

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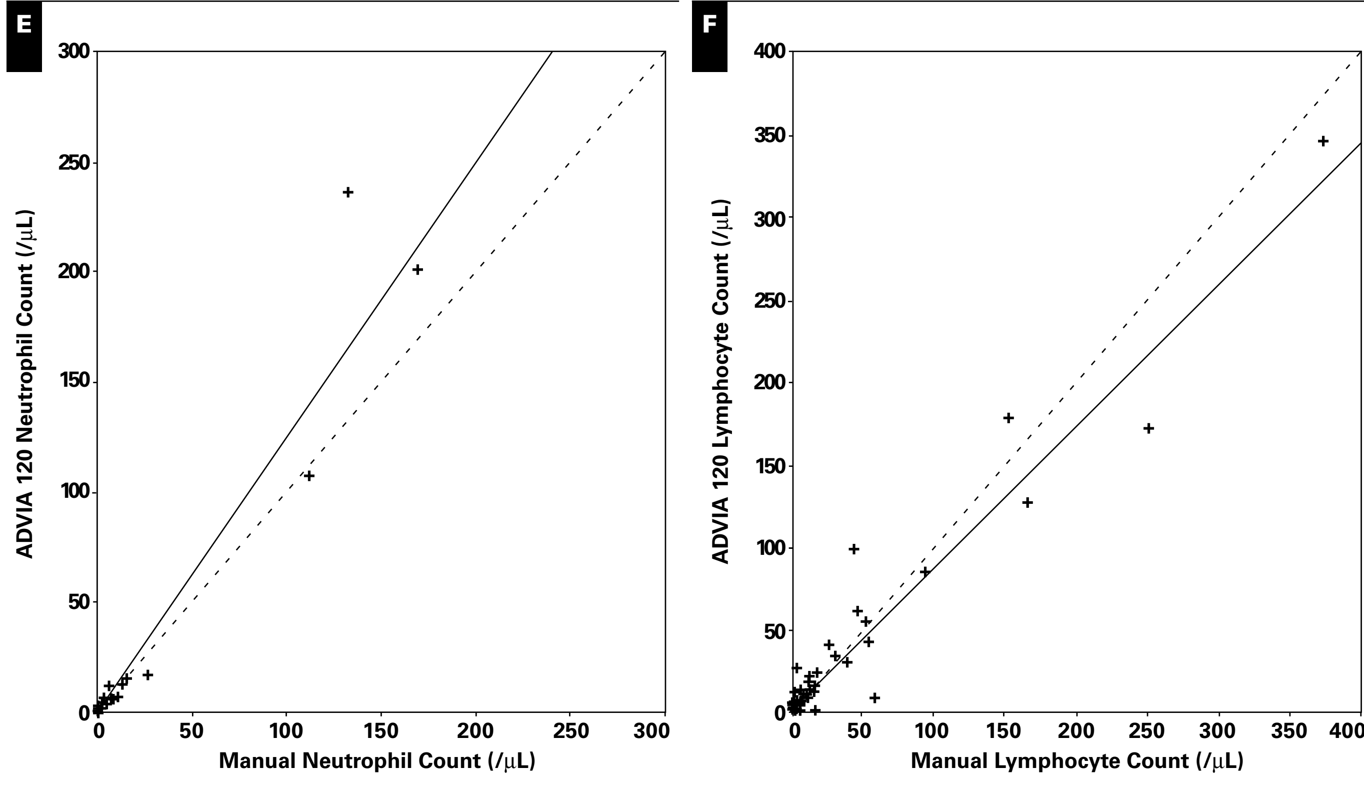

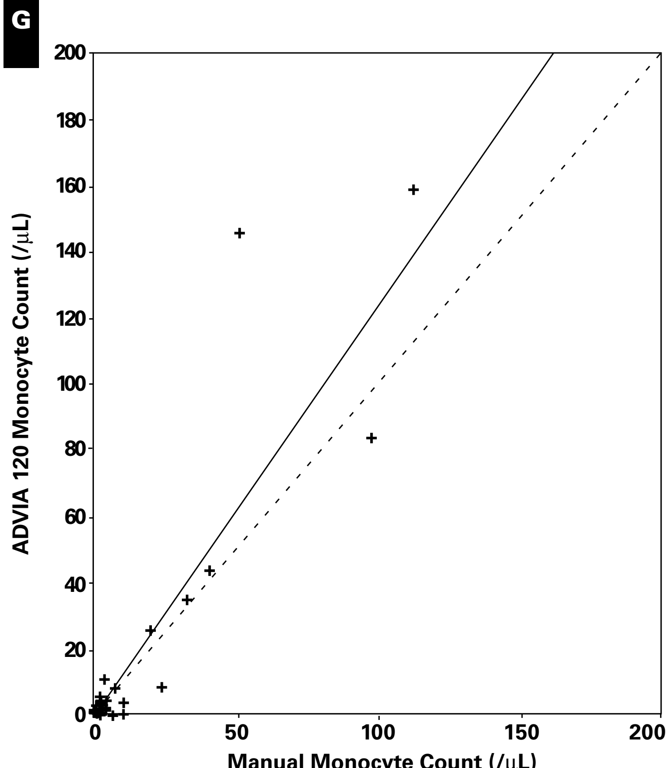

Figure 2 **E**, Neutrophil count. **F**, Lymphocyte count. **G**, Monocyte count. For regression data, see Table 1. For proprietary information, see the text.

ADVIA 120 Between-Run Precision

ADVIA TESTpoint CSF control materials were assayed daily at the trial sites and analyzed to determine the intra-assay precision of the ADVIA 120 CSF Assay. Results for the low-level control (ADVIA 120 TESTpoint CSF Level 1) demonstrated intra-assay precision of 19.5% and 20.0% for RBCs and WBCs, respectively **Table 5**. The mean WBC count for 10 clinical sites was 9/µL (SD ± 1.8), and the mean RBC count for the same sites was 22/µL (SD ± 4.3).

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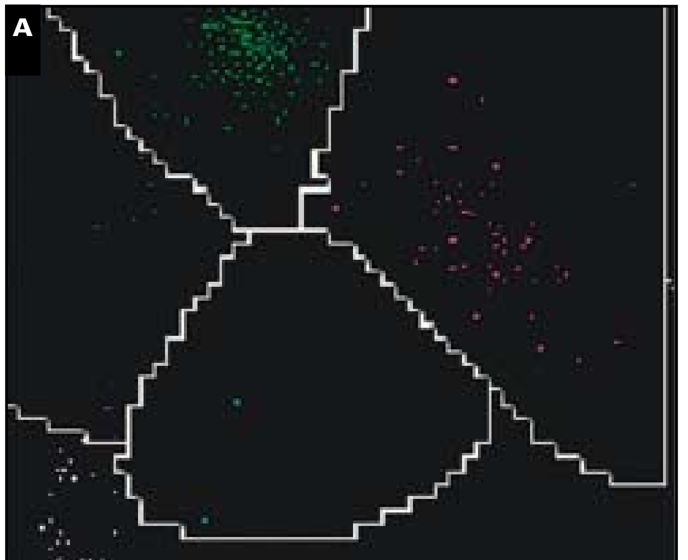
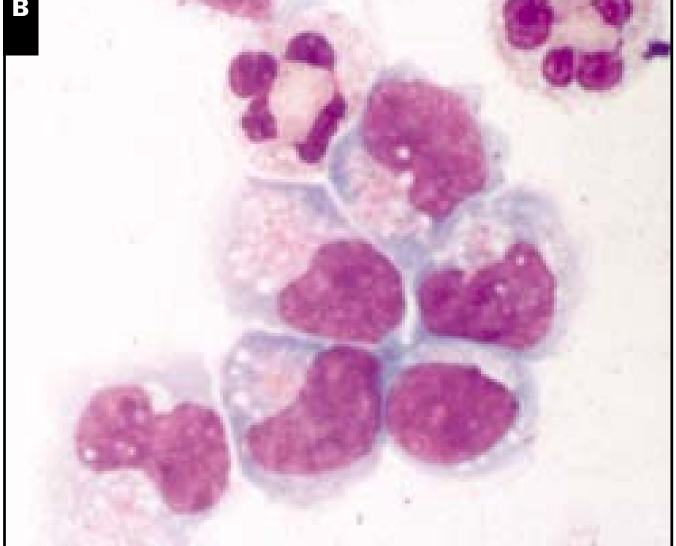
Table 2
Accuracy of Proportional Differential in Cerebrospinal Fluid Samples*

Parameter (%)	Manual Mean	ADVIA 120 Mean	*P*†	Within Binomial Limit (%)
Mononuclear cells	60.0	66.7	.102	98
Polymorphonuclear cells	37.8	33.3	.089	98
Neutrophils	21.4	17.7	.104	99
Lymphocytes	56.0	63.3	.108	96
Monocytes	20.6	18.0	.453	96
Eosinophils	0.5	1.0	.025	88

* Data were obtained using analysis recommended by the National Committee for Clinical Laboratory Standards H20-A standard.[5] Eosinophil counts are available for research use only.
† Paired *t* test.





	ADVIA 120	Manual
RBC (/µL)	1	1
WBC (/µL)	67	49
Mononuclear cells (%)	79	70
Polymorphonuclear cells (%)	21	30
Neutrophils (%)	21	30
Lymphocytes (%)	3	11*
Monocytes (%)	76	59†

* Atypical.
† Monocytic, 53; macrocytic, 6.

Image 1 Representative data from cerebrospinal fluid samples diagnosed as viral meningitis. Viral meningitis frequently is characterized by a predominance of mononuclear cells. The WBC distribution pattern on the cytogram from the automated analysis reflects the cell distribution seen on the stained blood smear. **A**, The monocyte population is predominant and is located high and slightly to the right in the monocyte region. The automated WBC population consists of 76% monocytes and 3% lymphocytes, which is similar to the manual proportional count, and reflects cell distribution seen on the stained blood smear in which the monocytes are large and heavily granulated (**B**, Wright-Giemsa, oil immersion at ×100).

Results for the high-level control (ADVIA 120 TESTpoint CSF Level 2) demonstrated intra-assay precision of 10.1% and 9.3% for the same parameters **Table 6**. Intra-assay precision for the WBC differential with the high-level control was well within the expected range, with CVs of 7.3% for neutrophils, 9.6% for lymphocytes, and 28.3% for monocytes (Table 6). The mean WBC count for 10 clinical sites was 88/µL (SD ± 8.2), and the mean RBC count for the same sites was 203/µL (SD ± 20.6).

Linearity

Linearity pools were prepared by making serial dilutions (0.00%, 0.05%, 0.10%, 0.20%, 1%, 10%, and 100%) of samples prepared at the high extreme of the proposed linearity ranges. Separate pools were prepared for the WBC and RBC counts. The results demonstrated that the ADVIA 120 CSF Assay is accurate up to 5,137 WBCs per microliter within 10% and accurate up to 2,165 RBCs per microliter within 10% **Table 7**.

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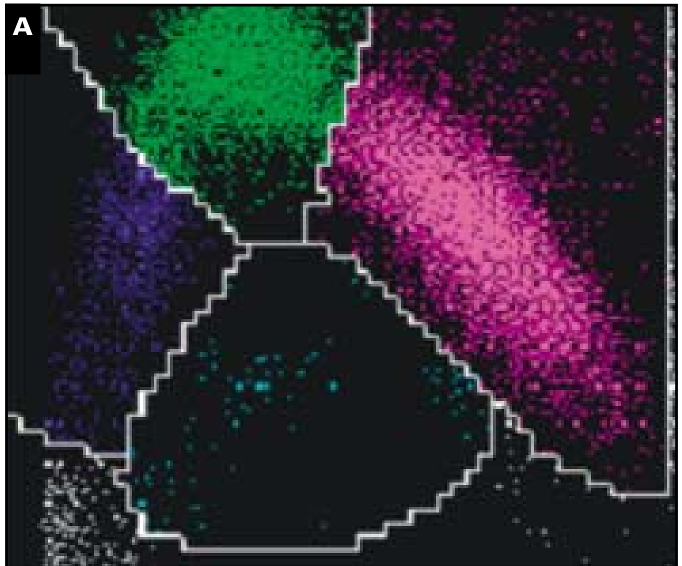

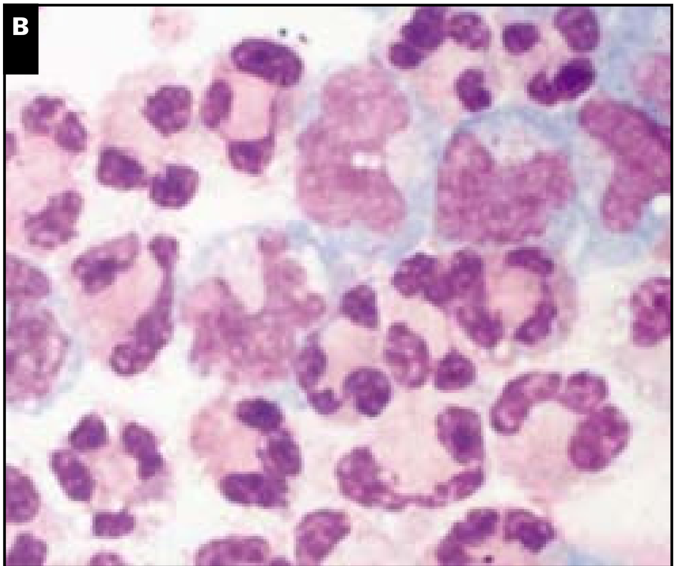

	ADVIA 120	Manual
RBC (/µL)	16	1
WBC (/µL)	3,838	3,089
Mononuclear cells (%)	31	22
Polymorphonuclear cells (%)	69	78
Neutrophils (%)	69	78
Lymphocytes (%)	3	3
Monocytes (%)	28	19*

* Hazy, xanthochromic.

❚Image 2❚ Representative data from cerebrospinal fluid samples diagnosed as bacterial meningitis. Bacterial meningitis often is consistent with a predominance of polymorphonuclear cells. The WBC distribution pattern on the cytogram from the automated analysis reflects the cell distribution seen on the stained blood smear. **A**, The WBC distribution pattern on the cytogram from the automated analysis reflects the cell distribution seen on the stained blood smear. The automated WBC population is predominantly neutrophilic (monocytes also are predominant), similar to the manual proportional count, and reflects the cell distribution seen on the stained cytocentrifuged blood smear (**B**, Wright-Giemsa, oil immersion at ×100).

❚Table 3❚
Within-Run Precision: Absolute and Proportional Counts in Cerebrospinal Fluid Samples

Parameter	Mean	SD	Coefficient of Variation (%)
Absolute counts (/µL)			
WBC	56	4.6	8.2
RBC	540	20.7	3.8
Mononuclear cells	25	3.7	14.8
Polymorphonuclear cells	5	0.9	18.0
Neutrophils	5	1.0	20.0
Lymphocytes	23	3.5	15.2
Monocytes	2	0.8	40.0
Eosinophils	0	0.4	NA
Proportional counts (%)			
Mononuclear cells	81	4.7	5.8
Polymorphonuclear cells	19	4.7	24.7
Neutrophils	17	4.2	24.7
Lymphocytes	74	5.0	6.8
Monocytes	7	1.6	22.9
Eosinophils	2	2.1	105.0

NA, not available.

❚Table 4❚
Within-Run Precision for Cerebrospinal Fluid Samples Containing 0 to 5 WBCs/µL

	Average Count (/µL)	
	RBC	WBC
Mean	253	2
SD	8.5	0.6
Coefficient of variation (%)	3.4	30.0

Carryover

Carryover was evaluated with the linearity pools by assaying the high-level pool followed by 3 assays of the low-level (cell-free) pool. This protocol, which was performed at 3 clinical sites, demonstrated insignificant carryover for CSF WBCs and CSF RBCs **❚Table 8❚**. Background counts were not performed in this phase of the study to demonstrate carryover. Background counts are recommended before acquisition of clinical samples.

Prepared Sample Stability

Prepared sample stability was evaluated by comparing automated RBC and WBC counts and the percentages of mononuclear and polymorphonuclear cells for 10 CSF samples analyzed at 4 minutes and 4 hours **❚Table 9❚**. No clinically significant differences were seen between the values at these 2 time points. Results of *t* tests (paired two-sample for means) comparing parameter values at 4 minutes and 4 hours showed no statistically

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Table 5
Total Precision of Low-Level Control in Cerebrospinal Fluid Samples at 10 Clinical Sites*

Site	N	Average Count (/µL)	
		RBC	WBC
1	8	22	11
2	18	21	9
3	15	23	10
4	10	23	9
5	44	22	8
6	25	25	10
7	27	25	10
8	24	21	9
9	8	24	10
10	29	20	8

* The mean, SD, and coefficient of variation (%) for the average RBC count were 22, 4.3, and 19.5, respectively, and for the average WBC count, 9, 1.8, and 20.0, respectively.

significant differences for the WBC count or for the percentages of mononuclear and polymorphonuclear cells ($P \geq .05$). A statistically significant difference was observed for the RBC count ($P < .05$). Although the RBC difference observed was statistically significant, it is not likely that a difference of this magnitude is clinically significant.

Effect of Increased Incubation Time on Samples Containing Hypochromic and/or Microcytic RBCs

It was observed that hypochromic and/or microcytic RBC populations in some samples crossed RBC counting thresholds in the CSF scatter/scatter cytogram as a result of incomplete RBC sphering after the standard 4-minute incubation with CSF reagent. In such samples, the RBC count was falsely decreased and the WBC count often was falsely increased compared with manual values. The surrogate CSF samples containing hypochromic and/or microcytic RBCs or normochromic and normocytic RBCs were prepared with CSF reagent as described and tested periodically during a period of 4 to 30 minutes. Automated counts for RBCs and WBCs were compared with initial manual counts **Table 10**. In all 3 samples, the total number of cells (RBCs + WBCs) remained similar at all time points. In samples containing hypochromic and/or microcytic RBCs, the RBC count increased over time and the WBC count decreased over time. The RBC and WBC

Table 6
Total Precision of High-Level Control in Cerebrospinal Fluid Samples at 10 Clinical Sites

Site/Statistical Data	N	RBCs (/µL)	WBCs (/µL)	Percentage of Cells					
				MN	PMN	Neutrophils	Lymphocytes	Monocytes	Eosinophils*
1	8	202	90	41.5	58.6	58.2	29.1	12.3	0.4
2	20	184	85	42.0	58.0	57.5	31.1	10.9	0.5
3	16	205	94	44.3	55.7	55.2	30.4	13.9	0.5
4	10	213	87	40.0	60.0	59.7	31.5	8.5	0.3
5	55	206	88	46.1	53.9	53.7	31.7	14.3	0.2
6	26	214	91	40.7	59.3	58.9	30.8	9.9	0.3
7	25	212	90	39.3	60.7	60.3	31.1	8.2	0.4
8	24	202	91	39.5	60.5	60.3	29.4	10.0	0.2
9	7	222	93	38.6	61.4	61.1	31.3	7.3	0.4
10	31	198	83	43.4	56.6	56.4	32.3	11.2	0.1
Mean	—	203	88	42.4	57.6	57.3	31.1	11.3	0.3
SD	—	20.6	8.2	4.2	4.2	4.2	3.0	3.2	0.3
CV (%)	—	10.1	9.3	9.9	7.3	7.3	9.6	28.3	100.0

CV, coefficient of variation; MN, mononuclear cells; PMN, polymorphonuclear cells.
* Eosinophil data are provided for research use only.

Table 7
WBC and RBC Linearity as Determined by Pooled Cerebrospinal Fluid Samples

Level (%)	WBC Linearity				RBC Linearity			
	Observed (/µL)	Expected (/µL)	Absolute Deviation (/µL)	Deviation (%)	Observed (/µL)	Expected (/µL)	Absolute Deviation (/µL)	Deviation (%)
0.00	0	0	0	—	0	0	0	—
0.05	2	3	–1	—	2	1	1	—
0.10	5	5	0	—	4	3	1	—
0.20	10	10	0	—	9	6	3	—
1	53	50	3	—	34	29	5	—
10	514	501	13	3	308	288	20	7
100	5,009	5,009	0	0	2,880	2,880	0	0

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counts for these samples were comparable to the manual values only after 20 to 30 minutes' incubation. In the normochromic and normocytic sample, the RBC and WBC counts remained stable throughout the testing period and were comparable to the manual values at all time points.

Table 8
WBC and RBC Carryover in Cerebrospinal Fluid Samples*

Site	Observed Carryover (%) WBC	Observed Carryover (%) RBC
1	0.04	0.12
2	0.02	0.00
3	0.04	0.04

* Before assaying each cerebrospinal fluid sample, the ADVIA 120 rinses all reagent and sample pathways in preparation for the next sample. Observed carryover is expressed as the average percentage of the high-level sample that is lost to the trailing low-level sample.

Discussion

The critical nature of CSF testing is compounded by limited sample volume, sample instability, and the need for immediate results. This is especially true for the hematologic portion of the analysis because the most commonly used method for hematologic analysis of CSF samples is a labor-intensive, technique-dependent, manual process of subjective enumeration and differentiation of cells in a counting chamber. This process typically requires 30 to 45 minutes to complete. The manual method also is prone to imprecision owing to interoperator variability and the relatively small number of cells counted.[3-5] This is especially true for the WBC differential count because it is a ratio of 2 counts, both of which are derived from counting a relatively small number of cells.[2] Whereas 1 μL of patient sample typically is analyzed in the manual method, approximately 4 μL of sample is analyzed in the automated method, resulting in better statistical precision of the results.

Table 9
Prepared Sample Stability: Comparison of ADVIA 120 Values at 4 Minutes and 4 Hours of Cerebrospinal Fluid Sample Incubation*

Sample No./Mean	WBCs/μL 4 min	WBCs/μL 4 h	RBCs/μL 4 min	RBCs/μL 4 h	MN (%) 4 min	MN (%) 4 h	PMN (%) 4 min	PMN (%) 4 h
1	1.0	2.0	5.0	5.0	100.0	100.0	0.0	0.0
2	5.0	4.0	12.0	12.0	100.0	100.0	0.0	0.0
3	9.0	7.0	23.0	23.0	100.0	93.3	0.0	6.7
4	9.0	9.0	13.0	14.0	100.0	100.0	0.0	0.0
5	20.0	15.0	18.0	24.0	98.8	100.0	1.2	0.0
6	23.0	22.0	78.0	91.0	90.2	88.5	9.8	11.5
7	23.0	21.0	70.0	96.0	98.7	98.5	1.3	1.5
8	32.0	33.0	34.0	40.0	99.2	96.6	0.8	3.4
9	40.0	35.0	111.0	127.0	94.6	91.3	5.4	8.7
10	102.0	101.0	133.0	162.0	98.6	97.9	1.4	2.1
Mean	26.4	24.9	49.7	59.4	98.0	96.6	1.9	3.9

MN, mononuclear cells; PMN, polymorphonuclear cells.
* P values for 4 minutes vs 4 hours were as follows: WBC, ≥ .05; RBC, < .05; %MN, ≥ .05; % PMN, ≥ .05.

Table 10
Effect of Incubation Time on RBC and WBC Counts in Cerebrospinal Fluid Samples Containing Hypochromic and/or Microcytic RBCs*

Incubation Time (min)	Sample 1 RBCs/μL	Sample 1 WBCs/μL	Sample 2 RBCs/μL	Sample 2 WBCs/μL	Sample 3 RBCs/μL	Sample 3 WBCs/μL
4	772	18	337	6	454	1
6	808	17	335	5	440	1
8	807	13	353	5	435	1
10	798	10	352	3	452	2
12	790	8	359	4	464	2
14	796	9	357	4	464	3
20	810	6	372	2	460	1
30	807	4	370	2	471	2
Manual	NA	4	377	2	446	1

NA, not available.
* Sample 1, hypochromic and microcytic; mean corpuscular hemoglobin (MCH), 24.4 pg; mean corpuscular volume (MCV), 78.2 fL; sample 2, hypochromic and microcytic; MCH, 20.2 pg; MCV, 69.4 fL; sample 3, normochromic and normocytic; MCH, 32.8 pg; MCV, 97 fL.

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In the present study, the automated ADVIA 120 CSF Assay demonstrated excellent correlation with the manual method, as seen by a greater than 98% correlation for absolute WBC and RBC counts. Paired *t* test values of .102 for mononuclear cells and .089 for polymorphonuclear cells showed that 98% of differential results fell within the binomial envelope. Automated WBC differential values compare well with manual reference values; however, in the absence of morphologic flagging, the differential should be confirmed for the presence of abnormal cells, especially for oncology samples. It has been observed that when the RBC count exceeds 1,500/µL, the WBC and the neutrophil results might be falsely elevated and hypochromic RBCs might interfere with the WBC and lymphocyte results. It was found that hypochromic and/or microcytic RBCs in some samples needed to be incubated with the CSF reagent for longer than 4 minutes to minimize the interference of RBCs in the WBC counting area. This suggests that laboratories that frequently receive such samples might consider increasing the incubation time of prepared CSF samples from the standard 4 minutes to 10 minutes.

The WBC differential count is a key component in the clinical differential diagnosis of meningitis. Viral meningitis frequently is characterized by a predominance of mononuclear cells (Image 1), whereas bacterial meningitis often has a predominance of polymorphonuclear cells (Image 2). WBC distribution patterns on cytograms from the automated analysis reflected the cell distribution seen on the stained blood smears.

The ADVIA 120 CSF Assay also demonstrated acceptable interassay precision for absolute WBC and RBC counts and linearity encompassing clinically relevant ranges. The published WBC count for normal CSF samples is 0 to 5 mononuclear cells per microliter.[1] WBC linearity for the automated method was demonstrated between 0 and 5,000 WBCs per microliter and between 0 and 2,880 RBCs per microliter.

Specimen stability is a key concern in the hematologic analysis of CSF specimens. While it is generally accepted that the half-life of neutrophils in CSF is about 2 hours,[1] pretreatment of the patient sample with ADVIA 120 CSF Reagent extends stability of the prepared CSF sample to 4 hours, regardless of the cell concentration. The prepared sample is ready to aspirate in 4 minutes, and a first result is available in 10 minutes.

Observed benefits of the automated assay include commercially available quality control material, elimination of interoperator imprecision, and reduction of the need for highly skilled technologists. Normal CSF samples, constituting the majority of CSF specimens received in the laboratory, can be identified quickly as such, and the automated results can be released without further manual intervention. Background counts performed before each CSF sample acquisition preclude the possibility of potential carryover and electronic interference, guaranteeing the accuracy of low cell counts.

Similar studies have been conducted comparing automated CSF results obtained on the CellDyn 3500 and CellDyn 4000 hematology analyzers (Abbott Laboratories, Abbott Park, IL) and the UF-100 urine flow cytometer (Sysmex, Kobe, Japan) with manual CSF results.[6-8] The results of this and other studies[9] have demonstrated that the automated ADVIA 120 CSF Assay compares well with the manual method with respect to accuracy, precision, and clinical usefulness. This multicenter evaluation shows that this method is an acceptable alternative to the labor-intensive and subjective manual method.

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Evaluation of the body fluid mode on Sysmex XE-5000

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Introduction

The haematology analyser, Sysmex XE-5000 (Sysmex Corp.,Japan), has recently introduced a body fluid mode on the instrument for counting white blood cells (WBC), polymorph nucleated cells, and mononuclear cells in biological fluids. Sysmex applies a flow cytometry method and differentiates blood cells on the basis of

1) scattered light depending on the size of the cell, shape of nucleus and the presence of granules.
2) fluorescent light from the staining of blood cells containing information mainly on the nucleic acid (DNA and RNA) content.

We wanted to compare absolute and proportional counts for the WBC on body fluids (cerebrospinal (CSF) and ascitic fluids (AF)) with those obtained on the haematology analyzer, Advia 120 (Bayer Corp., USA). In Advia WBC are detected and counted based on light scatter and myeloperoxidase activity and differentiated into neutrophils, eosinophils, basophils, lymfocytes and monocytes.

We also wanted to elucidate the imprecision on WBC and differential count on the body fluid mode on Sysmex XE-5000.

Methods and materials

CSF and AF from 87 patients (55 CSF and 30 AF) were collected in tubes with no anticoagulant. They were run simultaneously on the open mode on Sysmex and Advia for comparison analysis. AF was analysed within 24 hours and CSF within 1 hour.

Precision of WBC and differential counting on the Sysmex was evaluated by repeated counting of samples with different levels of WBC. The number of tests depended on the volume of available body fluid.

Results

The white blood cell counts in CSF and AF from Advia and Sysmex and their discrimination between mononuclear and polymorph nuclear cells in CSF with WBC>10x10E6/L were compared by performing Spearman correlation and Deming regression analysis. These results are shown in *Figure 1-4*.

Advia could not differentiate the cells in AF due to the presence of mesothelium cells and macrophages, so differential counts for AF were not compared.

Precision

The variability coefficient, %CV, on white blood cell counts >10x10E6/L was calculated from the repeated testing of 15 body fluid (CSF, AF and pleural fluid) samples on Sysmex. The results are shown in *Figure 5-7*, where a curve indicates the trend in %CV depending on the concentration of WBC.

Evaluation of the body fluid mode on Sysmex XE-5000

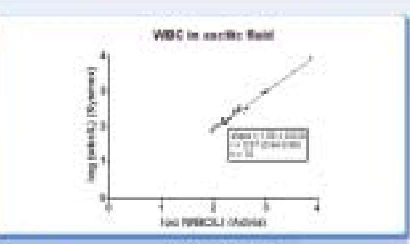
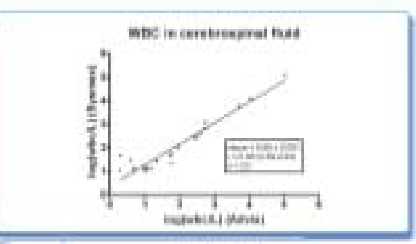


Figure 1.



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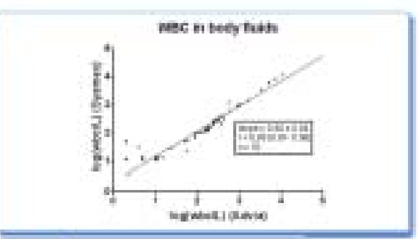
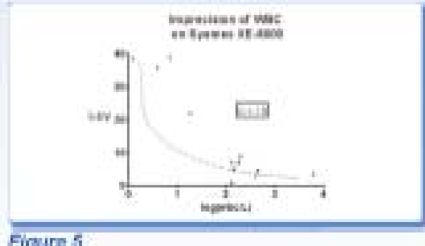


Figure 3.

Differential counts

Figure 4.



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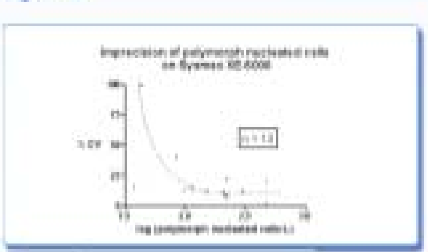

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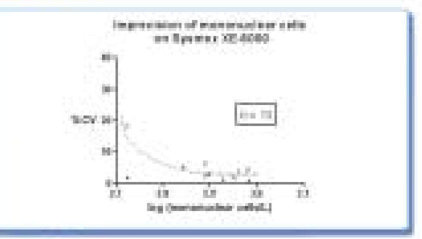

Figure 7.

Figure 5-7. Wbc>10E6/L. The curves show the trend of correlation between %CV and concentration.

Table 1. Divergent results of CSF on Sysmex XE-5000 and Advia 120.

Patient no	WBC ($x10^6$/L) Sysmex	Polymorph (%)	Mono (%)	WBC ($x10^6$/L) Advia	Polymorph (%)	Mono (%)	Remarks
1	48	56	44	2	11	89	[illegible]
2	6070	2	98	6220	89	11	[illegible]

Conclusion

The body fluid mode on Sysmex XE-5000 shows good precision and good agreement with Advia 120 when measuring WBC in bodyfluids at concentrations above 10x10E6/L.

The differential counts between the two methods vary with a relatively broad correlation coefficient confidence interval (r = 0.35-0,92).

The %CV on Sysmex XE-5000 improves by the increase in WBC concentration.

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Official Publication

Performance Evaluation of the Application of Body Fluids on the Sysmex XE-2100 Series Automated Hematology Analyzer

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ABSTRACT

Body fluid analysis on the Sysmex XE-2100 series automated hematology analyzer was evaluated at 4 hospitals (Baylor University Medical Center, Dallas, TX; St. John's Mercy, St. Louis, MO; Carle Clinic, Urbana, IL; and ACL Laboratories, West Allis, WI, USA). The total nucleated cell and red blood cell (RBC) counts of 493 samples were obtained with the Sysmex XE-2100 automated hematology analyzer and compared with results obtained by manual chamber counting. Seventy-eight samples were not suitable for evaluation because of the presence of clots, crystals, error messages related to white blood cell (WBC) and RBC parameters, and so on. Pearson correlation coefficients for the WBC parameter were 0.99 for cerebrospinal fluid, 0.95 for serous fluid, 0.99 for synovial fluid, and 0.99 for samples of combined body fluids. Ninety-six samples were used to compare RBC counting methods because these samples had RBC counts greater than $0.01 \times 10^6/\mu L$. The Pearson correlation coefficients for the RBC parameter were 0.96 for cerebrospinal fluid, 0.97 for serous fluid, 0.97 for synovial fluid, and 0.97 for samples of combined body fluids. Carryover, precision, and linearity studies also performed for WBC and RBC counts yielded very good results. *Lab Hematol.* 2005;11:24-30.

KEY WORDS: Performance evaluation · Body fluids · Automated hematology analyzer · XE series

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INTRODUCTION

Body fluid analysis in the clinical laboratory entails quantification and differentiation of cell populations present in synovial aspirates, cerebrospinal fluid samples, and various serous fluids (peritoneal and pleural fluids). Such analysis is an important procedure that provides valuable information for the diagnosis and treatment of a wide spectrum of diseases. Historically, this procedure had been performed manually by competent technologists using counting chambers. However, the reproducibility and accuracy of the manual method vary because of differences in the skills and experience of the technologists. The manual method is also labor intensive and time consuming [1-3]. In contrast, the reproducibility and accuracy of an automated method is more consistent because it is not subject to the variation inherent in the manual method. A large number of cells can be analyzed, and several parameters besides morphologic appearance alone, such as forward scatter, side scatter, and fluorescent labels, can be used to identify blood cells. These capabilities are found on the Sysmex XE series automated hematology analyzers (Sysmex Corporation, Kobe, Japan) and were applied in this study to the analysis of body fluids.

MATERIALS AND METHODS

Specimens

Four hospitals participated in this study: Baylor University Medical Center, Dallas, TX; St. John's Mercy, St. Louis, MO; Carle Clinic, Urbana, IL; and ACL Laboratories, West Allis, WI, USA. Of the 493 body fluid specimens analyzed,

78 samples were not suitable for evaluation because of the presence of clots, crystals, error messages related to white blood cell (WBC) and red blood cell (RBC) parameters, and so on. The data comprised 5% pediatric samples and 95% adult samples. Of the samples from study sites that reported the patient's sex, 52% of the samples were from male patients, and 48% were from female patients. Manual chamber counts and Sysmex XE-2100 automated counts of WBCs were evaluated for 415 body fluid specimens (205 cerebrospinal, 159 serous, and 51 synovial fluid samples). The serous fluids consisted of peritoneal, pleural, ascites, and dialysate samples. All of the samples were residual samples and contained either EDTA anticoagulant or no anticoagulant. Samples were tested within 8 hours of collection and were examined for the presence of clots. Synovial fluids were pretreated with hyaluronidase as needed. Ninety-six body fluid specimens (29 cerebrospinal, 44 serous, and 23 synovial fluid samples) with RBC counts $\geq 0.01 \times 10^6/\mu L$ were used for comparison with the manual chamber counts.

Sysmex XE-2100

The Sysmex XE-2100 is an automated hematology analyzer capable of providing a 26-parameter hemogram, a cell differential that includes immature granulocytes, hematopoietic progenitor cells, and nucleated RBCs, and a reticulocyte analysis that includes the immature reticulocyte fraction. The WBC count is determined by flow cytometry using forward-scattered and side-scattered light. The differential uses a specific nucleic acid dye to measure the cells by side-fluorescent light and side-scattered light. The RBC parameter is measured by direct current detection. XE pro software (Sysmex Corporation) is used to ensure consistent results. The XE series instruments with XE pro software express WBC counts to 3 decimal places and RBC counts to 2 decimal places. All samples were mixed by gentle inversion and run in the open mode.

Three levels of *e*-Check quality control material were used (levels 1, 2, and 3) throughout the comparison study. Start-up was performed each day with close observation of background counts before specimens were analyzed.

Manual Hemacytometer Chamber Counting

Cell counts of body fluids were carried out by loading undiluted fluid into both sides of a hemacytometer. For fluids with low cell counts, RBCs and WBCs were counted separately in 5 squares on each side of the hemacytometer. Counts were reported as the number of cells per microliter. For fluids with high cell counts, the cells within 1 central square on each side were counted, and the average of the 2 counts was multiplied by 10. A 1:10 dilution of the fluid was prepared with MLA pipettors (VistaLab Technologies, Mt. Kisco, NY, USA) and isotonic diluent for counts that were too high to be measured accurately without a dilution. When the fluid was bloody, the RBCs in 5 small squares in the large central square on each side were counted, and the average count was multiplied by 50. To count the WBCs in a bloody specimen, we lysed the RBCs by diluting the fluid 1:2 with 3% acetic acid. The WBCs in 5 large squares on both sides were then counted and multiplied by 2 to correct for the dilution.

Carle Clinic used 10 μL of prepared crystal violet stain (0.1 g crystal violet with 150.0 mL sterile distilled water) with 100 μL of fluid for their manual chamber counting.

Statistical Analysis

Statistical analyses included the calculation of regression statistics.

TABLE 1. Accuracy of White Blood Cell Counts Determined with the Sysmex XE-2100

Fluid Type	Mean, $\times 10^3/\mu L$	Minimum, $\times 10^3/\mu L$	Maximum, $\times 10^3/\mu L$	*r*	Slope	Intercept	*P*
Cerebrospinal (n = 205)							
XE-2100	0.257	0.000	18.398	0.99	0.9	0	.164
Manual	0.277	0.000	18.563				
Serous (n = 159)*							
XE-2100	1.521	0.000	24.678	0.95	0.9	0.15	.678
Manual	1.560	0.001	31.570				
Synovial (n = 51)							
XE-2100	16.611	0.060	173.929	0.99	1.0	−0.26	.549
Manual	16.208	0.041	164.000				
Body fluids combined (n = 415)							
XE-2100	2.751	0.000	173.929	0.99	1.0	−0.06	.785
Manual	2.726	0.000	164.000				

*Serous fluids included peritoneal, pleural, ascites, and dialysate samples.

TABLE 2. Accuracy of White Blood Cell Counts ≥0.050 × 10^3/μL Determined with the Sysmex XE-2100

Fluid Type	Mean, ×10^3/μL	Minimum, ×10^3/μL	Maximum, ×10^3/μL	*r*	Slope	Intercept	P
Cerebrospinal (n = 41)							
XE-2100	1.245		18.398	0.99	0.9	−0.028	.091
Manual	1.369	0.050	18.563				
Serous (n = 139)*							
XE-2100	1.734		24.678	0.95	0.9	0.071	.659
Manual	1.781	0.050	31.570				
Synovial (n = 50)							
XE-2100	16.942		173.929	0.99	1.0	−0.274	.550
Manual	16.532	0.050	164.000				
Body fluids combined ≥0.05 × 10^3/μL (n = 230)							
XE-2100	4.953		173.929	0.99	1.0	−0.12	.812
Manual	4.914	0.050	164.000				

*Serous fluids included peritoneal, pleural, ascites, and dialysate samples.

RESULTS

Background Count

Acceptable background counts for body fluids were <0.050 × 10^3/μL for WBCs and <0.01 × 10^6/μL for RBCs. Background counts were monitored and were within acceptable limits.

Accuracy

The performance of the body fluid application for the XE series was compared with the manual method with respect to the cellular enumeration of 3 body fluid types in the clinical laboratory setting. The test specimens included cerebrospinal fluid, serous, and synovial samples. Of 493 analyzed body fluid specimens, 78 samples were not suitable for evaluation because of the presence of clots, crystals, error messages related to WBC and RBC parameters, and so on. Clotted samples and synovial samples containing uric acid crystals, a high viscosity, or an error message related to WBC and RBC parameters may compromise results. Manual chamber WBC counts and Sysmex XE-2100 automated counts were compared for 415 body fluid specimens (205 cerebrospinal, 159 serous, and 51 synovial fluid samples). Manual and automated counts were also evaluated for 230 body fluid specimens (41 cerebrospinal, 139 serous, and 50 synovial fluid samples) with WBC counts ≥0.050 × 10^3/μL and for 96 specimens (29 cerebrospinal, 44 serous, and 23 synovial fluid

TABLE 3. Accuracy of Red Blood Cell Counts ≥0.01 × 10^6/μL Determined with the Sysmex XE-2100

Fluid Type	Mean, × 10^6/μL	Minimum, × 10^6/μL	Maximum, × 10^6/μL	*r*	Slope	Intercept	P
Cerebrospinal (n = 29)							
XE-2100	0.039	0.00	0.15	0.96	0.9	−0.002	.063
Manual	0.042	0.01	0.17				
Serous (n = 44)*							
XE-2100	0.092	0.01	0.65	0.97	1.0	−0.001	.164
Manual	0.092	0.01	0.70				
Synovial (n = 23)							
XE-2100	0.081	0.01	0.42	0.97	1.0	−0.006	.063
Manual	0.088	0.01	0.43				
Body fluids combined (n = 96)							
XE-2100	0.074	0.00	0.65	0.97	0.9	0.007	.302
Manual	0.076	0.01	0.70				

*Serous fluids included peritoneal, pleural, ascites, and dialysate samples.

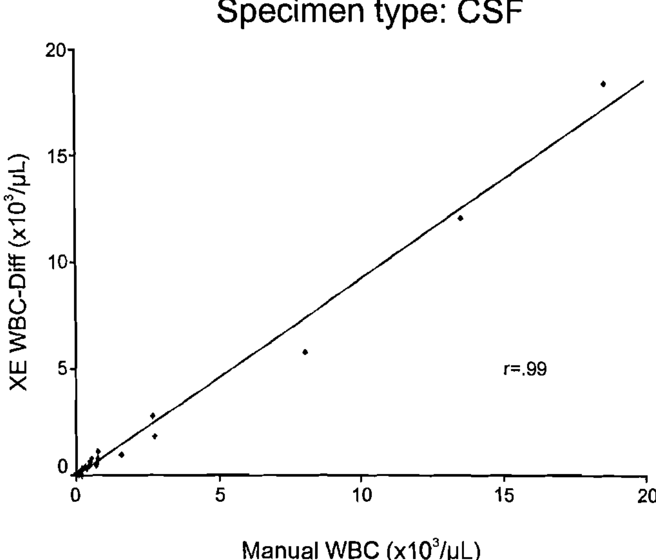

FIGURE 1. White blood cell (WBC) counts for cerebrospinal fluid (CSF) specimens determined with the manual method and with the Sysmex XE-2100 automated hematology analyzer (XE WBC-Diff).

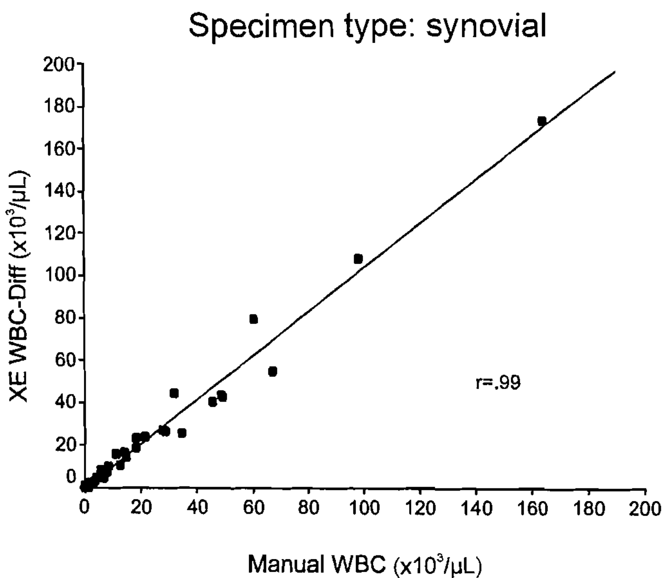

FIGURE 3. White blood cell (WBC) counts for synovial fluid specimens determined with the manual method and with the Sysmex XE-2100 automated hematology analyzer (XE WBC-Diff).

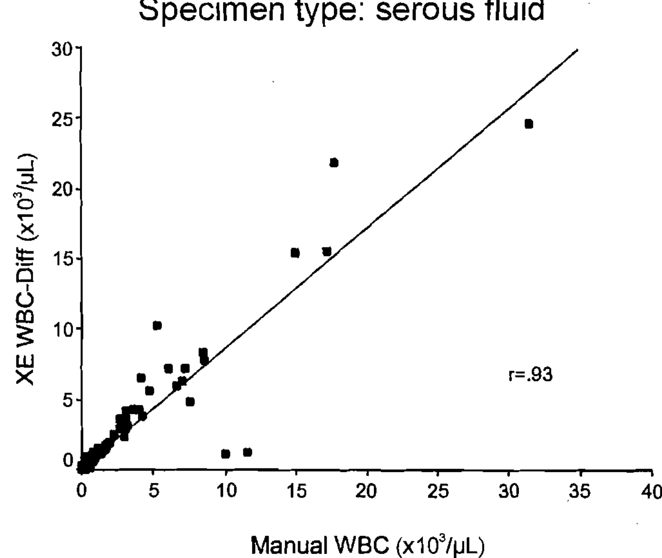

FIGURE 2. White blood cell (WBC) counts for serous fluid specimens determined with the manual method and with the Sysmex XE-2100 automated hematology analyzer (XE WBC-Diff).

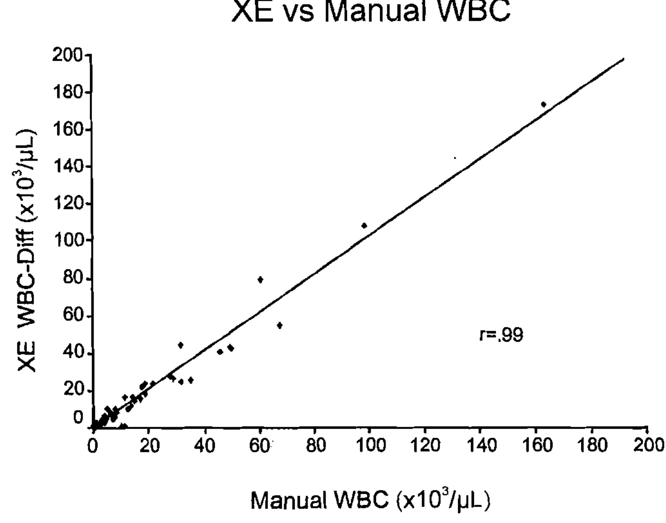

FIGURE 4. White blood cell (WBC) counts for combined body fluid specimens determined with the manual method and with the Sysmex XE-2100 automated hematology analyzer (XE WBC-Diff). The body fluid types combined for analysis were cerebrospinal fluid, serous, and synovial fluids.

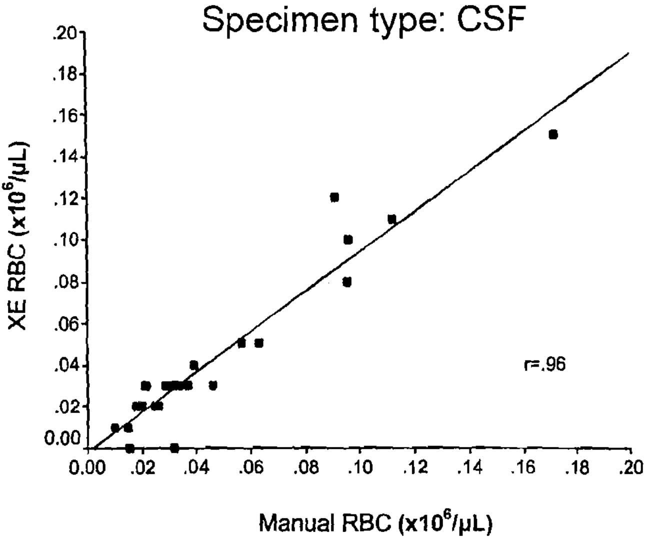

FIGURE 5. Red blood cell (RBC) counts for cerebrospinal fluid (CSF) specimens determined with the manual method and with the Sysmex XE-2100 automated hematology analyzer (XE WBC-Diff).

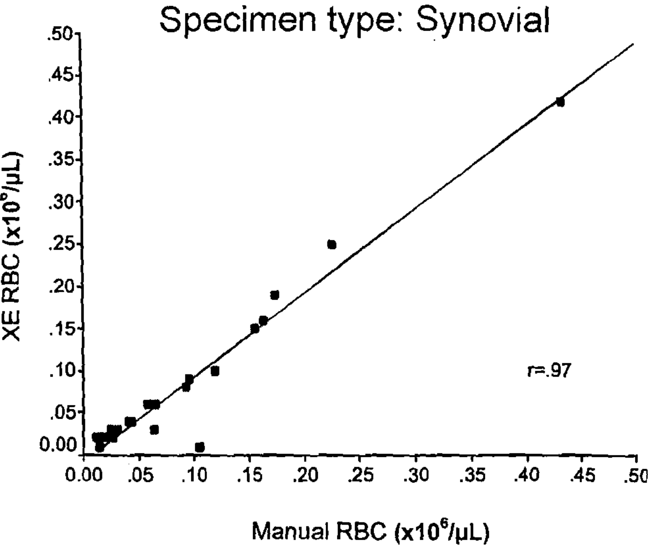

FIGURE 7. Red blood cell (RBC) counts for synovial fluid specimens determined with the manual method and with the Sysmex XE-2100 automated hematology analyzer (XE WBC-Diff).

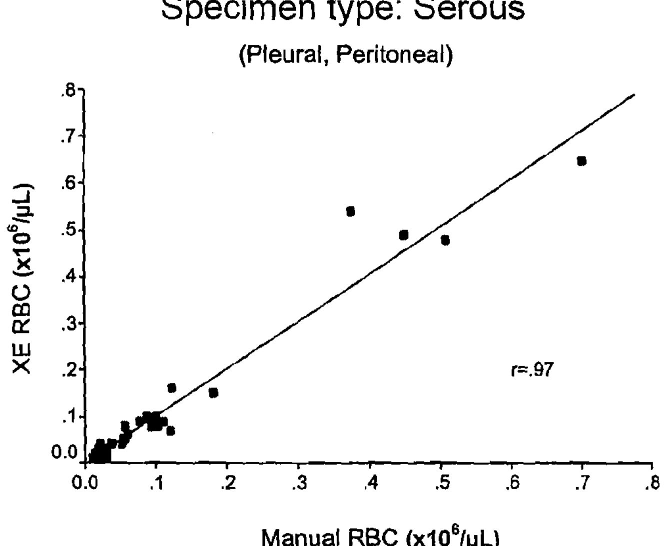

FIGURE 6. Red blood cell (RBC) counts for serous fluid specimens (pleural, peritoneal) determined with the manual method and with the Sysmex XE-2100 automated hematology analyzer (XE WBC-Diff).

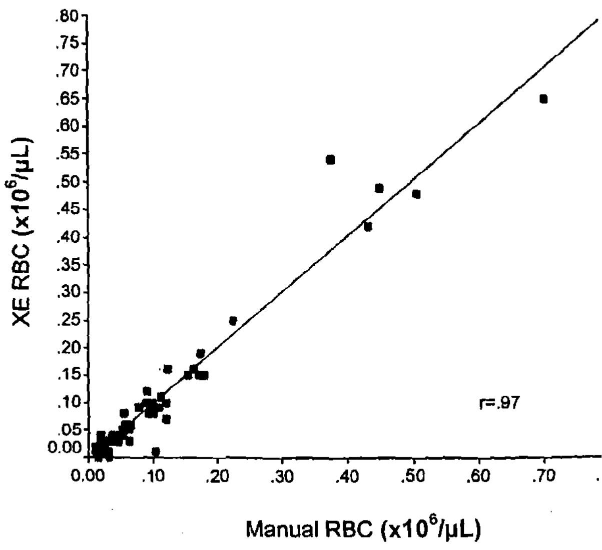

FIGURE 8. Red blood cell (RBC) counts for combined body fluid specimens determined with the manual method and with the Sysmex XE-2100 automated hematology analyze (XE WBC-Diff). The body fluid types combined for analysis were cerebrospinal, serous, and synovial fluids.

TABLE 4. Linearity

Parameter	Range Tested	r^2	r	Slope	Intercept
White blood cells	0.023-5.923 × 10^3/μL	0.9996	1.00	1.0068	0.0083
Red blood cells	0.01-3.50 × 10^6/μL	0.9998	1.00	1.0018	0.0088

samples) with RBC counts ≥0.01 × 10^6/μL. WBC counts <0.05 × 10^3/μL and RBC counts <0.01 × 10^6/μL should be confirmed with an alternative method. Tables 1 through 3 and Figures 1 through 8 summarize the accuracy data for WBC and RBC counts.

Carryover

Carryover analysis was performed with the procedure of the International Council for Standardization in Haematology [4]. A patient sample with a ~~high count~~ was analyzed 3 consecutive times (H1, H2, H3), and then a sample with a low count was analyzed 3 consecutive times (L1, L2, L3). Percentage carryover for each parameter was calculated according to the formula [3]:

$$\text{Percent Carryover} = \frac{L1 - L3}{H3 - L3} \times 100.$$

The WBC and RBC carryover results of high-to-low carryover testing were less than 1% and met manufacturer specifications.

Linearity

WBC and RBC count linearity was evaluated by diluting body fluid samples with instrument diluent to obtain results at low levels of detection. The WBC correlation coefficient (r^2) was 0.9996 (slope, 1.0068; intercept, 0.0083). The RBC correlation coefficient (r^2) was 0.9998 (slope, 1.0018; intercept, 0.0088) (Table 4). Linearity at low levels of detection was very good (Figures 9 and 10).

Precision

Body fluid samples representing different WBC and RBC ranges were evaluated. Each sample was run 10 consecutive times. Imprecision results were ≤30% for WBC samples with counts from ≥0.050 × 10^3/μL to 0.100 × 10^3/μL and ≤40% for RBC samples with counts from ≥0.01 × 10^6/μL to 0.20 × 10^6/μL (the lowest RBC value that the XE-2100 can detect is 0.01 × 10^6/μL). Table 5 exhibits the coefficients of variation for the samples in the precision study.

DISCUSSION

Simultaneous testing of body fluids by the manual method and the Sysmex XE-2100 automated method allowed the operating technologists to evaluate the potential advantages of the automated method. The results with all 3 body fluid types (cerebrospinal, serous, and synovial fluids) provided strong positive linear correlations between the manual counts and the automated counts. Additionally, no statistically significant differences between the 2 methods were noted when paired comparisons were performed with the 3 specimen types. All fluids should be tested as soon as possible after collection because body fluid cells will degenerate rapidly [1-2]. Each laboratory should perform its own testing of sample stability to determine the maximum time limit acceptable for the laboratory.

TABLE 5. Within-Run Precision (Open Mode)

Parameter	Mean	2 SD	CV%*	Minimum	Maximum
White blood cells, ×10^3/μL	0.059	0.013	11.13	0.049	0.07
	0.078	0.012	7.93	0.068	0.084
	0.084	0.019	11.11	0.071	0.099
	0.590	0.064	5.45	0.548	0.652
	0.631	0.095	7.56	0.576	0.712
	1.658	0.124	3.75	1.57	1.768
	3.945	0.131	1.65	3.870	4.046
	63.544	1.933	1.52	62.407	65.406
Red blood cells, ×10^6/μL	0.01	0	0	0.01	0.01
	0.02	0	0	0.02	0.02
	0.04	0	0	0.04	0.04
	0.1	0.01	4.98	0.09	0.1
	0.34	0.01	1.5	0.34	0.35
	1.00	0.01	0.702	0.99	1.01
	1.41	0.02	0.68	1.40	1.43

*CV% indicates coefficient of variation.

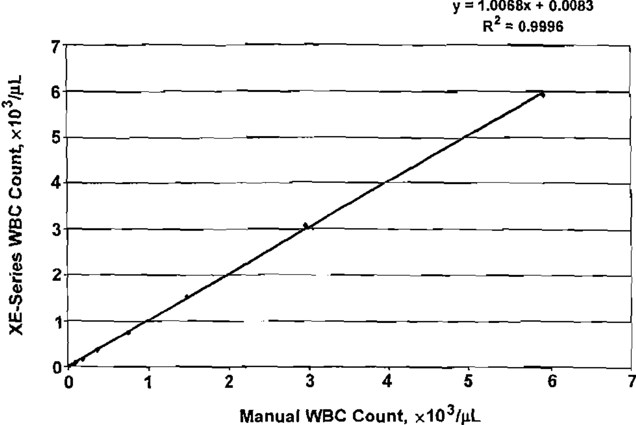

FIGURE 9. Linearity between white blood cell (WBC) counts determined with the manual method and WBC counts determined with the Sysmex XE-2100 automated hematology analyzer (XE Series).

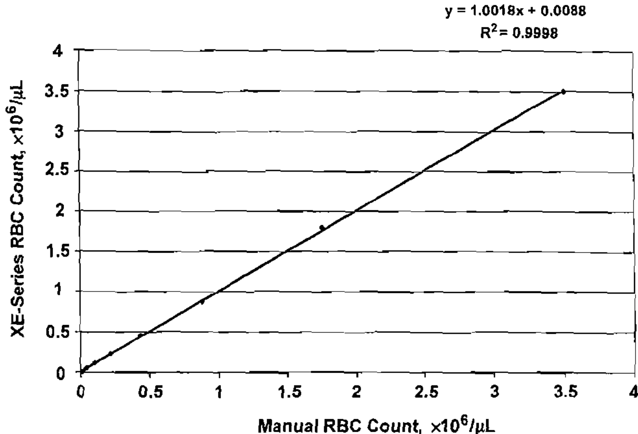

FIGURE 10. Linearity between red blood cell (RBC) counts determined with the manual method and RBC counts determined with the Sysmex XE-2100 automated hematology analyzer (XE Series).

This study showed the low-level sensitivity of the XE series to be $0.050 \times 10^3/\mu L$ for WBCs and $0.01 \times 10^6/\mu L$ for RBCs. The 3 decimal places for the WBC parameter allow automated counts at a low level of detection. RBC counts of body fluids other than cerebrospinal fluid are of limited diagnostic value [1-2]. WBC counts $<0.05 \times 10^3/\mu L$ and RBC counts $<0.01 \times 10^6/\mu L$ should be confirmed with an alternative method. Carryover was ≤1%. Imprecision results were ≤30% for WBC counts from $\geq 0.050 \times 10^3/\mu L$ to $0.100 \times 10^3/\mu L$ and ≤40% for RBC counts from $\geq 0.01 \times 10^6/\mu L$ to $0.20 \times 10^6/\mu L$. Linearity at low levels of detection was very good.

A significant advantage of the automated method was evident during the testing of bloody or otherwise highly cellular samples. When a manual analysis of such samples is performed, the laboratory technologist has to perform dilutions and the accompanying calculations. Making these dilutions introduces an additional degree of variability or inaccuracy. Use of the automated method eliminates the need for such calculations. Not only does the automated method remove a potential source of error, it also saves the time required to perform the dilution calculation. In addition to the advantage provided in cases of high cellularity, use of the Sysmex XE-2100 reduces the overall time of test performance while yielding a comparable level of reproducibility and accuracy for RBC and WBC measurements. The statistical evaluation offers evidence that the XE series provides a reliable automated cellular enumeration of body fluid specimens compared with the current manual method of chamber counting.

ACKNOWLEDGMENT

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Validation of Body Fluid Analysis on the Coulter LH 750

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ABSTRACT

The role of the hematology laboratory in the analysis of body fluid has been to provide accurate enumeration of red blood cells (RBCs), total nucleated cells (TNCs), and differentials by manual analysis. Three hospitals (London Health Sciences Centre, University of Pittsburgh Medical Center, and University of Michigan Health System) participated in the assessment of the performance of automated analysis of body fluid by the Beckman Coulter LH 750, an impedance-based hematology analyzer. We evaluated the accuracy of analysis results for both the TNCs and RBCs of 372 samples (158 serous fluid, 148 cerebrospinal fluid [CSF], 66 synovial fluid) run on the LH 750 compared to results obtained from manual chamber counting. Of the 372 samples, 152 were suitable for evaluation of accuracy of the automated TNC. The remaining 220 samples were either flagged for interfering substances or the reference results were $<0.2 \times 10^9$/L, below the background limit of the analyzer. Correlation coefficients for serous fluid were 0.895, $P = .88$; for CSF, 0.993, $P = .84$; and for synovial fluid, 0.836, $P = .94$. Of the 372 samples, 106 had RBC counts greater than 0.01×10^{12}/L and were used for method comparison. Correlation coefficients for serous fluid were 0.957, $P = .66$; for CSF, 0.849, $P = .55$; and for synovial fluid, 0.667, $P = .81$. Linearity and precision studies showed excellent agreement for both TNC and RBC parameters. Low-level sensitivity excluded the majority of cerebrospinal (119) and a small number of peritoneal dialysate fluid samples (8), which require accurate enumeration at clinical decision points between 0 to 100 cells/microliter. In the case of synovial and serous fluids, however, most clinicians are interested in TNC counts above 0.2×10^9/L, and RBC counts are relevant only if they are significantly increased ($\geq 0.05 \times 10^{12}$/L). Adopting the criteria of reporting TNC counts as $<0.2 \times 10^9$/L or accurate enumeration on counts $\geq 0.2 \times 10^9$/L, clinically relevant results could be provided by automated analysis in 93.8% of serous fluids and 85.8% of synovial fluids. *Lab Hematol.* 2003;9:155-159.

KEY WORDS: LH 750 · Hematology analyzer · Body fluids · Automated analysis

INTRODUCTION

The cellular analysis of body fluid such as cerebrospinal, pleural, peritoneal, or synovial fluid is important in discriminating between reactive, infectious, and malignant disease. The role of the hematology laboratory in this fluid analysis is to provide accurate enumeration of nucleated cells and differentials as well as the red blood cell (RBC) count. Historically, the cell counts have been performed by manual methods using a hemocytometer counting chamber. Despite the fact that the coefficient of variation of this procedure is estimated to be 45% [1], body fluid analysis has stubbornly refused to join the ranks of automated procedures. Maintaining proficiency and efficiency in manual cell counting in an environment of limited resources is a challenge for today's laboratories. As a result of fewer technical experts and increases in workload, laboratorians have been forced to reevaluate the traditional

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approach to body fluid analysis. The first descriptions of electronic counting of cerebrospinal fluid (CSF) appeared in 1970 [2] and then again in 1984 [3], but neither of these early papers generated any groundswell of interest. Automated analysis of body fluids was not enthusiastically embraced, because of issues of high instrument background, poor linearity, and interference from debris, all of which led to poor low-level sensitivity. More recently, published reports from several studies using modern hematology analyzers have suggested that automation of body fluid cell counts is an idea whose time has come [4-6]. Since the early 1990s, one of the centers in this study, London Health Sciences Centre (LHSC), has been performing automated analysis of serous and synovial fluids combined with a novel approach of reporting only clinically relevant results. In this multicenter study, we evaluated the feasibility of analyzing pathologic body fluids on the Coulter LH 750 (Beckman Coulter, Fullerton, CA, USA), a fully automated random-access analyzer, which provides a complete blood count (CBC) with correction of the white blood cell (WBC) count in the presence of interference. The study included an assessment of diluent background counts, linearity, carryover, accuracy, and precision.

MATERIALS AND METHODS

Specimens

Three centers participated in this study: London Health Sciences Centre, Ontario, Canada (LHSC); the University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania (UPMC); and the University of Michigan Health System Ann Arbor, Michigan (UMHS). A total of 372 body fluid specimens were analyzed (158 serous fluid, 148 CSF, 66 synovial fluid). The group of serous fluids comprised a collection of peritoneal (89), peritoneal dialysate (14), pleural (49), pericardial (1), and paranephritic (1) fluid. All of the samples were obtained from spent clinical workloads and depending on sample type were either anticoagulated with EDTA or heparin or did not contain anticoagulant. Samples were tested within 8 hours of collection, and if testing was delayed beyond 2 hours of collection, the samples were refrigerated until the time of analysis. Each specimen was closely examined for the presence of clot formation, and if a clot was found to be present it was removed with an applicator stick. All synovial fluids were pretreated with hyaluronidase (approximately 5 mg/mL).

Coulter LH 750

The Coulter LH 750 is a fully automated, high throughput random-access hematology analyzer. Enumeration of WBCs and RBCs by this instrument is based on impedance technology, with improved algorithms for identifying WBCs. These improvements allow for better detection of interference hovering at the WBC threshold that has the potential to erroneously elevate the WBC count results. The analyzer was calibrated at the beginning of the study, and calibration was verified weekly. Three levels of control material were run daily in both the automated and manual modes. The instrument was configured to express WBCs to 2 decimal places and RBCs to 3 decimal places. All samples were analyzed in the CBC test mode and aspirated through the secondary aspiration mechanism of the analyzer (250 μL). Prior to sample aspiration, an aliquot of fresh diluent was run and background counts of $<0.2 \times 10^9$/L (WBC) and $<0.01 \times 10^{12}$/L (RBC) were required before proceeding. In the event the background exceeded the maximum requirements, the diluent blank was run a second time. Samples were well mixed by gentle inversion and run in duplicate, and the results were averaged.

Neubauer Chamber Counting

At LHSC and UMHS, 100 μL of fluid was diluted with 10 μL of 0.1% toluidine blue diluting fluid. Following incubation at room temperature for 5 minutes, both sides of an improved Neubauer chamber were flooded. If required, front-end dilution of the sample was made with physiologic saline (0.9% NaCl). Enumeration of both TNCs and RBCs was based on counting a maximum of 11 1-mm squares (6 on one side, 5 on the other). Cell count calculation involved correction for dilution, area, and depth.

At UPMC, different diluents were used depending on the type of fluid and cellularity. For some CSF samples, dilutions were made with CSF diluting fluid (ENG Scientific, Clifton, NJ, USA), which lysed the red cells and stained the nucleated cells. Other CSF samples with high cell counts were diluted with WBC and RBC Unopettes (Becton Dickinson, Franklin Lakes, NJ, USA) or with 0.9% saline. Serous and synovial fluid samples were diluted with 0.9% saline. For CSF samples with low counts, cells in all 9 squares on both sides of the chamber were counted, whereas for fluids with higher cell counts, fewer squares were used.

Statistical Analysis

Statistical analysis included a nonparametric Mann Whitney rank sum test (P), correlation (R^2), and regression statistics.

RESULTS

Background Count

The acceptable limits of diluent background as specified by the manufacturer were $<0.2 \times 10^9$/L for the WBC (TNC) and $<0.01 \times 10^{12}$/L for the RBC. The average background count of the diluent aspirations was 0.07 (0.02-0.19) $\times 10^9$/L for the WBC (TNC) and 0.001 (0.000-0.009) $\times 10^{12}$/L for the RBC. The background was not subtracted from the specimen counts.

Accuracy

We obtained 372 samples for accuracy studies (158 serous, 148 CSF, 66 synovial). Samples flagged for cellular interference

TABLE 1. Accuracy of Total Nucleated Cell Counts (TNC) <0.2 × 10⁹/L

Sample Fluid Type	Analyzed	Method	Mean	Minimum	Maximum	R^2	Slope	Intercept	P
Serous	61	Manual	0.09	0.00	0.20				
	61	LH 750	0.23	0.05	0.73	0.244	1.046	0.130	<.001
Synovial	9	Manual	0.07	0.01	0.18				
	9	LH 750	0.32	0.18	0.61	0.080	-0.642	0.363	<.001
Cerebrospinal	119	Manual	0.02	0.00	0.12				
	119	LH 750	0.10	0.02	0.42	0.144	1.012	0.084	<.001

TABLE 2. Accuracy of Total Nucleated Cell Counts (TNC) ≥0.2 × 10^9/L

Sample Fluid Type	Analyzed	Method	Mean	Minimum	Maximum	R^2	Slope	Intercept	P
Serous	82	Manual	3.87	0.20	34.10				
		LH 750	3.37	0.25	27.19	0.895	0.768	0.395	.88
Synovial	47	Manual	9.10	0.25	47.25				
		LH 750	8.31	0.32	38.14	0.836	0.741	1.568	.94
Cerebrospinal	23	Manual	1.17	0.20	7.56				
		LH 750	1.14	0.23	6.77	0.993	0.890	0.103	.84

were discarded from analysis (15 serous, 6 CSF, 10 synovial). The accuracy of each parameter was examined above and below the diluent background threshold of the instrument. As predicted, the accuracy of both parameters was less than desirable below the threshold of 0.2 × 10^9/L (TNC) and 0.01 × 10^{12}/L (RBC). Those results exceeding the threshold, however, showed a high correlation with manual chamber count results, and there were no significant differences between the automated and reference results (Tables 1-4).

Linearity

TNC linearity was evaluated by diluting a body fluid sample (undiluted count, 3.57 × 10^9/L) with instrument diluent to obtain results approximating the lowest reportable result of 0.2 × 10^9/L. The parameter demonstrated excellent linearity with a correlation coefficient (R^2) of 0.9984 (slope, 0.9748; intercept, 0.1338). The linearity of the RBC parameter (undiluted count, 0.129 × 10^{12} /L) was evaluated in a similar fashion. This parameter also demonstrated excellent

TABLE 3. Accuracy of Red Blood Cell (RBC) Counts <0.01 × 10^{12}/L

Sample Fluid Type	Analyzed	Method	Mean	Minimum	Maximum	R^2	Slope	Intercept	P
Serous	115	Manual	0.002	0.000	0.010				
		LH 750	0.006	0.000	0.149	0.075	1.946	0.003	<.001
Synovial	34	Manual	0.002	0.000	0.010				
		LH 750	0.009	0.000	0.033	0.119	0.919	0.007	<.001
Cerebrospinal	117	Manual	0.001	0.000	0.009				
		LH 750	0.003	0.000	0.040	0.392	1.473	0.001	<.001

TABLE 4. Accuracy of Red Blood Cell (RBC) Count ≥0.01 × 10^{12}/L

Sample Fluid Type	Analyzed	Method	Mean	Minimum	Maximum	R^2	Slope	Intercept	P
Serous	43	Manual	0.217	0.010	2.335				
		LH 750	0.237	0.008	2.867	0.957	1.058	0.007	.66
Synovial	32	Manual	0.688	0.013	6.950				
		LH 750	0.703	0.009	4.694	0.667	0.734	0.198	.81
Cerebrospinal	31	Manual	0.062	0.010	0.325				
		LH 750	0.068	0.001	0.357	0.849	1.055	0.002	.55

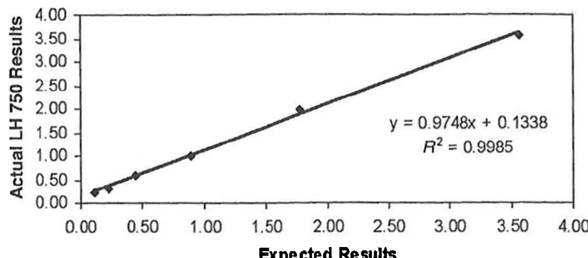

FIGURE 1. Total nucleated cell (TNC) count linearity. WBC indicates white blood cells.

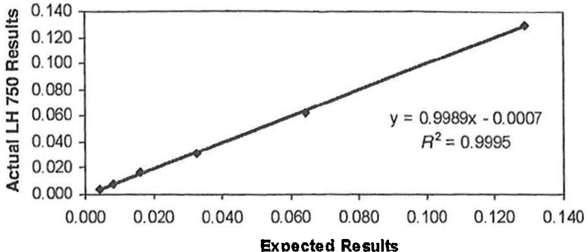

FIGURE 2. Red blood cell (RBC) count linearity.

linearity, with an R^2 of 0.9995 (slope, 0.9989; intercept, 0.0007) (Figures 1 and 2).

Precision

Six separate body fluid samples representing different TNC and RBC ranges were evaluated. Each fluid sample was run 11 consecutive times, and the results of the first run were universally discarded. Table 5 represents the corresponding coefficients of variation for each precision sample.

Carryover

Carryover was evaluated by processing 3 consecutive high body fluid counts (H1-H3) followed by 3 consecutive low body fluid counts (L1-L3). Carryover was calculated as [(L1-L3)]/[(H1-L3)] × 100%. TNC carryover was 0.64% and RBC carryover was 0.28%.

DISCUSSION

Manual analysis of body fluids remains a problematic area for most laboratories. This procedure requires a high level of expertise in cell identification and is labor-intensive, and results are poorly reproducible even with experienced observers [1]. New automated cell counters such as the Beckman Coulter LH 750 offer an alternative to the labor-intensive, imprecise manual methods of body fluid analysis. The LH 750, which offers technological advances in cellular analysis, particularly in the presence of interference, provides laboratorians with more accurate results as well as improved efficiency.

In this study, we compared the accuracy of the TNC and RBC counts in 372 body fluid samples run on the LH 750 to manual chamber counting. Of the 372 samples, only 106 had RBC counts greater than 0.01 × 10^{12}/L, and 142 had TNCs greater than 0.2 × 10^9/L measurable by automated analysis and suitable for method comparison. Low-level sensitivity (TNC <0.2 × 10^9/L and RBC <0.01 × 10^{12}/L) limits the utility of the LH 750 for analysis of many cerebrospinal and peritoneal dialysate fluids, which require accurate enumeration at clinical decision points between 0 and 0.1 × 10^9/L. Cellular CSF and peritoneal dialysate fluid samples with counts above the background limit of the analyzer, however, are excellent candidates for automated analysis. In the case of synovial and other serous fluids, most clinicians are interested in TNC counts above 0.2 × 10^9/L, whereas RBC counts are of limited significance [7-10]. If the criteria of reporting TNC counts as <0.2 × 10^9/L or accurate enumeration on counts ≥0.2 × 10^9/L are adopted, clinically relevant results could be provided by automated analysis in 93.8% of serous fluid specimens and 85.8% of synovial fluid specimens. Over this range of values for TNC and RBC counts, the LH 750 demonstrated high correlation with manual counts, low variation coefficients, and high reproducibility. Thus, with the exception of clear CSF and peritoneal dialysate fluid specimens, the majority of samples can be analyzed on the LH 750, resulting in significant time and cost saving for most laboratories.

TABLE 5. Precision Values Based on 6 Different Fluids at Various Cell Count Levels

Parameter	Mean	2 SD	Coefficient of Variation%	Minimum	Maximum
Total nucleated cells, ×10^9/L	0.41	0.04	4.44	0.39	0.45
	2.13	0.11	2.55	1.9	2.18
	14.1	0.10	0.39	13.99	14.15
Red blood cells, ×10^{12}/L	0.015	0.002	6.586	0.014	0.017
	0.307	0.005	0.891	0.303	0.312
	0.988	0.015	0.747	0.977	1.001

LHSC's 10 years of experience performing body fluid counts on automated instruments has had a highly favorable impact on the laboratory. As a result of a reduction in manual testing, the turn-around time dramatically improved and the level of anxiety on the part of the inexperienced technologist was significantly reduced. Not surprisingly, the change in reporting format was very well received by the clinicians. Consistency in unit expression and reporting of RBC counts only when appropriate, such as in the case of peritoneal trauma, helped to improve confidence in and accuracy of interpretation of the results. Currently, this particular sample group accounts for approximately 60% of the requests for body fluid analysis at this center. Automating the analysis has had a significant positive impact on the workload associated with this test.

This study confirms in a multicenter setting that automation of body fluid analysis on the LH 750 can result in a significant reduction in manual testing. This result should translate not only into improved accuracy but also improved efficiency, because the analysis time for manual methods is significantly longer than that for the automated method.

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The Clinical Reliability of Automated Cerebrospinal Fluid Cell Counts on the Beckman-Coulter LH750 and Iris iQ200

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Key Words: Cerebrospinal fluid; LH750; Iris iQ200; Body fluids

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Abstract

Although automation has improved the accuracy and precision of blood cell counts and is more rapid and less labor-intensive, cerebrospinal fluid (CSF) samples are still counted manually. We compared the IRIS iQ200 Body Fluids Module (Iris Diagnostics, Chatsworth, CA) and the Beckman-Coulter LH750 (Beckman-Coulter, Brea, CA) with manual counts and evaluated the impact of automation on the laboratory if clinically acceptable performance was to be maintained.

Automated counts were compared with manual counts on 313 specimens. Clinical reliability was estimated using the weighted κ coefficient and the impact of errors discussed in the context of a historic census of 3,653 samples spanning 19 months.

Nucleated cell counts had a reliability of 0.73 for the LH750 and 0.84 for the iQ200. However, our results showed unacceptable rates of error at counts less than 200/µL (200 × 10^6/L) for the LH750 and less than 50/µL (50 × 10^6/L) for the iQ200, representing 94% and 83% of the census specimens, respectively. If clinical reliability is to be maintained, neither the LH750 nor iQ200 would have a significant impact on improving the efficiency of the laboratory because of the high percentage of low CSF cell counts.

Since the inception of laboratory medicine, the goals have been to democratize the use of the laboratory by providing clinically accurate and relevant test results in a cost-effective manner. Cerebrospinal fluid (CSF) cell counts are a challenge because of low cell counts and limited sample volumes. Manual cell counts have provided clinically acceptable results for decades. It is inevitable and desirable that manual methods will be replaced by automated methods. However, it is imperative that this not occur without appropriate validation. Although several studies have attempted to show the usefulness of automated analysis of CSF, these studies also provide data showing unacceptable performance at low counts that are clinically important.[1-4] While emphasizing cost savings and enhanced productivity, they fail to provide or discuss the necessary demographic data to show the impact of automation on the workload.

Our study shows the distribution of CSF cell counts in a large hospital laboratory, has a large number of samples, and, in contrast with previous studies, compares manual and automated counts in the context of clinical decision-making thresholds. These directly model actual patient care, a connection that is omitted in parametric methods for assessing reliability, the result being these methods may overestimate the clinical reliability, particularly when the cell counts are low. Two automated instruments having different technology for enumerating cells were compared with duplicate manual counts.

Materials and Methods

Demographics

Rhode Island Hospital, Providence, is a 500-bed hospital that treats private and welfare inpatients and outpatients and is affiliated with a university medical school. Specimens were

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received from diverse specialty clinics, the emergency department, and hospitalized patients. Pediatric and adult patient specimens were included in our study.

Specimens

The study was done in 2 phases. Initially, all CSF samples sent to the clinical laboratory from April 2004 to October 2005 were tabulated to determine the distribution of cell counts. Subsequently, a total of 313 samples were collected for analysis to compare manual cell counts with automated cell counts on the Beckman-Coulter LH750 (Beckman-Coulter, Brea, CA) and Iris iQ200 Body Fluids Module (Iris Diagnostics, Chatsworth, CA). Each automated method was compared with the manual counts; however, this was not always possible because of insufficient sample volume, in which case automated counts were missing. All samples were analyzed at the time they were received on 2 shifts, 7 days a week by selected personnel. Permission for the study was granted by the institutional review board.

Manual Method

Total nucleated cells (TNCs) and RBCs were counted in an improved Neubauer hemocytometer chamber using bright-field microscopy with a 40× objective. A coverslip was placed on a clean, dry hemocytometer chamber that was placed in a moist Petri dish on 2 applicator sticks. Both sides of the chamber were filled and cells allowed to settle for 5 to 10 minutes. RBCs and TNCs were counted on each side of the chamber. If fewer than 200 cells were present, all 9 squares were counted. If more than 200 cells were present, the 3 middle squares were counted unless more than 200 cells were in 1 square. Then only the center square was also counted. The cell count was calculated using the area counted, depth of the chamber, and any corrections for dilution of the sample. The average count of the 2 sides was used for comparison with the automated result.

Coulter LH750

The specimens were analyzed using the manual aspiration mode. Before analysis, diluent was aspirated 3 times and the background count of the third aspiration was recorded. The CSF sample was then aspirated. Background counts were subtracted to determine the final TNC and RBC counts. Nucleated cells were counted using 2 decimal places and erythrocytes using 3 decimal places.

Iris iQ200 Automated Microscopy Analyzer Cell Counter

The iQ200 Body Fluid Module was used to count CSF nucleated cells and erythrocytes. The specimens were divided into 2 aliquots, 1 mixed with lysing reagent to count nucleated cells and 1 mixed with a buffered diluent to count total cells. Clear specimens were diluted 1:5, slightly bloody 1:10, and bloody 1:20.

Statistical Methods

All analyses and visualizations were conducted using SAS, version 9.1.3 (SAS Institute, Cary, NC), Excel 2003 SP3 (Microsoft, Redmond, WA), and Matlab R2007b (Mathworks, Natick, MA).

TNC counts were grouped into clinically relevant categories.[5] The reliabilities of the categories into which counts fell were estimated based on the weighted κ coefficient.[6] The Bowker test of symmetry was used to test for systematic overestimation or underestimation between the mean of manual counts and the automated counts.[7] Symmetry was not examined for the reliability of manual count duplicates because duplicate assignment was arbitrary.

RBC counts from the automated methods were compared with the mean of the manual counts via regression after logarithmic transformation holding the intercept at zero. Zero counts were excluded rather than adding a constant. A slope of 1.0 represents unbiased estimates. The higher the r^2, the less disagreement, and visualization was used in detection of systematic deviations that may occur even when the other parameters appear "good."

Results

Distribution of CSF Samples by Nucleated Cell Counts

During an 18-month period a total of 3,653 CSF samples were accessioned. Of the 3,653 specimens, nucleated cells ranged from 0 to 10/µL (0-10 × 10^6/L) in 76.2%. Only 10.4% had nucleated cell counts greater than 100/µL (100 × 10^6/L) **Table 1**.

Reliability of Manual Nucleated Cell Counts

There were 302 samples for which manual TNC counts were made from duplicate samples from the same patient. These counts showed a weighted κ value of 0.944 with 95% confidence limits of 0.907 and 0.981 **Table 2**. Where disagreements in clinical category occurred, the differences in the count were generally small relative to the count magnitudes, with one count often lying on the threshold value, particularly for the lower ranges.

There were 14 patients in whom one count was "normal" (TNC count, ≤5/µL) and the other count was in the category of more than 5 to 10/µL. The mean difference between these counts was 3.8/µL (SD, 1.1/µL), with 7 (50%) of the counts falling on the threshold with values of 6/µL. There were 13 patients in whom one count was in the category of more than 5 to 10/µL and the other was in the category of more than 10 to 50/µL. The mean difference between these counts was 6.2/µL (SD, 6.5/µL), with 4 (31%) of the counts falling on the threshold with values of 11/µL. There were 5 patients in

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Table 1
Distribution of Cerebrospinal Fluid Samples by Total Nucleated Cell Counts in 3,653 Specimens

	Total Nucleated Cells (/µL)				
	0	**1-10**	**11-100**	**101-200**	**>200**
No. (%) of specimens	1,113 (30.5)	1,672 (45.8)	488 (13.4)	141 (3.9)	239 (6.5)

Table 2
Interslide Manual Count Reliability*

	Manual TNC Count 2 (/µL)					
Manual TNC Count 1 (/µL)	**0-5**	**>5-10**	**>10-50**	**>50-200**	**>200**	**Total**
0-5	**113**	6	0	0	0	119
>5-10	8	**11**	10	0	0	29
>10-50	0	3	**54**	4	0	61
>50-200	0	0	1	**51**	2	54
>200	0	0	0	2	**37**	39
Total	121	20	65	57	39	302

TNC, total nucleated cells.
* Data are given as the number of cases in each clinically relevant group. Numbers in boldface indicate the number of cases in complete agreement by both counts in each clinically relevant group. Weighted κ, 0.944; 95% confidence limits, 0.907, 0.981.

whom one count was in the category of more than 10 to 50/µL and the other was in the category of more than 50 to 200/µL. The mean difference between these counts was 22.4/µL (SD, 8.8/µL). There were 4 patients in whom one count was in the category of more than 50 to 200/µL and the other was more than 200/µL. The mean difference between counts was 77.3/µL (SD, 58.8/µL).

Reliability of LH750 Compared With Manual Nucleated Cell Counts

The reliability of LH750 counts with the mean of the duplicate manual counts of 191 samples was 0.734 with 95% confidence limits of 0.649 and 0.820 **Table 3**. Of the patient samples, 43.5% were misclassified according to clinical thresholds **Table 4**, with agreement improving as mean manual cell counts increased to more than 200/µL (200×10^6/L). However, this "good" range would represent only 6.5% of the total specimens based on our hospital census (Table 1).

Table 4
Misclassified Cerebrospinal Fluid Nucleated Cell Counts

Manual (n = 302)	iQ200 (n = 300)*	LH750 (n = 191)*
0-5 cells/µL	33/123 (26.8)	49/76 (64.5)
>5-10 cells/µL	14/19 (73.7)	5/9 (55.6)
>10-50 cells/µL	20/63 (31.7)	18/43 (41.9)
>50-200 cells/µL	14/56 (25.0)	9/41 (22.0)
>200 cells/µL	1/39 (2.6)	2/22 (9.1)
Total	82/300 (27.3)	83/191 (43.5)

* Data are given as number/total (percentage).

Table 3
LH750 Reliability Compared With Manual Counts*

	LH750 (No. of Cells/µL)					
Manual TNC (/µL)†	**0-5**	**>5-10**	**>10-50**	**>50-200**	**>200**	**Total**
0-5	**27**	14	27	7	1	76
>5-10	2	**4**	3	0	0	9
>10-50	4	5	**25**	8	1	43
>50-200	1	0	5	**32**	3	41
>200	0	0	0	2	**20**	22
Total	34	23	60	49	25	191

TNC, total nucleated cells.
* Data are given as the number of cases in each clinically relevant group. Numbers in boldface indicate the number of cases in complete agreement by both methods in each clinically relevant group. Reliability: weighted κ, 0.734; 95% confidence limits, 0.649, 0.820; symmetry: S = 33.957; df, 10; *P* = .0002.
† Mean value for 2 counts.

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Reliability of iQ200 Compared With Manual Nucleated Cell Counts

The reliability of the iQ200 with the mean of duplicate manual counts of 300 samples was 0.836 with 95% confidence limits of 0.779 and 0.894 **Table 5**. Where the mean manual count was between 0 and 5/µL (0-5 × 10^6/L), 26.8% were misclassified by the iQ200. Of the normal samples, 11.4% ranged from 11 to 46/µL, a clinically significant misclassification.

RBC Counts

The comparisons of manual and automated RBC counts for the LH750 and iQ200 are shown in **Figure 1**. The slope of the LH750 regression function for the nonzero mean ± SE manual counts was 1.04 ± 0.0413 (top left). Although the r^2 value was more than 92.6% when all nonzero counts were included, there were clearly systematic deviations whereby the LH750 frequently overestimated the RBC count as 1,000/µL for values less than 1,000/µL. Further difficulties not illustrated here arose when subtraction of background produced negative values (n = 45), which were treated as zero counts. However, when samples having mean manual counts of 1,000/µL or less were excluded from analysis (top right), the systematic variation was removed, the r^2 increased to 99.9%, and the slope moved closer to unity, with a reduction in its SE despite the reduction in sample size (0.9911 ± 0.0060). The slopes for both were statistically significantly different from zero, and neither was significantly different from unity.

Similar, but less pronounced results were observed with the iQ200. However, the systematic deviation of the iQ200 from the mean manual counts less than 1,000/µL (bottom left, slope = 1.0047 ± 0.0138, r^2 = 96.7%) presented as overestimation and a general increase in variability, rather than a single repeating value. Again, analysis for samples with mean counts more than 1,000/µL improved performance (bottom right), with the slope remaining close to unity and with reduced variability (0.9945 ± 0.0035) and increasing the r^2 to near perfect agreement within the reported level of precision (100.0%). The slopes for both were statistically significantly different from zero, and neither was significantly different from unity.

Discussion

Clinical laboratories are in the unenviable position of having to provide cost-effective, rapid, and accurate test results to clinicians, often with reduced numbers of and/or minimally trained laboratory personnel. Automated methods have helped to successfully meet these goals in many areas of the laboratory, but CSF analysis has remained a challenge because nucleated cell counts that distinguish normal from abnormal test results are ultralow and the sample volume is limited.

Several studies have evaluated the applicability of automated cell counters commonly used in clinical laboratories for the analysis of CSF cell counts. The instruments have diverse principles of operation that include impedance, digital imaging, and uni-fluidics technology.[1-4,8] To evaluate the performance of automated counts, statistical analyses have traditionally included parametric methods such as regression analysis, precision, and linearity studies. These are described for the Coulter LH750 and iQ200 in several reports.[1,3,4] However, while parametric analyses provide estimates of the degree of concordance between manual and automated methods, they do not adequately determine clinical reliability. Our results highlight that parametric methods may overestimate the reliability of the applied system (ie, automated method) as a whole.

Diseases of the CNS have different patterns of pleocytosis that influence clinical decisions, additional test selection, and follow-up.[5,9-12] Thus, to determine the clinical implications of automated compared with manual nucleated cell counts, the weighted κ statistic was used as a measure of clinical reliability, with supplemental information provided as to the types and rates of errors produced.

Table 5
iQ200 Reliability Compared With Manual Counts*

	iQ200 (No. of Cells/µL)					
Manual TNC (/µL)†	**0-5**	**>5-10**	**>10-50**	**>50-200**	**>200**	**Total**
0-5	**90**	18	14	1	0	123
>5-10	3	**5**	11	0	0	19
>10-50	3	1	**43**	16	0	63
>50-200	1	0	2	**42**	11	56
>200	0	0	0	1	**38**	39
Total	97	24	70	60	49	300

TNC, total nucleated cells.
* Data are given as the number of cases in each clinically relevant group. Numbers in boldface indicate the number of cases in complete agreement by both methods in each clinically relevant group. Reliability: weighted κ, 0.836; 95% confidence limits, 0.779, 0.894; symmetry: S = 45.388; df, 10; P = < .0001.
† Mean value for 2 counts.

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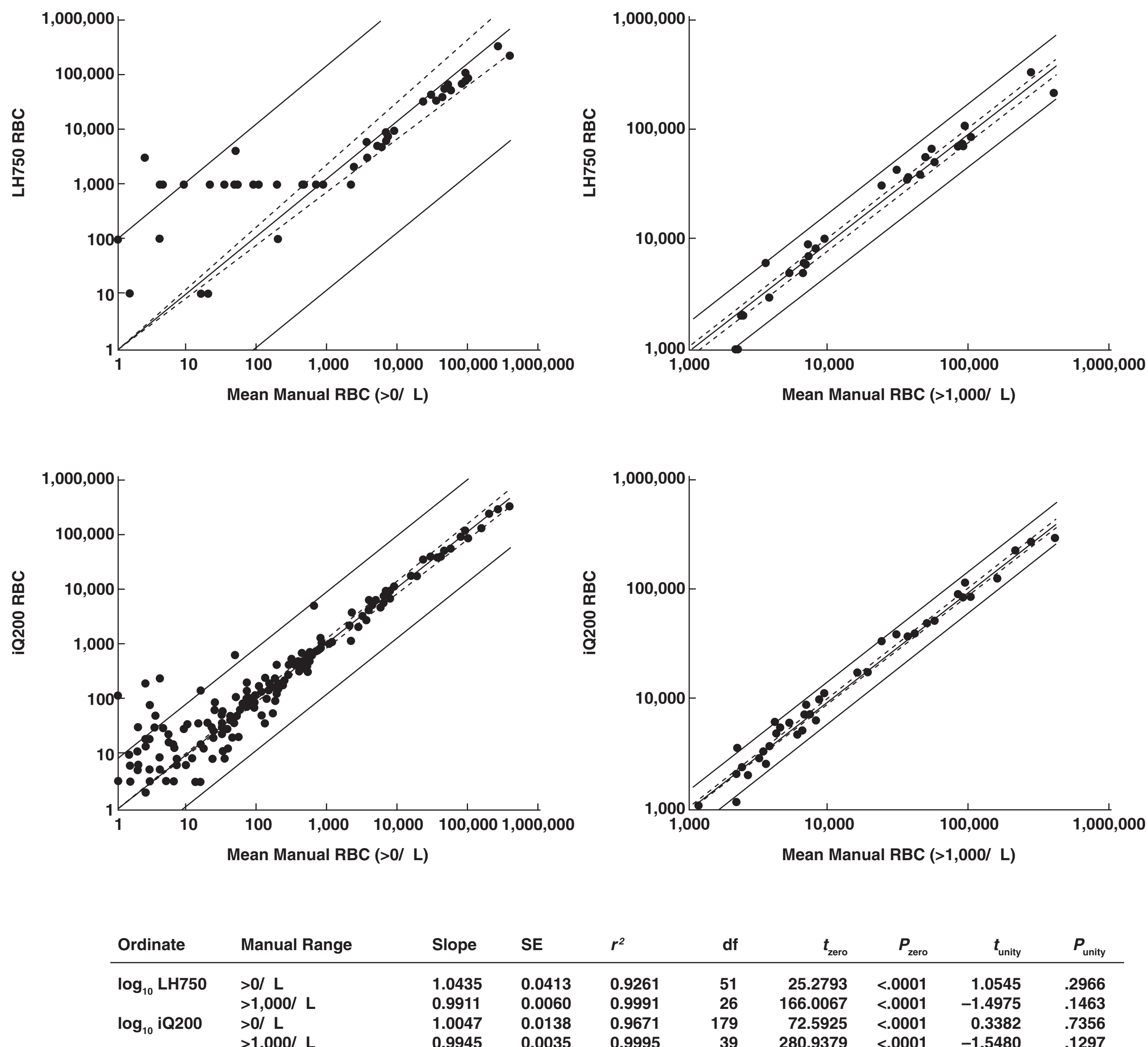
Our results for the LH750 showed poor reliability of automated nucleated cell counts less than 200/μL (200 × 10^6/L). In our laboratory, this would exclude about 94% of CSF specimens for analysis. This finding is similar to findings of other studies.[1-3] Barnes et al[3] reported a cutoff value of 300/μL, excluding more than 90% of the CSF samples and restricting the use of the LH750 to grossly bloody or cloudy fluids.

Results of the iQ200 automated digital imaging system were more encouraging. It is clear that differences between normal and abnormal results determine clinical decisions that, in some cases, would avoid medical misadventures. In the



Ordinate	Manual Range	Slope	SE	r^2	df	t_{zero}	P_{zero}	t_{unity}	P_{unity}
$\log_{10}$ LH750	>0/ L	1.0435	0.0413	0.9261	51	25.2793	<.0001	1.0545	.2966
	>1,000/ L	0.9911	0.0060	0.9991	26	166.0067	<.0001	–1.4975	.1463
$\log_{10}$ iQ200	>0/ L	1.0047	0.0138	0.9671	179	72.5925	<.0001	0.3382	.7356
	>1,000/ L	0.9945	0.0035	0.9995	39	280.9379	<.0001	–1.5480	.1297

Figure 1 Automated RBC count as a function of the mean manual RBC count. Upper left, Comparison of the automated LH750 RBC count with the manual RBC count. The LH750 frequently overestimated the RBC count as a single repeating number of 1,000/μL for manual counts ≤1,000/μL. Upper right, Comparison of LH750 RBC counts with manual counts when manual counts ≤1,000/μL are excluded. Lower left, Comparison of iQ200 and manual RBC counts. The iQ200 RBC counts do not show a single repeating value when manual counts are ≤1,000/μL but are overestimated and show an increase in variability. Lower right, Comparison of iQ200 RBC counts with manual counts ≤1,000/μL excluded. The concordance improved with less variability and near perfect agreement.

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normal range, about 27% of the samples were misclassified. However, interpretation of results also differs among physicians. Whereas Fishman[5] considered values greater than 5/µL definitely abnormal, Merritt and Fremont-Smith[9] considered values of TNCs in the range of more than 5 to 10/µL (5-10 × 10^6/L) "suspicious." By this criterion, only 12% of the samples would be misclassified, a value that is also clinically unacceptable. It is less clear that misclassifications in other groups would alter clinical decisions. In only 1 sample was there a serious misclassification of the TNC count in the more than 50 to 200/µL group. Thus, 50 TNCs/µL (50 × 10^6/L) is our recommended lower limit of detection for the iQ200, a value similar to the lower limit of detection of TNCs of 30/µL (30 × 10^6/L) reported by Butch et al.[4]

There are conflicting data on the Advia 120 hematology analyzer (Bayer HealthCare, Tarrytown, NY). Aulesa et al[2] found the lower limit of detection for leukocytes to be 47/µL (47 × 10^6/L) and established their limit of reliability at 150/µL (150 × 10^6/L), which was equivalent to 3 SD. Aune et al[8] claim accurate results between leukocyte counts of 0 and 10/µL (0-10 × 10^6/L) in a study supported by Bayer Healthcare. It would be of interest to statistically evaluate data using the weighted κ statistic.

The importance of the clinical laboratory to report accurate CSF nucleated cell counts is concisely summarized by Fishman[5]: "Finally, clinicians are at the mercy of the clinical laboratories upon which they depend, some of which fail to meet a high standard of performance for the chemical, bacteriological, serological, and cytological study of the CSF. Caveat emptor!"

RBC counts are of importance in diagnosing pathologic bleeding in the central nervous system. This includes cerebral hemorrhage, subarachnoid hemorrhage, cerebral trauma, and subdural hematoma. There are usually numerous RBCs present in these clinical conditions, and their enumeration is not a problem for the clinical laboratory. The counts are also useful in determining if bleeding is secondary to the trauma of the procedure. Decreasing RBC counts in serial tubes are indicative of a traumatic tap. If RBC counts are less than 1,000/µL, the automated instruments would not be able to reliably distinguish RBC counts in serial tubes. If there is a traumatic tap, the RBC count can also be used to calculate the baseline CSF nucleated cell count because 1 leukocyte contaminates the CSF fluid for every 500 to 1,000 RBCs. The LH750 and iQ200 reliably measured RBC counts of more than 1,000/µL.

Clinical laboratories are under intense pressure to replace labor-intensive manual methods with automation. Comparative studies emphasize the benefits of precision, consistency, and reduction in the need for highly trained skilled personnel with automation. As indicated previously, clinicians are held captive to the administrative and scientific decisions implemented by clinical laboratories. Our study demonstrates the importance of using measurements of reliability sensitive to clinical interpretation in addition to parametric analyses to evaluate the needs of clinicians and the impact on patient care. It also demonstrates the importance of evaluating demographic data to determine the actual cost savings of automation, if clinical reliability is to be maintained.

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Hematology

Leukocyte Counts in Cerebrospinal Fluid with the Automated Hematology Analyzer CellDyn 3500 and the Urine Flow Cytometer UF-100

Reinhard Ziebig,* Andreas Lun, and Pranav Sinha

Background: **The counting of leukocytes and erythrocytes in cerebrospinal fluid (CSF) is still performed microscopically, e.g., using a chamber in most laboratories. This requires sufficient practical experience, is time-consuming, and may constitute a problem in emergency diagnostics. Specific automated systems for CSF cell counting are not available at present.**
Methods: **We tested the hematology analyzer CellDyn 3500 (CD) and the urine flow cytometer UF-100 (UF), which are not designed for CSF analysis. We studied >104 samples with both analyzers, and the counts obtained were compared with the reference method (Fuchs-Rosenthal chamber).**
Results: **Good linearity in the medically relevant range of 15×10^6 to 1000×10^6 leukocytes/L and a high degree of within-run accuracy were seen for both analyzers. Cell counting on the UF was excellent, especially when low cell counts were encountered (CV, 4.9% compared with 28% observed for the CD). Method comparison showed that identical results could be detected for a majority of the count pairs. For a few samples, there was a discrepancy between the results from the analyzers and the counting chamber. In most cases, these were CSF samples containing a high proportion of lymphocytes. For these samples, the CD result led to a false-positive high leukocyte count, and on the UF these cells were not allocated to the leukocyte population, thus leading to false-negative counts.**
Conclusions: **Both analyzers should not be used for CSF cell counting in all cases at present. However, once the technical and software problems have been solved, routine use of the two analyzers for CSF analysis should be seriously contemplated.**
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The counting of leukocytes and of erythrocytes in cerebrospinal fluid (CSF)[1] has not been automated. Cell counts are still performed microscopically, e.g., using the Fuchs-Rosenthal chamber in most laboratories. Fully automated analyzers meet time and quality requirements and are objective in material handling. On the other hand, laboratory technicians frequently must squeeze microscopic chamber counting into tight laboratory schedules (with increasing workloads), additionally needing to consider several other factors (e.g., number of samples and quality of the cells). Furthermore, insufficient practical experience in microscopic chamber counting and the subjectivity of individual laboratory workers adds to the unreliability of the results frequently observed.

The reason for the lack of more specific CSF diagnoses, including, e.g., a cell differentiation, often is insufficient sample volume and/or too few cells in a sample. Analyses performed with the modified sedimentation chamber technique according to Sayk *(1)* or with centrifugation *(2)* have contributed to the optimization of CSF cytology. They do not, however, solve the primary problem of the accurate determination of the number of cells.

Specific automated systems for CSF cell counting are not available at present. The following requirements, among others, would be necessary for such a system: (*a*) the ability to count small numbers of cells (e.g., 10×10^6 cells/L); (*b*) the ability to differentiate leukocytes into polymorphonuclear and mononuclear cell populations; and (*c*) the use of small sample volumes. On the basis of these requirements, we considered two analyzers appro-

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Dedicated to Professor Eckart Köttgen on his 60th birthday.

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[1] Nonstandard abbreviations: CSF, cerebrospinal fluid; CD, CellDyn 3500; UF, UF-100 urine flow cytometer; WOC, white blood cell optical count; FWBC, fragile white blood cell; and KWOC, kinetically corrected white blood cells in optical channel.

priate for a test to determine cells in the CSF: the Abbott CellDyn 3500 (CD) and the Sysmex UF-100 (UF).

The automated hematology analyzer CD permits, according to the manufacturer, the counting and differentiation of leukocytes in <50 × 10^6 cells/L. This analyzer requires a sample volume of 150–200 μL. The urine flow cytometer UF was designed for the analysis of particles in urine and thus also of small numbers of leukocytes and erythrocytes. The sample volume required by this analyzer is 800 μL.

Materials and Methods

CSF SAMPLES

One hundred four CSF samples were examined for leukocytes in a paired comparison on the CD, the UF, and in a microscopic counting chamber (Fuchs-Rosenthal).

Routine CSF samples were brought from the ward on ice-water and were stored in the laboratory until the analyses were performed. The samples were obtained from patients after brain surgery (n = 76), patients suffering from viral and bacterial meningitis (n = 12) and/or encephalitis (n = 5), and patients in remission who had undergone control examinations (n = 11). Because only the material that was leftover from routine analyses was used, special consent from the patients was not required.

Initial analyses in defined cell suspensions prepared in our laboratory were used to check the accuracy and linearity. For this purpose, cell-rich plasma (buffy coat) was obtained from EDTA-blood samples after their spontaneous sedimentation, and its composition was checked on the H3 hematology analyzer (Bayer Diagnostics) in repeated tests. Thereafter, these samples were diluted with physiological saline solution to cell concentrations typical in the CSF in patients suffering from encephalitis and meningitis.

A special CSF pool was prepared to test the within-run imprecision in CSF. Ten samples were mixed and analyzed.

ANALYZERS

The counting principles, specifications, and evaluations of the CD and the UF have been published previously *(3–8)*. For counting and differentiation of leukocytes, the CD uses a multiangle polarization scatter separation technology in the optical channel [white blood cell optical count (WOC)], combined with a second channel with impedance count (white blood cell impedance count). The erythrocyte counting is based on the impedance principle *(3–5)*. On the UF, cells in the urine are determined by light scatter (small-angle and wide-angle scattering) and the fluorescence of the cell membrane and the chromatin after staining with phenanthridine and carbocyanine as well as by impedance *(6–8)*.

In preparation for CSF cell counting, the CD was flushed three times with saline to obtain cell counts of <3 × 10^6 cells/L. Only the leukocytes counted in the WOC channel were taken into consideration for the evaluation. All scattergrams including lobularity of 90 degrees and complexity of 10 degrees that showed the separation of polymorphonuclear cells from mononuclear cells were scrutinized carefully. In addition, the counts rate summary for leukocytes in the optical channel was printed. This indicated the stability of leukocytes.

As with the CD, the UF was flushed three times to obtain cell counts of <3 × 10^6 cells/L. The CSF samples were measured either directly or were first diluted with a physiological saline solution when the sample volumes were <800 μL. Samples were also prediluted when the number of cells (particles) counted was flagged by the analyzer as >40 000 particles.

INTERPRETATION OF COUNTING PROTOCOLS

When interpreting the findings, it was helpful to take the following alarms as well as some other results into account: For the CellDyn 3500, "VARIANT LYM" indicates atypical lymphocytes and/or pathological cells; "FWBC" indicates fragile white blood cells (WBCs); "KWOC" indicates a kinetically corrected value of the optical counting channel (WOC) caused by a nonlinear counting pulse rate. For the UF-100, the only alarm that was considered was "Other Particles", which indicates particles that can not be allocated to any population in the scattergram.

STATISTICAL EVALUATION

The test results were evaluated by the regression method of Passing and Bablok *(9)* as well as by demonstrating the count difference of two methods in relation to the mean value of the two value pairs, according to the Bland-Altman method *(10)*.

Results

The within-run imprecision for the CD and the UF for three series of tests with different cell suspensions and a special CSF pool (UF only) are shown in Table 1. The UF had surprisingly low within-run CVs (4.9–3.5%) for the investigated cell suspensions ($\bar{x}$ = 28.9 × 10^6, 82.8 × 10^6, and 387.4 × 10^6 WBC/L). The measurement in the CSF pool produced a CV of 6.8% ($\bar{x}$ = 37.4 × 10^6 WBC/L). With the CD, the cell suspensions with low leukocyte

Table 1. Within-run imprecision of leukocyte counting on the CellDyn 3500 (CD) and the UF-100 (UF).

CD			UF		
n[a]	$\bar{x}$[b]	CV, %	n[a]	$\bar{x}$[b]	CV, %
10	28.1	28[c]	10	28.9	4.9[c]
10	86.6	16[c]	10	82.8	3.8[c]
10	363.7	3.3[c]	10	387.4	3.5[c]
			5	37.4	6.8[d]

[a] Number of measurements.
[b] Mean (WBC × 10^6/L).
[c] Cell suspension.
[d] CSF pool.

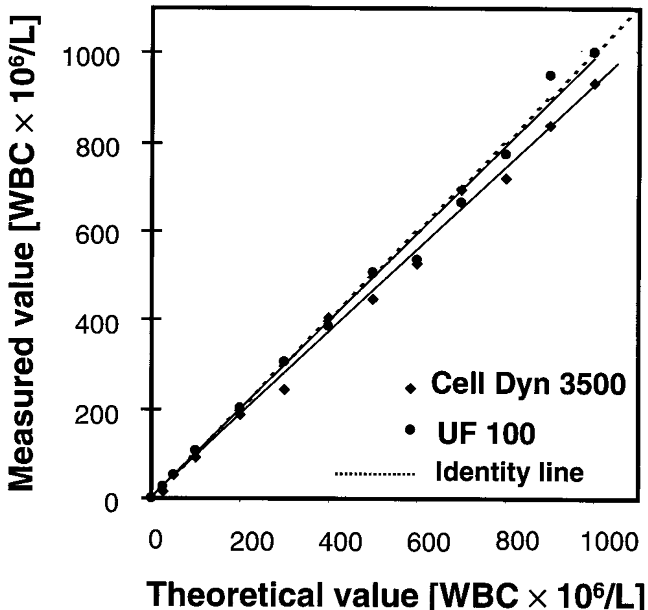

Fig. 1. Linearity of leukocyte counting of cell suspensions on the CellDyn 3500 and the UF-100.

◆, theoretical leukocyte value vs leukocyte counts on the CD 3500 (y = 0.93x + 0.19); ●, theoretical leukocyte value vs leukocyte counts on the UF-100 (y = 0.99x + 1.31).

counts ($\bar{x}$ = 28.1 × 10^6 and 86.6 × 10^6 WBC/L) showed acceptable CVs (28% and 16%), and the CV of the third test series was 3.3% ($\bar{x}$ = 363.7 × 10^6 WBC/L), identical to that of the UF.

The linearity of leukocyte counting was carried out in serial dilutions prepared from a defined cell suspension. Fig. 1 shows that of the leukocyte counts on the UF were linear even in the lower range. Furthermore, the CD also produced leukocyte counts with acceptable linearity in this range. The Cusum test produced no significant deviation in both cases.

The results of the comparison of the methods used for the determination of leukocytes in the CSF are shown in Fig. 2. Leukocyte counts >1000 × 10^6/L were not taken into account for statistical evaluation so that the examinations were narrowed to the medically critical range for decisionmaking.

The comparison of the CD, UF, and microscopic chamber counting methods yielded correlation coefficients (r) between 0.87 and 0.91, and the slope of the regression line in the comparison between CD and UF was almost identical with the identity line (Fig. 2a). In contrast, the regression lines between the chamber and CD (Fig. 2b) and the chamber and UF (Fig. 2c) methods show larger deviations from the identity line. In Fig. 2c, it is the slope of 1.35 (intercept = −1.39 × 10^6 WBC/L) that is primarily responsible for the difference in the leukocyte values between chamber and UF. It should be critically stated that the extended range of leukocyte counts (0 to 980 × 10^6 WBC/L) makes the correlation look more favorable than it is.

A simple graphic presentation of pair differences in method comparison (difference plot after Bland and Altman) clarifies this fact. Paired values between CD and UF were chosen for this presentation. In Fig. 3, the differences between the value pairs are depicted on the y-axis and the mean is depicted on the x-axis. The mean difference of the comparative counting as well as the standard deviation (± 2 SD) of the differences is shown parallel to the x-axis. From Fig. 3, it is apparent that some of the value pairs show larger differences in different directions. Based on these differences, medical acceptance is impossible. Because the results obtained with microscopic chamber counting were similar to the results obtained with CD or UF, they are not shown here.

The following results obtained for one sample illustrate the problems with the validity of the cell counts and the impact of interfering factors on CSF cell counting with automated analyzers. The macroscopic appearance of this sample was without any apparent abnormalities. Cell counts yielded the following results for leukocytes: cham-

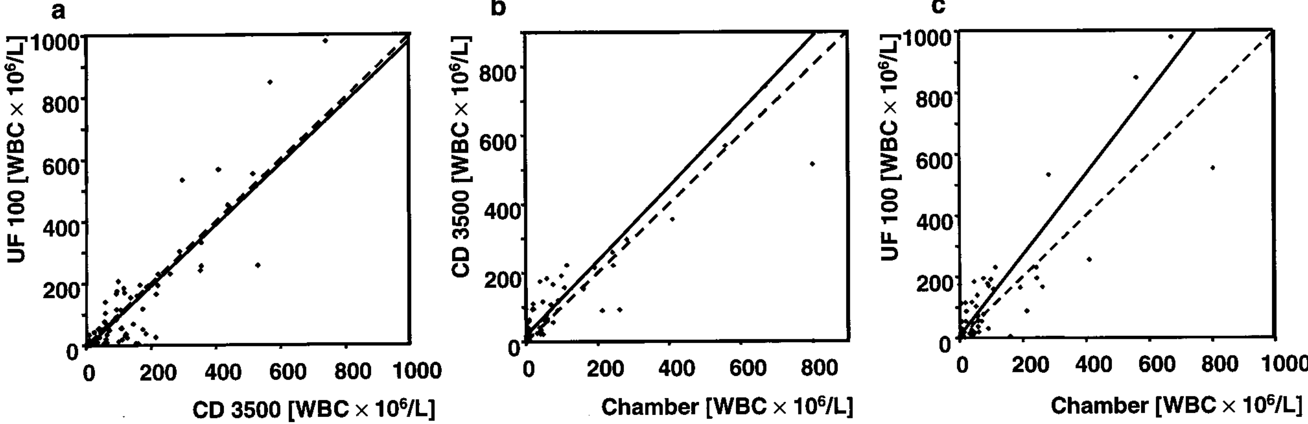

Fig. 2. Regression analysis between leukocyte counting by the UF-100 and CD 3500.

(a), UF-100 vs CD 3500; regression line: y = 0.99x − 4.09; r = 0.91; n = 104. (b), CD 3500 vs counting chamber; regression line: y = 1.11x + 5.68; r = 0.92; n = 73. (c), UF-100 vs counting chamber; regression line: y = 1.35x − 1.39; r = 0.87; n = 73. n, number of value pairs.

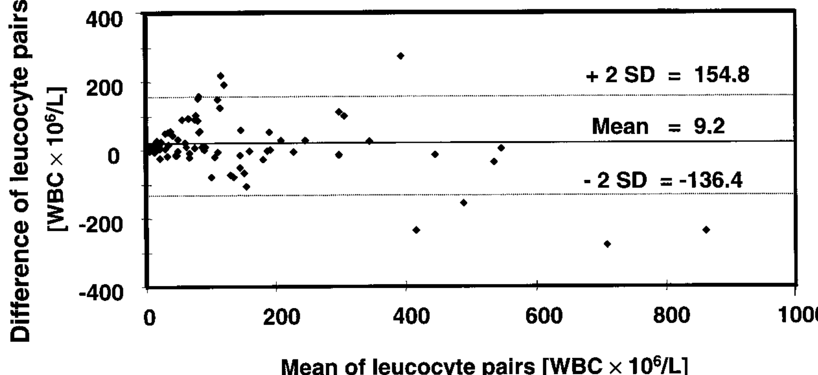

Fig. 3. Comparison of CellDyn 3500 and UF-100 counts of leukocytes for 104 CSF cell samples.

The Bland-Altman difference plot depicts the difference of the value pairs on the *y-axis* and the mean on the *x-axis*. The mean difference of the comparative counting (*solid line)* as well as the SD (± 2 SD) of the differences (*dotted lines*) is shown parallel to the *x-axis*.

ber counting, 150 × 10^6 WBC/L; CellDyn 3500, 215 × 10^6 WBC/L; UF-100, 24 × 10^6 WBC/L.

The protocols of the CD indicated an abnormal lymphocyte population with KWOC, VARIANT LYM, and FWBC flags. KWOC in this case means the mathematical extrapolation of the number of leukocytes with assumed cell degeneration during the count period. This mathematical correction algorithm leads to a higher number of leukocytes. The percentage of the lymphocyte population registered here was 97%.

The same sample yielded a lower leukocyte count (24 × 10^6 WBC/L) on the UF. The scattergram (forward scattered light intensity vs fluorescent light intensity; Fig. 4) shows in addition to the blue cluster representing the leukocytes, a second cluster marked in yellow. These cells (169 × 10^6 WBC/L) are not assigned to any of the populations defined in the UF scattergram. The addition of these 169 × 10^6 cells to the leukocyte count would produce a corrected value of 193 × 10^6 WBC/L. A strictly defined yellow cluster in the scattergram such as the one shown for this sample was always found in the presence of atypical lymphocytes.

Six additional samples yielded similar results. Method comparisons between the leukocyte value obtained with the chamber and the UF revealed poor correlation (r = 0.20). A comparison of the corrected leukocyte value and chamber counting showed good correlation (r = 0.94). Fig. 5 shows the corresponding regression line for seven CSF samples (circles).

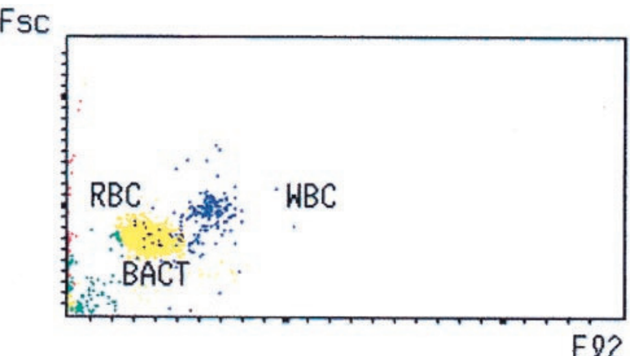

Fig. 4. UF-100 scattergram for a CSF cell sample with atypical lymphocyte characteristic.

Fsc, forward scattered light intensity; *Fl2*, fluorescent light intensity; *RBC*, erythrocytes (*red*); *WBC*, leukocytes (*blue*); *Bact*, bacteria (*green*). The cells in the *yellow clusters* are not allocable to any of the population defined in the UF scattergram.

To study the influence of lymphocytes on the leukocyte count obtained with the UF, defined cell suspensions whose leukocyte concentration and composition had been determined earlier on the H3 were analyzed on the UF (Fig. 6). We can demonstrate that an increase in the lymphocyte population leads to reduced leukocyte counts. Typically, these lymphocytes are not allocated to any defined cell population.

If one correlates leukocyte differences (between CD and UF, CD and chamber, or UF and chamber) with the percentage of lymphocytes in the CSF sample, the correlation coefficients (r) are between 0.42 and 0.71 (Table 2). Evidence of the same correlation was found when cell suspensions were used instead of CSF (Table 2, values in parentheses).

Better correlations between CD and the counting chamber for CSF samples high in lymphocytes were

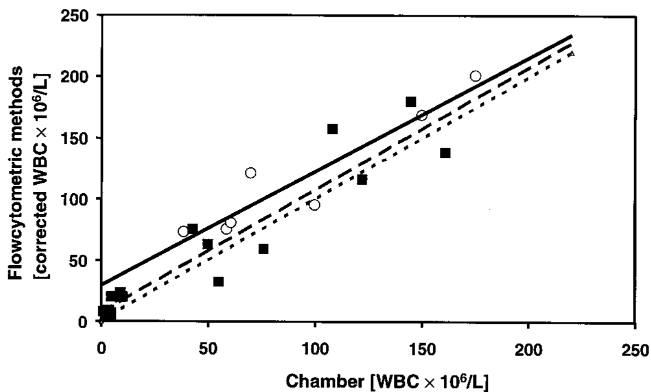

Fig. 5. Comparison of leukocyte counts between chamber and UF-100 (corrected value: summary of original leukocyte counts plus "other particles") and comparison of leukocyte counts between chamber and CD 3500 (raw data of measurements as corrected values).

○, value pairs of chamber and UF-100; (———), regression line between chamber and UF-100: $y = 0.929x + 29.515$; $r = 0.94$; n = 7. ■, value pairs of chamber and CD 3500; (— — — — —), regression line between chamber and CD 3500: $y = 1.005x + 6.572$; $r = 0.95$; n = 20. (········), line of identity. n, number of value pairs.

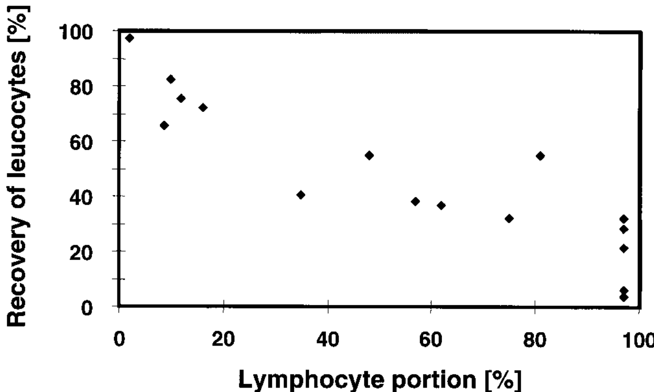

Fig. 6. Dependence of recovery of leukocytes (%) by the UF-100 on lymphocyte portion (%) in defined cell suspensions.

The leukocyte concentration and composition had been determined earlier on the H3 analyzer.

achieved when raw uncorrected values measured on the CD were used. Fig. 5 shows the regression line resulting from the comparison (squares) of CD (raw leukocyte values) and counting chamber results for 20 CSF samples high in lymphocytes (50–97%). It is evident from these results that in this way, it is possible to considerably minimize the differences of the counts. This is, however, a tedious way of obtaining correct results.

The evaluation of the erythrocyte counts obtained with the UF (y) and by chamber counting (x) showed a correlation coefficient (r) of 0.83 ($n = 48$) and the following regression line: $y = 0.873x + 1.518$. Erythrocyte values of $>1000 \times 10^6$/L were not included in the analysis. Because the CD does not print erythrocyte values $<1000 \times 10^6$/L, no correlation in the relevant erythrocyte range could be determined between the CD and the UF as well as between the CD and the counting chamber.

Discussion

The examinations of the accuracy and linearity of the two analyzers showed that it was not irrelevant to test them for CSF analyses although they had not been designed specifically for that purpose. The comparison of the methods used for CSF samples produced correlation coefficients (r) between 0.87 and 0.91 that further corroborate our approach.

The high degree of the scattering of the residuals around the identity line and/or the high degree of the scattering of the count differences with a still acceptable mean difference (bias) manifests the fault liability of the automatic cell counting. One possible fault was indicated during analyses of CSF samples with high lymphocyte populations and was confirmed in analyses of defined cell suspensions obtained from sedimented EDTA samples.

As far as CD is concerned, there is a spurious extrapolation of the number of leukocytes in the WOC channel with a recorded high lymphocyte percentage of the CSF sample in most cases indicated by the alarms VARIANT LYM and KWOC. The CD performed this erroneous extrapolation of the leukocyte count when certain samples containing atypical lymphocytes were measured repeatedly.

Thus, it must be assumed that the algorithm of the CD is sensitive to certain conditions (CSF) and/or that is susceptible to certain coincidences. In some subsequent examinations, it was possible to show that in counting atypical lymphocytes, the rough counts of the WOC channel lead to a better conformity with the chamber counts.

The UF does not classify certain lymphocyte populations in CSF and allocates them to a different particle population ("Other"). The number of leukocytes that is determined is falsely low. In some cases, it was possible to correct the leukocytes count by simple addition (leukocytes $\times 10^6$/L + number of cells $\times 10^6$/L in the "Other" population). This method of correction, however, requires additional confirmation before being used.

If the leukocytes count determined for sample mentioned in the *Results* is considered from a medical point of view, the possible diagnoses may range from suspected herpes simplex encephalitis (24×10^6 WBC/L on the UF) to meningitis (215×10^6 WBC/L on the CD).

The difference shown in this specific case is reflected in the described series of tests by a considerable number of clinically relevant differences in the number of leukocytes. The critical difference between two counts may be derived roughly from three times the time-dependent standard deviations ($d_k \cong 3\ SD_T$). With 100×10^6 WBC/L and a SD_T of 15×10^6 WBC/L, the resulting difference of almost 50×10^6 WBC/L would still be acceptable.

The use of automated analyzers for cell counting in CSF is certainly feasible. The material to be analyzed is handled by the fully automated analyzers, meeting time and quality requirements, and in an objective manner. There is a good correlation between the three methods studied (Fuchs-Rosenthal chamber, CD, and UF) in most cases. In low ranges, the UF performs better than the CD (CV, 4.9% as opposed to 28%). Difficulties will be encountered when fragile cells, especially lymphocytes, are present in the sample. In these cases, the Fuchs-Rosenthal chamber is regarded as the reference method (although it is not known how many cells are destroyed during the preparation procedure). Here, the leukocyte cell counts on

Table 2. Comparison of the differences[a] of counted leukocytes (WBC 10^6/L) with the lymphocyte portion (%) indicated on the CellDyn 3500 in CSF cell samples.

	n	r
(CellDyn 3500 − UF-100)/Lym[b] %	17 (10)[c]	0.71 (0.88)[c]
(CellDyn 3500 − chamber)/Lym %	13 (10)	0.42 (0.32)
(UF-100 − chamber)/Lym %	13 (10)	0.58 (0.84)

[a] CellDyn 3500 − UF-100; CellDyn 3500 − chamber; and UF-100 − chamber.
[b] Lym, lymphocyte.
[c] Results for cell suspensions given in parentheses.

the CD lead to falsely high values. In contrast, the UF leads to falsely low values. In both analyzers, the presence of the atypical fragile lymphocyte population can easily be suspected.

Regression analyses showed from the high degree of scattering of the data points around the line of identity that both analyzers do not offer a sufficient degree of safety for analyzing CSF as yet.

The high degree of accuracy and linearity that is offered by both analyzers should prompt us and the manufacturers to remedy the interfering factors as described by improving the algorithms these analyzers have to offer. Once this is done, these analyzers may be very useful for cell counts in CSF.

We thank the Sysmex Corporation for financial assistance and for helpful advice during the course of the study.

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Automated Counting of Cells in Cerebrospinal Fluid Using the CellDyn-4000 Haematology Analyser

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Counting of cells in cerebrospinal fluid is currently performed manually. Because of the inherent analytical and economical disadvantages, we attempted to introduce a fully automated method. Therefore, we validated the Abbott CellDyn-4000 haematology analyser for counting cells in cerebrospinal fluid. The analyser was used in its standard configuration with the simple precaution of a preceding blank sample. As for leukocyte counting the analyser yielded high precision (CV ~5% above the upper reference limit), good linearity, low limit of detection (2/µl) and excellent correlation (r > 0.99) with the counting chamber method. The differential leukocyte count was equally accurate and precise, even in the low concentration range. Performance of the erythrocyte count was impaired by its high limit of detection (6/nl) and it appeared satisfactory only for detecting blood admixture due to traumatic puncture. The specificity of the analyser is excellent, since it correctly classified non-viable leukocytes and excluded yeast cells from the leukocyte count in a patient with cryptococcal meningitis. We conclude that the CellDyn-4000 is well suited for quickly and reliably counting leukocytes in cerebrospinal fluid. Developing some software modifications might make the analyser useful also for performing erythrocyte counting in cerebrospinal fluid. Clin Chem Lab Med 2002:40(11):1168–1173

Key words: Cerebrospinal fluid; Differential count; Erythrocytes; Haematology analyser; Leukocytes.

Abbreviations: CSF, cerebrospinal fluid; RBC, red blood cells (erythrocytes); WBC, white blood cells (leukocytes).

Introduction

Automated haematology analysers belong to the standard equipment of clinical laboratories. These analysers have completely replaced microscopic counting chambers for enumerating cells in blood. However, microscopic techniques are still widely in use for counting cells in other body fluids, since current haematology analysers are not designed for enumerating low-cell concentrations as, for example, in cerebrospinal fluid (CSF). Yet, several attempts have been described of automated haematology analysers being used for this purpose. One of the very first reports on electronic CSF cell counting concluded that the then used haematology counters produced satisfactory results, but the lower limit of detection was rather high, varying from 100 to 200/µl, depending on the specific instrument (1). Lower detection limits could be obtained upon modification of the analysers and by increasing the sample volume, although this approach was considered not very practical (1). In addition, the early instruments were not specific enough; for example, yeast cells were incorrectly identified as leukocytes (2).

Recently, results of CSF cell counting applying more modern haematology analysers have been published. In a study using the Bayer H*2 analyser the authors concluded that this instrument yielded clinically acceptable results (3). The Abbott CellDyn-3500 haematology analyser was found to yield satisfactory precision and good linearity, but there were problems in accurately classifying fragile lymphocytes (4). These authors concluded that at present this analyser should not be used for CSF cell counting in all cases. They also concluded that a modification of algorithms might solve the problems and then make the instrument very useful for cell counting in CSF (4).

An entirely different approach for counting and differentiating CSF cells is the application of a urine flow cytometer, Sysmex UF-100 (4, 5). Although the results appear promising, the software in this instrument, too, needs to be improved before clinically reliable counts can be obtained.

One of the most advanced haematology analysers presently on the market is the Abbott CellDyn-4000. It is a 5-parameter clinical flow cytometer that combines excellent precision at low white blood cell (WBC) concentrations with superior classification of cells, including recognition of non-viable WBC and nucleated red blood cells (RBC) (6). These characteristics potentially make this analyser well suited for CSF cell counting. The aim of our study was to investigate the analytical performance of the CellDyn-4000 in cell counting and differentiating WBC in CSF. For comparison we used the conventional microscopic counting chamber method, whereas differential counting was performed on cytospin slides.

Materials and Methods

Materials and patient samples

We used 129 CSF samples from 104 patients that were collected for normal diagnostic purposes. According to the regu-

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lations from the hospital's Ethical Review Committee, no special approval is required for using material that is leftover after the requested tests have been completed. CSF was collected into sterile glass tubes without anticoagulant and the assays were performed as soon as possible, but always within 1 hour from the time of sample collection.

For method evaluation studies we also used whole blood, washed erythrocytes or isolated leukocytes (see below) that were added to CSF, which was rendered free of cells by centrifugation (4300 *g* for 10 min). In carry-over and linearity experiments we used NaCl solution (0.15 M) buffered with phosphate (0.1 M) and containing human albumin (0.5 g/l) as a diluent. Polymorphonuclear granulocytes were isolated from the blood of a patient with bacterial infection and lymphocytes from a patient with chronic lymphocytic leukaemia by density centrifugation over Polymorphoprep™ and Lymphoprep™, respectively (both from Nycomed, Oslo, Norway). The purity of the isolated cells was at least 97%.

Methods

CSF samples were analysed in the CellDyn-4000 (Abbott Diagnostics Division, Santa Clara, California, USA) in the manual mode with one background sample (normal saline) inserted immediately prior to it for reducing carry-over (see Results). The instrument was always switched into the so-called extended count mode for providing increased precision. In this mode the counting time is extended to 32 s, whereas in the standard mode it is only 9 s. The sample volume aspirated was 0.1 ml. The CellDyn-4000 was operated in accordance with the manufacturer's recommendations and checked daily following our standard operating procedures.

In the conventional method, erythrocytes (RBC) and leukocytes (WBC), the latter after staining with Türck's acidic methyl violet solution, were counted in a Fuchs-Rosenthal chamber using 400 × magnification in a standard microscope. Differential WBC counts in CSF were performed in preparations on glass slides made using a cytocentrifuge (10 min at 500 rpm and low acceleration in a Cytospin II; Thermo Shandon, Runcorn, Cheshire, United Kingdom) and stained with Wright's stain. Experienced observers examined at least 100 leukocytes, if present. For assessing the differential count, we added the percentage of lymphocytes and monocytes determined by the CellDyn-4000 and compared this sum with the mononuclear cells found in the cytospin. The remaining WBC (classified as neutrophils, eosinophils and occasionally basophils by the CellDyn-4000) were regarded as polynuclear cells.

Statistical analysis

Linearity of the automated method was assessed using linear regression analysis. Method comparison was done using Passing-Bablok regression. We used the MedCalc software package (version 6.14) for analysing data (MedCalc; Mariakerke, Belgium).

Results

Carry-over

Since the haematology analyser is normally running blood samples in which the cell concentrations are at least 100 to 1000-fold higher than in CSF, elimination of carry-over is of paramount importance. Therefore, a series of samples containing buffer only was measured after a normal blood sample. Figure 1 shows that in the second blank sample the WBC count drops to background level. Consequently, all further measurements were carried out with one blank sample immediately preceding the CSF sample to be analysed. As for RBC, the first blank still contained 22.9±8.9 RBC/nl (mean ± SD, n = 14), and only after four blanks the count became similar to the background (1.0±0.4 RBC/nl). So, at least four blank samples are necessary for eliminating erythrocyte carry-over.

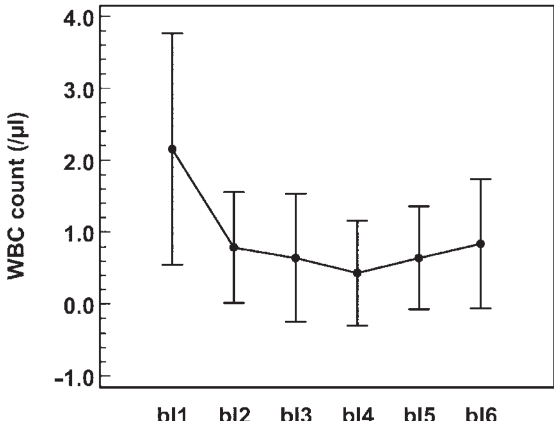


Figure 1 Carry-over between whole blood and CSF cell counts. A sample of blood with normal WBC count was followed by a series of six blank samples (buffered saline) designated as bl1 to bl6, in order to estimate the carry-over. Means ± SD of replicates (n= 14) are shown.

Detection limit

The lower limit of detection, defined as the mean +3 SD of cell-free CSF measured after one blank, was found to be 2.1/µl for WBC and 6.3/nl for RBC. There was no difference in the lower limit of detection between cell-free CSF and albumin-containing saline solution (data not shown).

Linearity

The linearity of the WBC count was investigated using a CSF sample from a patient with pleocytosis, which was diluted with saline. Over the range from 2 to 300/µl, excellent linearity was found as shown by the regression equation $WBC_{found} = 2.7 + 0.973\ WBC_{expected}$ ($r = 0.998$). Series of diluted whole blood, suspensions of isolated neutrophils and isolated lymphocytes all yielded identical results (data not shown).

Precision

The interassay precision of the WBC and RBC counts was determined at a range of concentrations encompassing both the normal and the pathological levels. Table 1 shows the precision as a function of the cell count.

Comparison with counting chamber

Figure 2 shows the correlation in WBC counts between the CellDyn-4000 and the counting chamber as a refer-

ence. The slope was 1.01 (95% confidence interval (CI): 0.99 to 1.06) and the intercept +1.7 (95% CI: 1.4 to 1.8). The coefficient of correlation was 0.991 (95% CI: 0.987 to 0.994). The correlation in RBC counts was very weak: the coefficient of correlation was only 0.132, which is not significant ($p = 0.146$). In the counting chamber the majority of samples (87 out of 129) contained a detectable amount of RBC, whereas the RBC count in the CellDyn-4000 was above the detection limit in 14 samples only.

Table 1 Precision (coefficient of variation, CV) of WBC and RBC counts in CSF over the relevant concentration range, calculated from 10 measurements at each point.

WBC count (/μl)	CV (%)	RBC count (/nl)	CV (%)
5	29.8	3	15.7
10	23.3	10	8.6
30	8.4	30	5.3
100	6.9	100	1.6
300	5.2	300	1.8
560	6.1	1000	3.0
1800	4.3		

Differential count: linearity and precision

For investigating the linearity and precision of the CellDyn-4000 differential count we prepared a range of dilutions of normal blood samples in buffer. An example of a dilution experiment is shown in Figure 3. The coefficients of variations of the mononuclear and polynuclear counts ranged between 2.2 and 9.6%. In addition, we mixed isolated lymphocytes and neutrophils in varying amounts with buffered saline to mimic a range of CSF samples covering a relevant range of WBC counts and, simultaneously, all possible mononuclear to polynuclear cell ratios. These experiments confirmed the reliability of the differential counts down to the WBC detection limit (data not shown).

Differential count: comparison with cytospin

Although a differential count was performed on all 129 samples, only those samples with an increased WBC count (> 10/μl) were considered acceptable for sta-

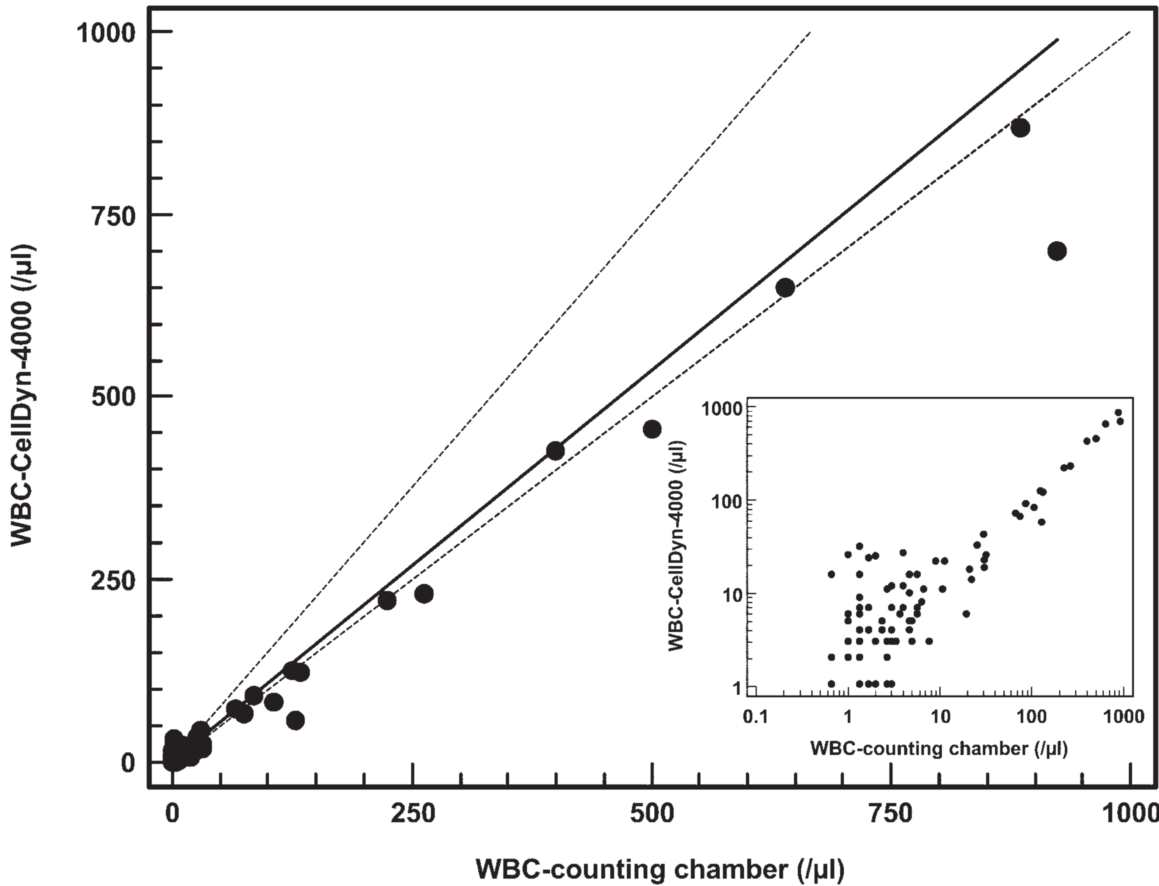


Figure 2 Passing-Bablok regression analysis comparing the WBC cell counts performed on the CellDyn-4000 with the conventional counting chamber method in 129 CSF samples. The parameters of the regression equation are: slope 1.01 (95% CI: 0.99 to 1.06); intercept 1.7 (95% CI: 1.4 to 1.8) and coefficient of correlation 0.991 (95% CI: 0.987 to 0.994). For clarity reasons, 12 samples with extremely high WBC counts were omitted from the plot, but they were included in the analysis. The dotted lines indicate the 95% confidence limits of the regression (solid line). The line of identity is invisible, since it exactly coincides with the lower confidence limit line. Insert: Plot for highlighting the samples with WBC in the reference range (note logarithmic scale).

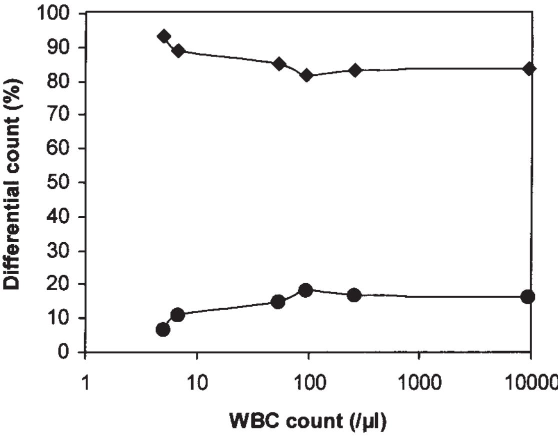
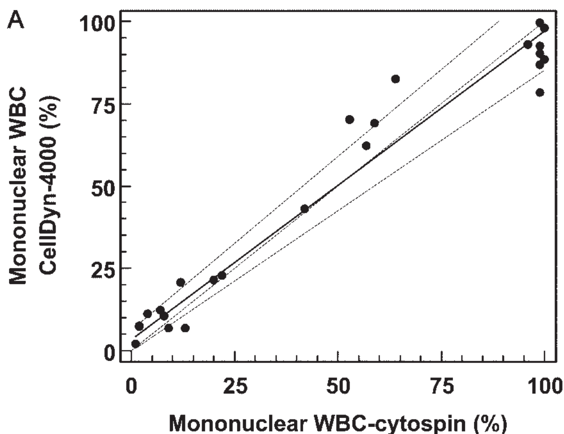


Figure 3 Linearity of differential cell count in CSF. A normal blood sample was diluted until WBC counts were in the CSF range. The percentages of mononuclear (●) and polynuclear cells (◆) are given as a function of WBC count (note logarithmic scale).



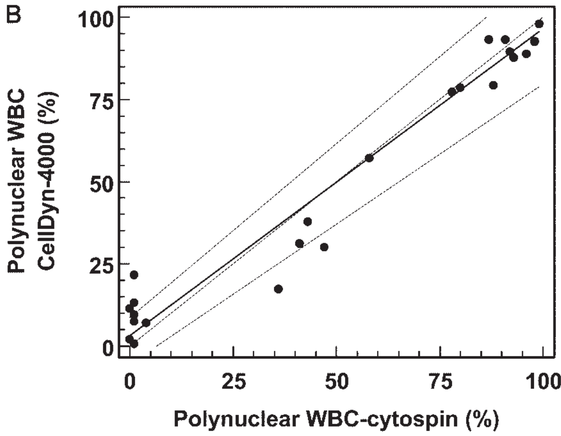


Figure 4 Regression analysis comparing the differential count by the CellDyn-4000 with the microscopic count in 24 samples with WBC > 10/µl. A: mononuclear cells with slope 0.93 (95% CI: 0.85 to 1.06), intercept 3.5 (95% CI: −0.2 to 6.1) and coefficient of correlation 0.977 (95% CI: 0.948 to 0.990). B: polynuclear cells with slope 0.93 (95% CI: 0.85 to 1.06), intercept 3.1 (95% CI: −5.5 to 8.6) and coefficient of correlation 0.978 (95% CI: 0.948 to 0.990). The utmost dotted lines indicate the 95% confidence limits of the regression (solid line); the middle line indicates identity.

tistical analysis because of the inherent high imprecision of differentials at low WBC counts. Figure 4 shows the correlation of both the mononuclear and the polynuclear cells between the CellDyn-4000 and the cytospin differential. The confidence intervals of the slopes included unity and the correlation coefficients were close to 0.98 in both cases.

Specificity

Apart from the CellDyn-4000's ability to count and differentiate WBC, it is of interest to know how other CSF cells will be recognised. During the evaluation period we encountered a single patient who had an infection with *Cryptococcus neoformans*. The CellDyn-4000 correctly classified the yeast cells in his CSF as unknown events and excluded them from the WBC count. In the experiments with lymphocytes isolated from blood of a patient with chronic lymphocytic leukaemia, the fragile lymphocytes were accurately classified as lymphocytes (data not shown).

Discussion

One of the main items of importance for measuring CSF samples in a standard haematology analyser is the risk of carry-over, since the cell concentration in blood is considerably higher than in CSF. Remarkably, this item has not been addressed in the available literature (3–5) but is of evident importance. Our experiments show that at least one preceding blank sample is needed for eliminating carry-over in WBC counts (Figure 1). For RBC as many as four blank samples are required before the background level is reached. Since the detection limit of RBC count is rather high (see below), we focused on WBC counting and therefore we ran routine CSF samples in the CellDyn-4000 after a single blank sample. Using this procedure, the lower limit of detection was as low as 2 WBC/µl, which is comparable to the counting chamber and adequate for discriminating normal from pathological CSF. In contrast, the lower detection limit of RBC is insufficient for accurately measuring low RBC counts, even after as much as four blank samples. As a consequence, increased RBC counts resulting from cerebral bleeding will remain undetected as long as they are below the limit of 6.3/nl. Therefore, RBC counts in the CellDyn-4000 will only be increased in patients with severe bleeding. However, the RBC detection limit is sufficient for detecting admixture of WBC from peripheral blood caused by traumatic lumbar puncture. For example, 1 RBC/nl of normal blood is accompanied by approximately 1–2 WBC/µl. This implies that as long as the RBC count remains below the detection limit of the CellDyn-4000, any increased WBC count in CSF indicates true WBC pleocytosis. If the RBC are increased above their limit of detection, the WBC count may need to be corrected for admixture by comparing with RBC and WBC counts in blood (1).

As expected, the precision of the CellDyn-4000 com-

pares favourably with the counting chamber method. In the reference range, the imprecision of the Fuchs-Rosenthal counting chamber is between 18 and 56% (expressed as CV, as calculated using Poisson statistics). The imprecision of the analyser is lower, around 25–30%. In addition, in the low pathological range the imprecision of the CellDyn-4000 significantly decreased to between 5 and 8% CV (Table 1), which is half that of the counting chamber (calculated CV 12–17%). The precision found in the present study is at least as good as published by others using different analysers (3, 4).

The lower limit of detection has been reported in one study only (3). These authors could detect 5 WBC/µl, which is higher than 2.1 WBC/µl found here. For RBC, the H*2 analyser was one order of magnitude more sensitive, 0.5/nl as compared with 6.3/nl in the CellDyn-4000. It should be taken into account that these limits could only be obtained by analysing raw data in the research mode of the H*2 (3), while the CellDyn-4000 was operated in its standard routine mode and used one blank sample only. The linearity of the WBC count was excellent in the CellDyn-4000, which is identical with other haematology analysers, as well as with the urine flow cytometer (3, 4).

The correlation of WBC counts between the CellDyn-4000 and the counting chamber method was satisfactory over the entire concentration range relevant to CSF (Figure 2). At very low WBC counts there was evident scatter around the regression line. This is most likely the consequence of the high imprecision of both methods. In the very high range there is some scatter as well, but the precision of the automated analyser is beyond any doubt here, because it is very similar to that in blood (6).

Our results indicate that the CellDyn-4000 is capable of accurately and precisely performing a differential count in CSF. The dilution experiments show that the mononuclear to polynuclear ratio remained constant over a broad range of WBC counts and it was identical to that in the original blood sample (Figure 3). This is similar to the results by Aune and Sandberg, who described that the level of WBC count did not influence the reliability of the differential count (3). Only if the WBC count became very low in the dilution experiment, we found some underestimation of the lymphocyte percentage, because lymphocytes disappeared from the area that is predefined for this cell cluster in the analyser. If we would have used the normal count mode instead of the extended mode, the apparent loss of lymphocytes would have occurred already down from 200 WBC/µl (data not shown). It is therefore mandatory to run CSF samples in extended count mode, where better precision and accuracy can be obtained. In contrast to the apparent lymphocyte loss in the dilution experiments, the correlation between mononuclear cells in native CSF samples is very good even in the low range (Figure 4A). Probably the artificial dilution fluid induces changes in the light scatter of the lymphocytes, moving them out of their window of analysis.

From the experiments with cells isolated from a patient with chronic lymphocytic leukaemia we conclude that the CellDyn-4000 correctly counted and classified even these fragile lymphocytes, due to its ability to recognise non-viable WBC (7, 8). This is in contrast with other analysers studied, where the accuracy was hampered by the fact that non-viable lymphocytes were omitted from the WBC and lymphocyte counts (4, 5).

The CellDyn-4000 is equipped with advanced optics and multi-parameter algorithms for classifying the different cell types in blood. Theoretically, one may therefore expect that other cells, not originating from blood, will be recognised and flagged by the analyser. Indeed, in a CSF specimen containing *Cryptococcus* the yeast cells were not counted as WBC but flagged as non-classifiable. This is in contrast to older instruments, where spurious pleocytosis has been reported (2). As for bacteria we have no experience, but interference is highly unlikely because the optical properties of bacteria differ too much from blood cells to be recognised by the CellDyn-4000. During our study we did not encounter sufficient number of samples containing rare CSF cells, such as tumour cells and cells from the epithelial lining. However, the CellDyn-4000 is certainly incapable of correctly classifying these abnormal cells. Depending on their physical properties, the analyser might include such cells in the lymphocyte or monocyte counts or might exclude them from the WBC count and report them as non-classifiable events. Therefore, it is necessary that each request for a CSF differential count be accompanied by adequate clinical information as to enable the laboratory to decide whether to perform additional microscopic analysis.

Although in its present state the CellDyn-4000 offers clear advantages over the traditional microscopic methods, improvement clearly lies within the technical possibilities of the analyser. Modifications in the internal sample dilutions might further reduce the imprecision and decrease the detection limit, particularly in RBC counting. Creation of a special CSF mode would further enhance the CellDyn-4000's performance as an automated analyser for CSF cell counting. Then, the analyser would meet the modern requirements for quick and reliable CSF analysis still better (9).

The general conclusion from the present study is that the CellDyn-4000 is very well suited for leukocyte analysis in CSF. Provided some simple precautions are taken into account (a blank sample preceding a CSF specimen and analysis in extended count mode), the analyser yields highly precise and accurate results for WBC and differential count. In addition, the results are available within 1 minute, which minimises artefacts by the well-known rapid deterioration of cells in CSF. Laborious and time-consuming processing of CSF samples is no longer needed and remains limited to those cases that require special expertise.

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CSF ANALYSIS ON A ROUTINE HEMATOLOGY ANALYZER

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INTRODUCTION

The Hematology laboratory at Upstate Medical University, Syracuse, NY, supports a teaching hospital and runs 180,000 CBC analyses per year, as well as other body fluids, of which there are approximately 1,050 Cerebrospinal Fluid (CSF) annually

CSF cells counts are important in the diagnosis of many diseases. A higher than normal red blood cell (RBC) count in CSF can be due to a hemorrhage or a traumatic puncture. Moreover, a high white blood cell (WBC) count in CSF can be a result of infections like meningitis, or some types of leukemia[1].

An opportunity exists to automate some of these body fluid counts that are traditionally done by hemocytometry, by utilizing existing automated hematology cell counters that are already present in laboratories. These analyzers have already replaced nearly all the manual cell counting procedures on whole blood samples. The aim of our feasibility study was to assess the capabilities of the Abbott CELL-DYN Sapphire to quantify WBC and RBC counts in low cell concentrations as seen in CSF.

MATERIALS AND METHODS

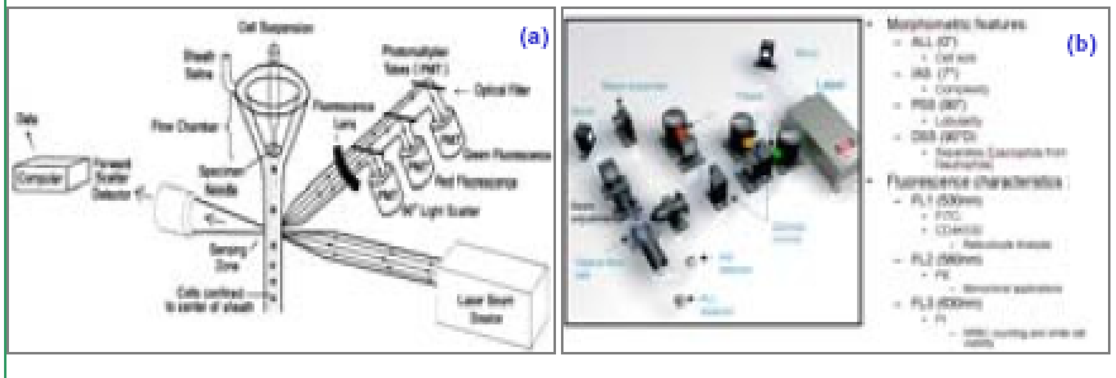
CELL-DYN Sapphire: The CELL-DYN Sapphire *(figure 1)* is an example of an automated hematology counter that has the capability beyond the processing of whole blood samples. The CELL-DYN Sapphire incorporates multiple analytical methods that include MAPSS (Multi-Angle Polarized Scatter Separation), three-color fluorescence detection, hemoglobinometry, and focused flow impedance analysis. In addition to providing the complete blood count, the CELL-DYN Sapphire employs immuno-fluorescence analysis technology, similar to that used on a dedicated fluorescence flow cytometer *(figure 2)*, for analysis of monoclonal antibody applications.

The CELL-DYN Sapphire utilizes a pumped- diode solid-state laser to provide 20 mW of 488-nm light. The system's optical bench detects up to four light scatter signals and has the ability to collect up to three fluorescent signals corresponding to 530nm, 580nm, and 630nm wavelengths permitting the measurement of three-color fluorescent detection. With the CELL-DYN Sapphire's current hardware and software design, the opportunity exists to explore the feasibility of performing analysis of other body fluids that are conventionally analyzed by hemocytometry.

Figure 1: The CELL-DYN Sapphire Hematology Analyzer.

Patient Samples: Residual patient CSF samples were obtained from the routine laboratory after being processed by the laboratory's standard protocols for producing WBC, spun smear differential, and RBC on CSF specimens. Sample age was less than 2 hours from time of collection. One hundred specimens were initially analyzed, as reported in the abstract, however, the study was ongoing and an additional 80 specimens were included in this reported data set. Depending on residual sample volume, the CSF was processed at least one time on one of the two CELL-DYN Sapphires in the lab, and up to four times or twice on each of the two CELL-DYN Sapphires.

Figure 2: (a) Typical layout of a immuno-flow cytometer. (b) Optical bench of the CELL-DYN Sapphire including the Blue (488 nm) diode pumped laser.



Work Flow: The concept of having a background assessment run before each CSF specimen is processed is very important. The intent of this action is to ensure the CSF sample to be run will not be significantly influenced by background or carryover issues, and the state of the instrument prior to a CSF specimen is always exactly the same (CSF is always preceded by a background, not any other cycle).

For the CELL-DYN Sapphire's CBC mode, the background specification for WBC is less than 0.100 K /µL, or 100 cells /µL. The CELL-DYN Sapphire also has a carryover specification of < 0.1% for whole blood samples. That means that slight carryover (< 0.1%) from a prior CBC run of 10.0 x 10^3 / µL would add approximately 10 cells / µL to the next test. This level of carryover is inconsequential for a CBC sample that may follow, but this level already represents an abnormal (high) for a CSF specimen.

The reference interval (normal range) for WBC in CSF samples is 0 – 5 cells/µL. Since the normal range of CSF is so much less than the background specification (<100 cells /µL), and what could be anticipated as a contribution of carryover (~5-10 cells / µL), a method had to be developed to handle this.

Typical performance of a CELL-DYN Sapphire systems for WBC backgrounds in the 0 – 5 cells /µL level. Backgrounds of this level are acceptable to have any CSF sample run after it. However, it has to be verified before each CSF analysis. Therefore, prior to any CSF specimen being processed on the CELL-DYN Sapphire for this study, background cycles were processed until the reported WBC value was 5 cells / µL or less. This process was repeated, even when the CSF was processed in duplicate, which ensures that the hydraulic condition of the instrument is similar to how it was for the first sample.

Once the background levels were determined to be acceptable by the operator, the CSF sample was processed by the CELL-DYN Sapphire's CBC+C mode, which enables the system to perform an extended counting cycle for the WBC portion. This mode allows 2.3 µL of the CSF fluid to be counted, which is 27% more fluid than is counted by a neat preparation of CSF in a Neubauer counting chamber. This allows for slightly better Poison counting statistics, and the visualization of more events on the dot plot that the system presents to the user either via printout or on the data terminal.

RESULTS

Statistical Analysis: Data were analyzed by linear regression performed with Microsoft Excel 2000 (Microsoft, Redmond, WA, USA).

The results obtained on CSF samples processed through the CELL-DYN Sapphire have numerous flags associated with them. Per the CELL-DYN Sapphire Operator's Manual, these displayed results have been 'INVALIDATED'. On inspection, many of these flags are associated with anticipation of whole blood samples being processed. Every specimen run in the study was indicated as a 'Short Sample', which is an artifact of the clear CSF fluid, and not an actual short sample.

Other flags have similar issues and were seen on a great majority of the samples. However, ignoring all of the flags is not appropriate. In cases where significant amount of debris or yeast were seen, the CELL-DYN Sapphire over estimated the WBC values, but flagged the result with an 'OUT OF BOUNDS' (*figure 8*) flag. This flag in particular was useful in catching outliers, and was used to either include or exclude data from the analysis. A more typical representation of CSF samples can be seen in figure 9, with a 200 white cell count, that appears to be mostly lymphocytic.

Figure 3: CSF White Blood Cell Count – Full Range All Samples

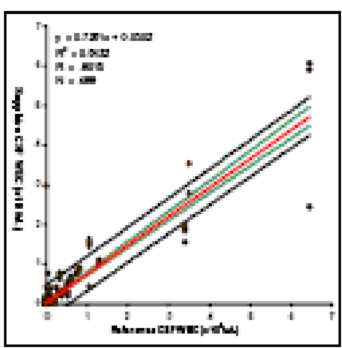


Figure 4: CSF White Blood Cell Count – Full Range w/ Flagged Samples Removed

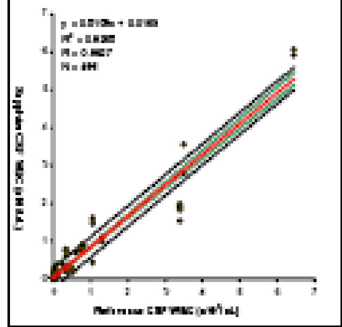
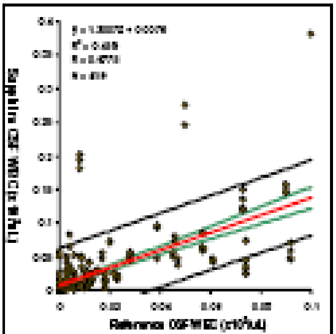


Figure 5: CSF White Blood Cell Count – Below 100 Cells / micro liter



CELL-DYN, CELL-DYN Sapphire and MAPPS are trademarks of Abbott Laboratories.

A linear regression analysis for WBC (n= 499) was performed yielding a correlation coefficient (R) of 0.883, a slope of 0.7, and intercept of 35 cells (*figure 3*). This certainly shows some over-estimation of the CELL-DYN Sapphire on CSF counts. Upon eliminating samples that were flagged for OUT OF BOUNDS, the linear regression analysis of the remaining 466 samples yield a correlation coefficient of 0.962 with a slope of 0.8 and an intercept of 16 cells (*figure 4*).

Unlike the WBC for a CBC, there is no such thing as a low WBC for CSF. With the normal range being 0 to 5 cells per microliter, statistics such as correlation and linear regressions are not as helpful in assessing the diagnostic utility of CSF counts, like they are for WBC in whole blood. Figure 5 shows the same data as figure 4, but with the scale of the data focused on the 0 –100 cell range.

To access how well the CELL-DYN Sapphire distinguishes normal from abnormal samples, Bayesian statistics were utilized. Comparing the manual hemocytometer count for TRUTH, and the range of 0 – 5 cells / microliter as normal, yielded the following:

TRUE Negatives = 179
TRUE Positives = 150
FALSE Negatives = 5
FALSE Positives = 135

For a sample set with a prevalence of 33.0%, this yields poor specificity (57.0%) and Predictive Value of a Positive Result (PVP) of 52.6%. However, the CELL-DYN Sapphire does show good sensitivity (96.8%) and this data set yielded a result of 96.8% for Predictive Value of a Negative Result (PVN) 97.3%.

To access how these Bayesian statistics of CELL-DYN Sapphire verses a manual hemocytometer compare current practices, a follow-up study was performed. This study consisted of twenty CSF specimens being analyzed (prepared and counted) by two technicians. This smaller sample set had exactly the same results regardless as to which technician (A or B) was considered TRUTH. This study had prevalence 25.0%, yielded a specificity of 93.3%, a PVP of 80.0%, which were both substantially higher than the Manual to CELL-DYN Sapphire numbers. However, the sensitivity of 80%, and PVN of 93.3%, were both lower than the Manual to CELL-DYN Sapphire values, on this small study.

For the RBC counts on the Sapphire, the same logic of data exclusion was used. As the CELL-DYN Sapphire was optimized around counting of RBC in whole blood, its use of RBC counting of CSF has some significant limitations:

- The dilutions and counting volumes are designed for counts that are in the millions of cells per µL.
- The lowest reportable value that the CELL-DYN Sapphire can currently display is 0.001 x 10^{12} / L which is 1000 cells/ µL.
- The existing analysis algorithm for the RBCi channel does not deal with any interference from WBC that are also present in the RBC dilution.
- The RBCo is just a count above a hardware gate, and digitized data of the scatter from the cells is not currently available for inspection.

Figure 6: CSF Red Blood Cell Count – Full Range (a) RBCo, (b) RBCi

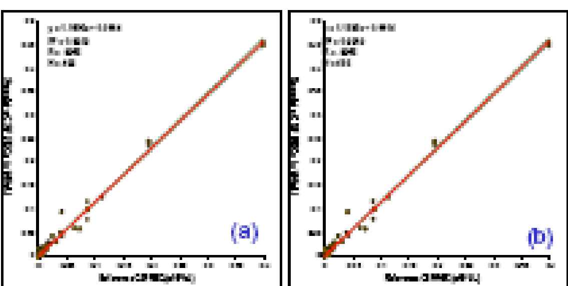
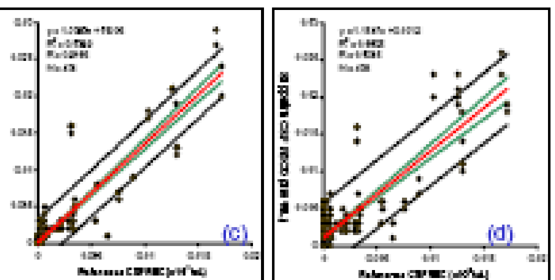


Figure 7: CSF Red Blood Cell Count – < 20,000 Cells (c) RBCo, (d) RBCi



With all of these issues, the Sapphire cannot be utilized to determine normal CSF RBC values (< 1 cell / µL) from abnormal (>= 1 cell / µL) CSF results in the same fashion that the WBC results can be delivered.

Even with all of the issues mentioned above, the results show excellent agreement with the manual methods, with correlations (R) of 0.992 (*figure 6b*) over the full range of RBC values that were obtained in the study (N=466 runs). It is on the low end of the scale (< 20,000 cells / microliter) that the CELL-DYN Sapphire has significant limitations (*figure 7c,d*), due in part to Poisson counting statistics, residual background, and white cell interference.

The CELL-DYN Sapphire does have two methods to determine RBC values, an impedance method and an optical method. The impedance method is the value that reports the RBC in whole blood mode. While the optical method may have several advantages over the impedance method, these advantages do not make the RBC values below 20,000 cells per microliter useful to the laboratory.

DISCUSSION

Within the laboratory, CSF testing is an arduous process secondary to limited sample size, variability in counting on the hemocytometer, and the need for immediate results for providers. It is a labor intensive and subjective counting process. With the number of samples tested, results have indicated that the enumeration of WBC counts >100 correlate very well with the manual method and showed statistical precision. WBC counts on CSF below 100 showed unacceptable agreement in results between the hemocytometer chamber counting method and the CELL-DYN Sapphire instrumentation, except when the values from the CELL-DYN Sapphire were in the very low range of < 10. At these low levels, clinical agreement between the methods was as good or better than that of two technicians.

Upon evaluating the RBC method above 20,000 cells per microliter, the CELL-DYN Sapphire has excellent correlation, yet, below this value the instrument has significant limitations which include counting statistics, residual background, and white cell interference.

We conclude that the CELL-DYN Sapphire can be useful in screening all CSF samples for white blood cells at any level, and in accurately enumerating WBC and RBC in visibly cloudy and bloody samples. This should assist in reducing the need for increased technical time for these labor-intensive methods. With future software upgrades to the Cell-DYN Sapphire, these limitations of the low-end analysis could be significantly reduced.

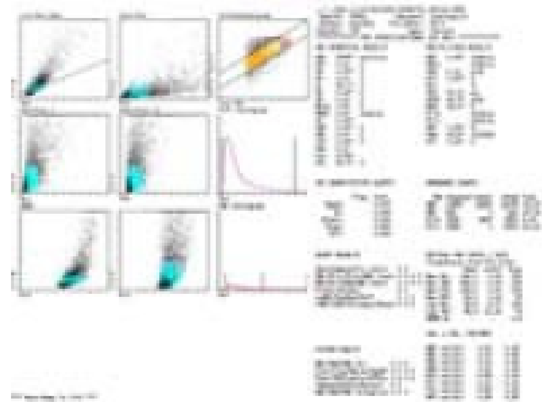


Figure 8: Example of Specimen Deleted from Study – Hyphsyted Yeast

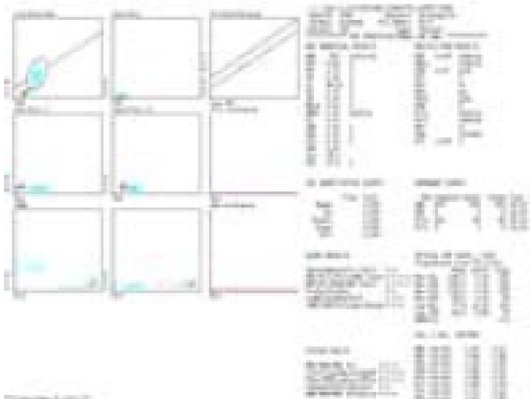


Figure 9: Example of a Typical Specimen Included in Study

References:

1. Rodak, Bernadette F. Diagnostic Hematology. Philadelphia, Penn: W.B. Saunders Company; 1995:633-643.

Body Fluid Analysis on the Cell-Dyn Sapphire™, Multi-Angle Scatter and Fluorescent Hematology Analyzer

Young Ran Kim,[1] Albert Huisman,[2] and Willie Cowart[1]

[1] Abbott Laboratories, Santa Clara, CA, U.S.A.; [2] University Medical Center Utrecht, Utrecht, The Netherlands

Introduction

Timely generation of accurate data from body fluid analysis (BFA)* has clinical importance in diagnosis and treatment of patients. The conventional chamber counting method is time-consuming and the reproducibility and accuracy vary because of differences in the skills and experience of the technologists. To resolve these limitations, we evaluated the performance of a rapid and precise multi-angle scatter and fluorescence hematology analyzer, Cell-Dyn Sapphire™ (CD-Sapphire), for BFA.

Method

The CD-Sapphire system was standardized and calibrated for routine hematology analysis and the quality control was performed daily. Background check, low-end linearity, precision and carryover studies were performed. Then, 114 BF specimens (61 CSF, 18 serous fluid, 26 dialysates, 7 drains, 1 amniotic fluid and 1 synovial fluid) were analyzed in the instrument using an extended count mode, CBC+C, to extend cell counting time by 3.6-fold of that of the routine CBC mode. All samples were run in duplicate within 2 hours of receipt. The optical RBC (RBC°) was chosen because a greater number of cellular events are counted than the impedance measurement. Since RBC° counts include white blood cells (WBC), WBC counts were subtracted from RBC° before performing linear regression analysis.

Results

WBC background count was consistently <0.002 x 10^3/µL and RBC background was 0.000 x 10^6/µL. One amniotic fluid was eliminated from the data set due to over 60% epithelial cell contamination. Another point was also removed because of very high non-cellular debris present in the specimen from the regression analysis. Linear regression and correlation of the CD-Sapphire data with the manual reference method in a Fuchs-Rosenthal chamber resulted in excellent agreement for both WBC and RBC (WBC: r=0.995, slope=1.14x, Y-int=0.016 and RBC°: r=0.995, slope=1.15x, Y-Int=136). Low-end WBC (0.00 ~ 1.113 x 10^3/µL) and RBC (0.000 ~ 0.987 x 10^6/µL) linearity with the theoretical cell counts generated correlation of 0.999, slope of 1.023 and Y-int of 0.005 for WBC and correlation of 0.999, slope of 0.999 and Y-int of 0.005 for RBC. Carryover for WBC was 0.156% and for RBC was 0.079%. The precision of WBC and RBC at low, normal and high level (n=10) is presented in **Table 2**.

Results (cont.)

Table 1. CD-Sapphire CBC+C Mode Low-end WBC[a] and RBC[b] Linearity

	WBC x 10^3/µL	RBC° x 10^6/µL
Correlation coefficient (R^2)[c]	0.999	0.999
Slope	1.02	0.999
Y-Intercept	0.005	0.005

[a] WBC range tested: 0 ~ 1 x 10^3/µL
[b] RBC range tested: 0 ~ 1 x 10^6/µL
[c] Against theoretical WBC and RBC counts based on the dilution factors

Table 2. CD-Sapphire WBC and RBC Counts and Within-Run Precision (n=10)

WBC Level (cells x 10^3/µL)	WBC CV (%)	RBC° Level (cells x 10^6/µL)	RBC CV (%)
0.073	4.62	0.072	5.07
0.238	3.15	0.230	2.58
3.29	1.96	2.75	0.96
8.13	1.15	4.20	0.80
16.70	0.98	5.24	1.11

Table 3. Correlation of WBC and RBC: CD-Sapphire vs. Rosenthal Chamber Counts

	WBC x 10^3/µL[a]	RBC° (–WBC) x 10^6/µL[b]
Correlation coefficient	0.995	0.995
Slope	1.14	1.150
Y-Intercept	0.016	0.000

[a] WBC range tested: 0 ~ 350 x 10^3/µL
[b] RBC° range tested: 0 ~ 1.200 x 10^6/µL

Out of 114, only 50 specimens had >50 nucleated cells/µL for differential analysis. Linear regression statistics are presented in **Table 4**.

Table 4. Comparison of WBC Differential: CD-Sapphire vs. Wright-Giemsa Stained Slide Review

	Correlation Coefficient	Slope	Y-Intercept
Polymorphonuclear cells (%)	0.897	0.945	6.09
Lymphocytes (%)	0.930	0.926	3.98
Total Mononuclear cells (%)	0.865	0.880	0.89

Figure 1. The CD-Sapphire distribution of a diluted normal blood for low-end linearity study. WBC and RBC concentrations were set at 1.000 x 10^3/µL and 1.000 x 10^6/µL, respectively. The CD-Sapphire WBC reagent is designed to lyse all RBCs, expose NRBC nuclei by stripping off their membrane and preserve all intact WBCs.[1] The reagent contains an intact membrane impermeant FL3 fluorescent dye (PI) to stain DNA of NRBC nuclei and damaged cells for cell viability test.[2] For WBC differential analysis, the CD-Sapphire utilizes axial light loss, multi-angle light scatter and FL3 fluorescence. The colors of each cluster represent: orange for neutrophils, blue for lymphocytes, purple for monocytes, green for eosinophils and black for basophils. Cell debris, if existing, are white on the screen but black on the print out. NRBC cluster is not shown here since this blood does not contain any NRBCs.

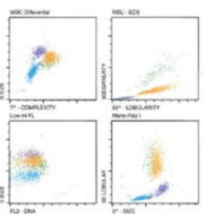


Figure 2. The CD-Sapphire distribution of a CSF from a patient with no suspicion of infection. The CD-Sapphire WBC count was 0.002 x 10^3/µL and both impedance (RBC) and optical (RBC°) RBC counts were 0.000 x 10^6/µL.

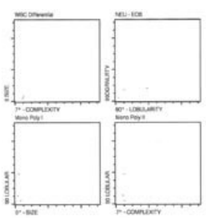


Figure 3. The CD-Sapphire distribution of a CSF from a 56-year-old female patient diagnosed for meningo-coccal sepsis. The CD-Sapphire results: WBC 5.06 x 10^3/µL, RBC 0.003 x 10^6/µL, Neuts 86.8%, Eos 0.18%, Lymph 5.40%, Monos 7.54% and total mononuclear cells (L+M) 12.90%. The black dots in the circle at lower-right corner of the cytogram may represent the bacteria whose DNA stained brightly (FL3+) with NRBC dye in the WBC reagent.

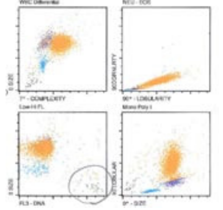


Figure 4. CD-Sapphire distribution of an intraperitoneal dialysate from a 57-year-old male with peritonitis. The CD-Sapphire results: The CD-Sapphire results: WBC 1.43 x 10^3/µL, RBC 0.002 x 10^6/µL, Neuts 83.4%, Eos 0.89%, Lymph 8.75%, Monos 6.95% and total mononuclear cells (L+M) 15.70%. The black dots in the circle at lower-right corner of the cytogram may represent the bacteria whose DNA brightly (FL3+) stained with NRBC dye in the CD-Sapphire WBC reagent.

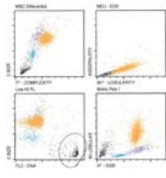


Figure 5. CD-Sapphire distribution of CSF from a 13-day-old female baby with perinatal post-hemorrhagic ventricular dilation (bleeding into one of the brain cavities). The CD-Sapphire WBC/diff dot plots revealed very low cell viability and very high non-cellular debris as shown. Manual microscopic method confirmed very high level of cell damage. This specimen was not included in the regression analysis.

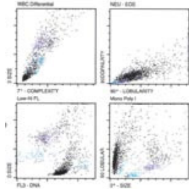
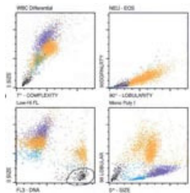


Figure 6. CD-Sapphire distribution of an intra-peritoneal dialysate from a 60-year-old female CAPD-peritonitis with acinobacter infection. The CD-Sapphire results: WBC 10.30 x 10^3/µL, RBC 0.0014 x 10^6/µL, Neuts 60.4%, Eos 0.69%, Lymph 11.80%, Monos 8.32% and total mononuclear cells (L+M) 20.12%. The black dots in the circle at lower-right corner of the cytogram may represent the bacteria whose DNA brightly stained (FL3+) with NRBC



References

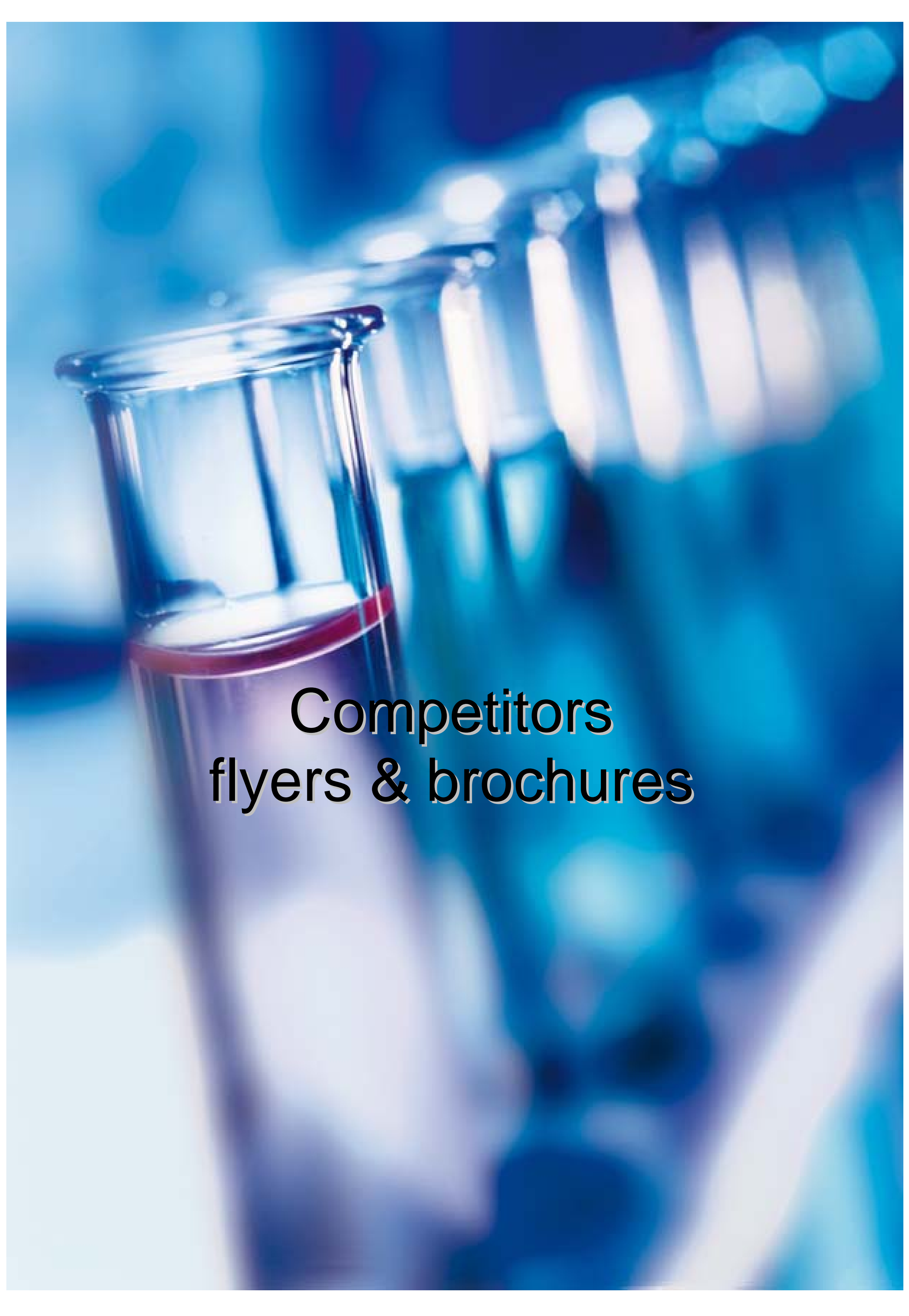
1. U.S. Patent No. 5,516,695. May 14, 1996. Multipurpose reagent system for rapid lysis of whole blood. Kim YR, Kantor J, Gill J, and Luptovic S.
2. U.S. Patent No. 5,559,037. September 24, 1996. Method for rapid and simultaneous analysis of nucleated red blood cells. Kim YR, Yee M, Mehta S, Sagala J, and Kantor J.

Conclusion

BFA can be performed on the CD-Sapphire hematology analyzer for WBC and RBC with reliability of cell counts >0.150 x 10^3/µL for WBC and >0.001 x 10^6/µL for RBC. A two-part WBC differential can be reliably performed on BF specimens with >50 cells/µL. The multi-angle scatter and fluorescence analysis, combined with the triple-triggering circuitry,[2] of the CD-Sapphire system permits the detection of FL3+ small particles, such as NRBCs and bacteria, whose DNA are stained with a bright nucleic acid stain in the WBC reagent.

* BFA is in development.

60388_Poster2


Competitors
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ADVIA 2120 CSF Assay

An Instant Hit

Another first, as Siemens Hematology debuts an FDA-cleared, automated CSF assay on a routine analyzer.

Performance Specifications
Reportable Results

- Automated WBC on all samples
- Automated RBC on samples <1,500 RBC/µL
- Differential, PMN, and MN (absolute and %) on samples with >20 WBC/µL
- WBC Differential includes: Neutrophils, Lymphocytes, Monocytes, Eosinophils*

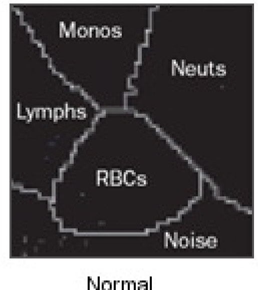
Linearity

Parameter	Range	Max. Deviation
WBC	0-50/µL	5 cells
	50-5,000/µL	10%
RBC	0-50/µL	5 cells
	50-1,500/µL	10%

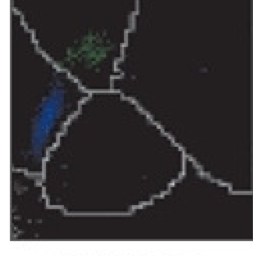
Within Run Precision and Accuracy
(Nominal 100 cells/µL; 150 samples, at least 50% abnormal)

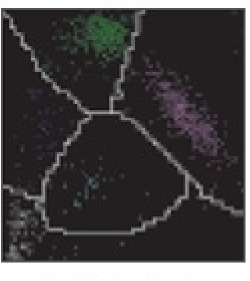
Parameter	Stan. Dev./Bias	%CV/%Bias
WBC	15	15%
RBC	15	15%
#PMN	20	20%
#MN	20	20%

Sample Stability
The prepared sample is stable between 4 minutes and 4 hours when stored at 18°-30°C.



Normal


Viral Meningitis


Bacterial Meningitis

Typical Cytogra (for illustrat purpos only)

CSF Assay Kit Components

CSF Assay Reagent Kit
1 bottle of reagent
- 25 aspirations
- 1-year shelf life (unopened)
- 30-day open bottle stability
- store at room temperature, 18°-30°C

CSF Assay Control Kit

2 levels: Low and High
- 1 vial of each level
- 10 aspirations/vial
- 105-day shelf life (unopened)
- 10-day open vial stability
- store refrigerated, 2°-8°C

Summary of ADVIA 2120 CSF Assay:

- Reduces manual, labor-intensive procedure, optimizing efficiency, productivity, and resource management.
- Improves turnaround time, providing critical results to clinicians for faster diagnosis and treatment.
- Allows results to be available 24 hours per day, regardless of staffing constraints.
- Increases consistency and confidence in results, minimizing technique variations between technologists.
- Provides QC for previously uncontrolled method.
- Expands platform for future development.

*For research use only.

Numération et formule du LCR en routine sur Advia 120

Le système d'hématologie Advia® 120 est le premier appareil agréé par la FDA pour l'analyse du LCR en routine.

Résultats disponibles

- Numération des leucocytes
- Numération des hématies (<1500 GR/µl)
- % et valeur absolue des mononucléaires et des polynucléaires sur les échantillons contenant plus de 20 leucocytes/µl
- Formule leucocytaire comprenant:
  - Neutrophiles
  - Lymphocytes
  - Monocytes
  - Eosinophiles*

Performances

Linéarité

Paramètres	Valeurs	Déviation Max.
GB	0 - 50/µl	5 cellules
	50 - 5 000/µl	10%
GR	0 - 50/µl	5 cellules
	50 -1 500/µl	10%

Précision intra-série
(100 cellules/µl ; 150 échantillons, dont au moins 50% d'anormaux)

Paramètres	Ecart-type/déviation	%CV/%Déviation
GB	15	15%
GR	15	15%
Polynucléaires**	20	20%
Mononucléaires**	20	20%

Stabilité

L'échantillon préparé est stable entre 4 minutes et 4 heures, conservé entre 18° et 30°C

*A titre de recherche uniquement.
** valeur absolue

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Le kit LCR permet

- De réduire les manipulations
- De diminuer le temps de rendu des résultats
- La standardisation de l'analyse du LCR
  - Propose un contrôle pour une analyse qui jusqu'alors n'en possédait pas
  - Ne nécessite pas la présence d'un spécialiste
- Propose une plateforme pour des développements futurs

Bénéfices

- Augmentation de la fiabilité et la productivité de l'analyse du LCR
- Facilité d'utilisation
- Diminution du stress de l'urgence
- Augmentation de la confiance dans les résultats
- Un même niveau de confiance quelque soit l'opérateur

Composition du kit LCR

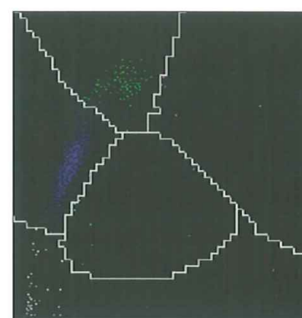
Description	Référence
Clé logiciel	06842206
Réactif LCR 1 flacon – 25 tests – Conservation flacon non entamé : 1 an – Conservation flacon ouvert : 30 jours – Stockage entre 18°C et 30°C	04274197
Contrôle LCR Deux niveaux (Haut et Bas) – Un flacon pour chaque niveau – 10 aspirations par flacon – Conservation flacon non entamé : 105 jours – Conservation flacon ouvert : 10 jours – Stockage entre 2°C et 8°C	00872863

Nota : La version 3 (ou supérieure) est requise sur l'ADVIA120 pour supporter le kit LCR

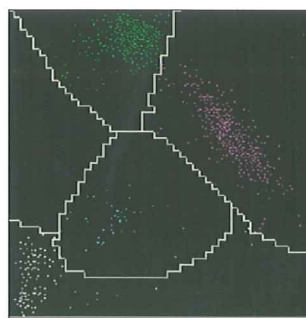
Cytogrammes caractéristiques (pour illustration uniquement)

Monos
Neuts
Lymphs
RBCs
Noise

Normal



Méningite virale



Méningite bactérienne

La disponibilité des produits peut varier en fonction du pays, et est soumise aux réglementations locales. Veuillez contacter votre représentant local pour toute question relative a la disponibilité d'un produit.

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XT-Series

Body Fluid Application

XT-SERIES
Body Fluid Application

Principles & Technologies	WBC: Fluorescent Flow Cytometry RBC: DC-sheath flow
Specimen Types	• Body Fluid Applications including CSF (cerebrospinal fluid), serous fluid, synovial fluid • Specimens collected in EDTA are acceptable
Precision	WBC: CV% <= 40% (WBC: 0.050-0.10 x 10^3/µL) RBC: CV% <= 40% (RBC: 0.01-0.20 x 10^6/µL)
Low Level of Linearity	WBC >=0.050 x 10^3/µL RBC >=0.01 x 10^6/µL
Carryover	WBC <=1% RBC <=1%
Sample Volumes	85 µL open mode
Quality Control (Total QC Management)	The same technology is used for both body fluid and complete blood cell (CBC) counting; therefore, a separate control is not necessary.

A logical step in blood cell analysis is the application of automated body fluid testing. The XT-Series analyzers with XT pro software now brings the power of fluorescent flow cytometry to body fluid analysis.

The XT-Series Body Fluid Application is a quantitative, automated procedure for analyzing cerebrospinal fluid, serous fluid and synovial fluid, providing WBC and RBC enumeration.

Increases Efficiency and Productivity

- Built on the robust fluorescent flow cytometry platform
- Reduces time consuming, labor intensive manual counts
- Accurate counts in the most clinically significant ranges

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Document Number 10-1071 08/06 2M

XE-Series

Body Fluid Application

XE-SERIES
Body Fluid Application

Principles & Technologies	WBC: Fluorescent Flow Cytometry RBC: DC-sheath flow
Specimen Types	• Body Fluid Applications including CSF (cerebrospinal fluid), serous fluid, synovial fluid • Specimens collected in EDTA are acceptable
Precision	WBC: CV% <=30% (WBC: 0.050-0.10 x 10^3/µL) RBC: CV% <= 40% (RBC: 0.01-0.20 x 10^6/µL)
Low level of Linearity	WBC >=0.050 x 10^3/µL RBC >=0.01 x 10^6/µL
Carryover	WBC <=1% RBC <=1%
Sample Volumes	130 µL open mode
Quality Control (Total QC Management)	If the same instrument is being used for Complete Blood Cell (CBC) counting in peripheral blood, a separate control for body fluid cell counting is not necessary.

A logical step in blood cell analysis is the application of automated body fluid testing. The XE-Series analyzers with XE pro software now brings the power of fluorescent flow cytometry to body fluid analysis. The XE-Series Body Fluid Application is a quantitative, automated procedure for analyzing cerebrospinal fluid, serous fluid and synovial fluid, providing WBC and RBC enumeration.

Increases Efficiency and Productivity

- Built on the robust fluorescent flow cytometry platform
- Reduces time consuming, labor intensive manual counts
- Accurate counts in the most clinically significant ranges

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Document Number 10-10 05/06 2M 2M

XE-5000

Managing body fluid analysis

Cerebrospinal fluid analysis within one hour...

In most laboratories the counting and differentiation of white blood cells in cerebrospinal fluid is cumbersome and time consuming. Even in the hands of an experienced technologist the current manual procedure using microscopy easily takes one hour to perform. On the other hand cerebrospinal fluid (CSF) analysis needs to be performed quickly since fast diagnosis and treatment is mandatory if a viral or a bacterial infection is suspected. Furthermore, such samples do not always come to the lab at convenient times, but may also require urgent reporting during night shifts.

... or in just about a minute?

The patient's situation demands a fast and reliable CSF result available on a 24-hour basis. This is achievable by an automated analysis. Based on the well-established fluorescence flow cytometry technology XE-5000 offers a method helping to provide these results fast, reliably and with easy sample handling. Approximately 1 minute after aspiration in the specific body fluid mode the counts of white blood cells, mononuclear cells, polymorphonuclear cells and red blood cells are available. Extended count volumes ensure an increased precision in the very low concentration range.

Analysis of further body fluids

Besides cerebrospinal fluid other body fluids can also be analysed in the same dedicated body fluid mode of XE-5000:

- peritoneal effusions (ascites)
- continuous ambulatory peritoneal dialysis (CAPD) fluid
- pleural effusions
- synovial fluid

In these samples increased leucocyte counts may indicate the status of infection even if the number of detectable cells is extremely low. By preventing potentially interfering substances like micro air bubbles or other non-cellular particles from being counted via the fluorescence signal, the resulting background count achieves values as low as 0 to 1 white blood cell per µL.

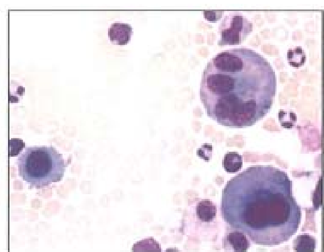
Key path main unit XE-5000

Sampling on XE-5000 in body

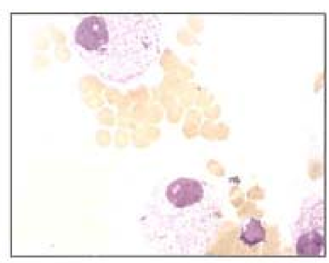
XE-5000

Manage your body fluid cases as well

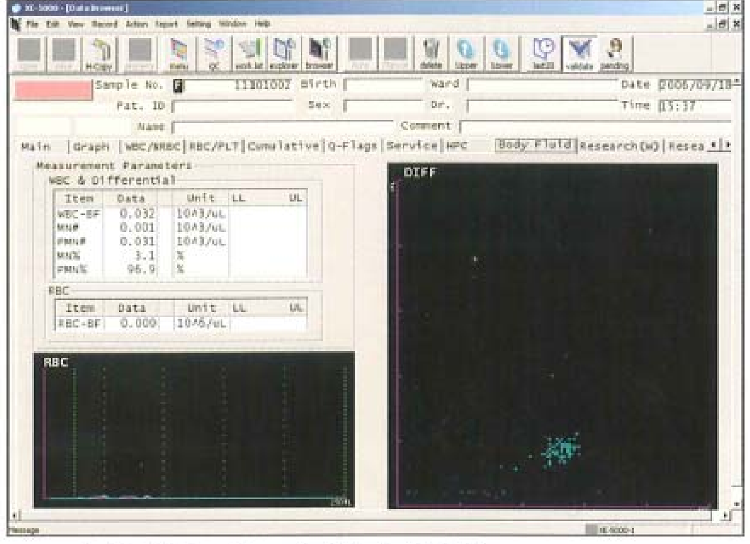
Additionally mesothelial cells and macrophages (so-called 'high fluorescence body fluid cells'; HF-BF) are excluded from the leukocyte count and differential by a special algorithm. Their number can be reviewed in the research display of XE-5000. Via a user-definable setting program flagging of samples with increased numbers of mesothelial cells, macrophages or tumour cells becomes possible.



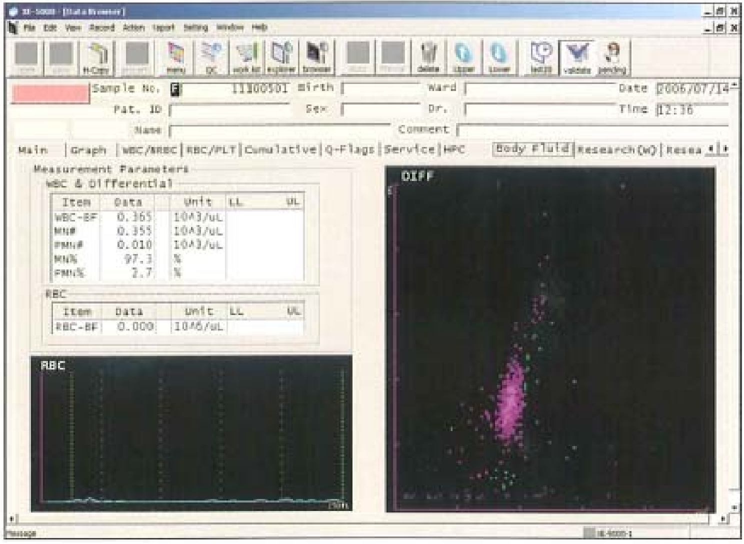
Mesothelial cells



Macrophages



XE-5000 body fluid display: CSF sample with bacterial infection



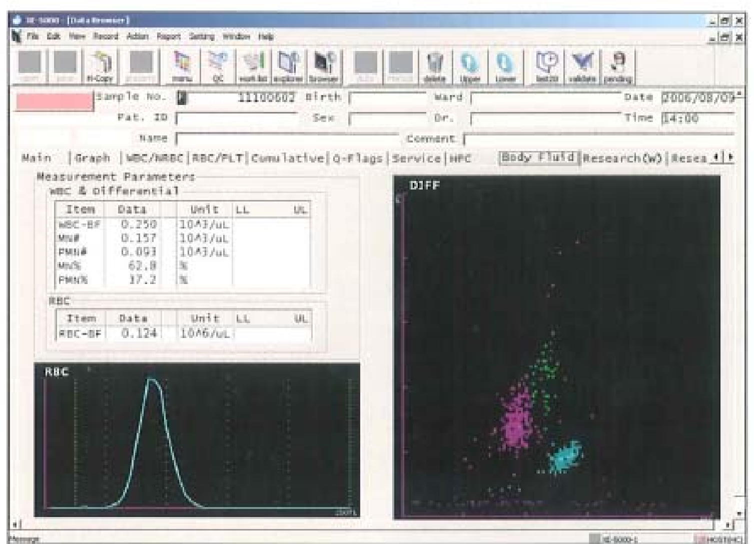
XE-5000 body fluid display: CSF sample with viral infection

Simplicity in operation

Switching from whole blood mode to body fluid mode requires just a simple push on the respective key. A rinse program is then automatically executed to ensure the required low WBC background count (0–1 cells/µL). In routine conditions, this procedure takes less than 2 minutes. The specific body fluid mode of XE-5000 also virtually eliminates sample-to-sample carry-over by an additional, extended rinse program performed automatically after each sample analysis. The direct sampling of CSF is an outstanding feature as this means there is no need for manual dilution and incubation of the sample with specific reagents prior to analysis. Moreover, interferences with the WBC count or differential by RBC are eliminated as merely cells containing nucleic acids are stained by SYSMEX' unique fluorescence dye. As a result even single white blood cells can be seen easily in the DIFF scattergram which is displayed in the body fluid screen of XE-5000.

Synovial fluid analysis: an easy task for XE-5000

Analysis of this body fluid is a challenge for instruments due to its very viscose nature and content of hyaluronate as its major component. Acidic reagent systems (e.g. RBC lyse reagents) can induce polymerisation of the polysaccharide hyaluronate causing falsely decreased WBC counts. Furthermore, there is always the risk of clogging the



CSF sample with RBCs included

instrument's hydraulic lines which may require tedious maintenance procedures thereafter. However, these phenomena cannot occur in the body fluid mode of XE-5000 as its reagent system operates in neutral pH, thus avoiding potential polymerisation.

Cost-effective with only 4 reagents

By offering body fluid analysis in a dedicated mode, XE-5000 operates highly economically at a lower cost level than for a whole blood CBC+DIFF profile. There is no need to purchase any additional control material for body fluid analysis since all body fluid parameters are checked by SYSMEX' *e*-CHECK (XE) control blood. The analytical performance of XE-5000 in body fluid mode and whole blood mode is monitored simultaneously using *e*-CHECK (XE).

Cost-effective operation

The robust instrument leaves the operator with a minimal daily maintenance of only 20 seconds for aspiration of the cleaning solution and is ready-to-use for 24 hours and 7 days a week due to high-quality mechanical and optical components. Moreover, a calibration by the operator is not required.

XE-5000

Body fluid mode specifications

Technologies Body fluid mode	■ fluorescence flow cytometry: WBC, DIFF ■ DC sheath flow method: RBC
Diagnostic parameters	WBC-BF, RBC-BF MN (%, #), PMN (%, #)
Histogram	RBC cell size distribution
Scattergram	WBC DIFF channel
Throughput	body fluid mode: 38 samples/h (max.), manual mode
Sample volume	130 µL (open manual mode)
Display data range	WBC-BF 0.000 – 999.999 x 10^9/L MN % 0.0 – 100 % MN # 0.000 – 999.999 x 10^9/L PMN % 0.0 – 100 % PMN # 0.000 – 999.999 x 10^9/L RBC-BF 0.000 – 99.999 x 10^{12}/L
Reproducibility *	typical performance characteristics CV%: < 25 % (WBC 10 – 15 cells/µL) CV%: < 20 % (WBC 15 – 25 cells/µL) CV%: < 15 % (WBC 25 – 50 cells/µL) CV%: < 10 % (WBC > 50 cells/µL)
Carry over	WBC: ≤ 0,3% or less than or equal to 1 x 10^6/L RBC: ≤ 0,3% or less than or equal to 3 x 10^9/L
Data storage (IPU: information processing unit)	10,000 samples incl. graphics
Quality control	20 QC files, 300 data points, 51 parameter 1 XbarM file, 9 body fluid parameters IQAS ONLINE
Interfaces	serial (e.g. LIS, line printer) parallel (e.g. graphic printer) LAN (gigabit Ethernet – e.g. LIS, further SYSMEX components) USB (e.g. graphic printer, bar code reader, memory sticks) SUIT (SYSMEX UNIVERSAL INTERFACE) protocol for LIS connection
Dimensions/weights wxhxd [mm]/[kg]	main unit (MU): 706 x 711 x 535 / 81 MU incl. sampler: 706 x 711 x 912 / 93 pneumatic unit: 195 x 333 x 395 / 16 IPU: 338 x 101 x 379 / 8
Configurations	stand-alone or twin module of HST-N, XE-AMS or EXPERT LINE
Reportable research parameters (for laboratory use only)	high fluorescence count (HF-BF) (%, #) total nucleated cells (TC-BF) (#)

* *Typical performance characteristics for reproducibility were determined during evaluation on a typical XE-5000 instrument, using 14 body fluid samples which were counted 10 times consecutively in the body fluid mode and are based on the precision profile graph.*

Design and specifications may be subject to change due to further product development.

Fast analysis

- all body fluid parameters ready for review 1 min. after sample aspiration
- direct sampling of CSF and all other body fluids – no need for manual dilution and preincubation of the sample with specific reagents
- switching from whole blood mode to body fluid mode in less than 2 min.

Decisive results

- automatic rinse program to ensure a low WBC background count (0 – 1 cells/µL)
- newly designed count sequences with extended count volumes for increased precision in the very low cell concentration range
- a special algorithm to exclude mesothelial cells and macrophages from WBC and DIFF

XE-technology made for body fluid analysis

- through fluorescence staining of RNA/DNA inside the white blood cells, effective separation from RBC
- XE-5000's body fluid channel shows no interference caused by hyaluronate from synovial fluid
- ascites, CAPD-, cerebrospinal-, pleural- and synovial fluid: all analysed in the same mode

Body fluid analysis – the economical and automated way

- cost per test is lower than that of a whole blood CBC+DIFF profile
- SYSMEX e-CHECK (XE) control blood surveys all parameters of XE-5000's body fluid mode and whole blood mode at the same time
- ready-to-use analyser by robust mechanical and optical components requiring only a minimal daily operator's maintenance of 20 sec for aspiration of the cleaning solution

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XE-SFS N 6040 ICE 8/07

UF-1000*i* SERIES

UF-1000*i* Series

A New Generation Automated Urine Particle Analyzer

Convenient, Accurate and Fast

UF-1000*i* SERIES

Technology	- Urine flow cytometry - Two stains with fluorescent dye - Separate bacteria channel for improved discrimination - Modern IT environment
Throughput	Up to 100 sample per hour
Sampler	50 samples on board processing
Sample Volume	Manual mode : 1mL Sampler mode : 4mL
Parameters	RBC, WBC, Epithelial Cells, Casts, Bacteria
Flagging Parameters	Pathological Casts, Crystals, Small Round Cells, Sperm, Yeast, Mucus
Data Storage	10,000 samples (incl. Scattergrams)
Peripheral Interfaces	Host line printer Graphic printer Hand-held bar code reader
Quality Control	24 files with 300 data points each Sysmex control material: UF II CONTROL Levey-Jennings and X-bar quality control programs
Accessories	Graphic printer
Dimensions / Weight w x d x h [inches] / [lb]	Main unit: 22.8” x 27.0” x 24.2” / 148

Simplicity

- Fully automated sample preparation (mixing, aspirating, diluting, staining)
- Objective particle characterization and identification based on detection of forward scatter, fluorescence and on adaptive cluster analysis
- Automatic sample validation supported by extensive user-definable flagging system
- Automatic sample validation for review or output to host computer/printer according to user-definable system setting

Efficient workflow

- Ease-of-use with Windows® XP operating system
- Fast sample processing
- Batch analysis with sampler or immediate analysis of STAT samples
- Minimal exposure to hazardous sample material
- Review only by exception

High quality screening

- Use of uncentrifuged urine reduces the inherent errors of sediment analysis
- Urine flow cytometry provides outstanding precision for urine particle analysis
- Comprehensive information on a wide range of parameters raises confidence

Design and specifications may be subject to change due to further product development.

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UF-SERIES

UF-100™ and UF-50™ When time matters as much as quality

Fully Automated Urine Cell Analyzers
Efficiency Made Simple

Urinalysis is one of the highest volume and least automated testing areas in the clinical laboratory. The UF-Series fully automated urine cell analyzers bring simplicity, efficiency and flexibility to this labor intensive test.

Simplicity

Operation of the UF is simple and familiar, just like many other automated systems in the laboratory. Simply load tubes onto the sampler and press *Start*. Then walk away. The analysis is automatic and completely unattended. You return to final results.

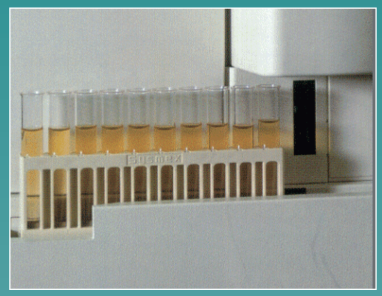
RBC	22.5	[/μL]	4.1	[/HPF]
WBC	279.3	[/μL]	50.3	[/HPF]
EC	4.3	[/μL]	12.5	[/LPF]
CAST	0.26	[/μL]	0.75	[/LPF]
BACT	1497.2	[/μL]	3+	

Efficiency

Now, final urinalysis results are available in about a minute. Samples with significant pathological formed elements are automatically identified with a series of flags. The efficiency of the UF-Series analyzers frees technologists from the repetitive, time-consuming task of visually identifying urinary formed elements. Automated, immediate analysis improves work flow resulting in faster turn around time of critical patient results.

Flexibility

Workstation design can be customized to further improve urinalysis work flow. You choose the UF autosampler configuration that complements your automated chemistry strip reader. The UF gives you the flexibility to create the complete workstation that best fits your laboratory's needs. The SYSMEX UF-Series is the automation alternative that provides fast efficient testing, your way.



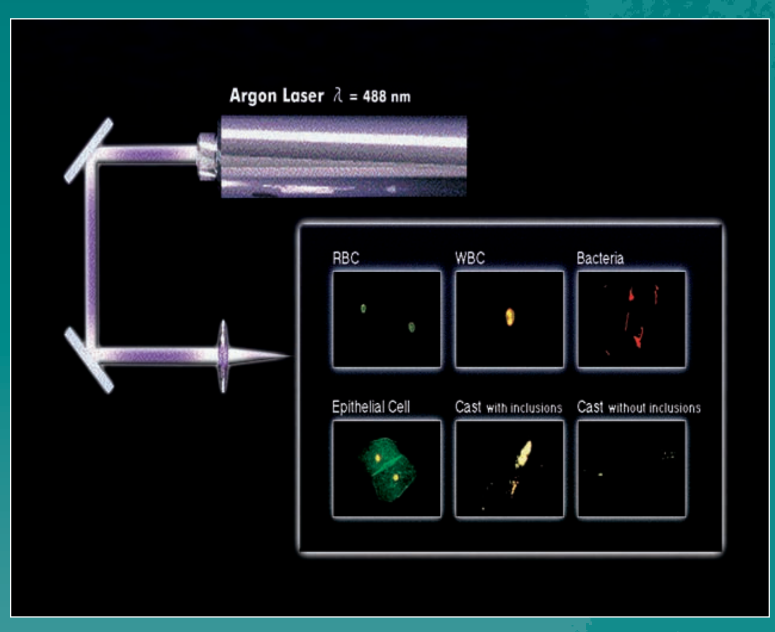
Innovative Application, Technology You Trust

Using advanced, laser-based fluorescent flow cytometry, the UF brings unparalleled sensitivity and standardization to urinalysis. Measurement of fluorescence and forward scatter of light are both used for urine cell counting and classification. Urinary formed elements are stained with two fluorescent dyes specific for nucleic acids and membranes. As these par-ticles, hydrodynamically focused in a sheath flow, pass an argon laser beam, laser light is scattered and the particles emit fluorescent light.

Automatic and Objective

Each formed element has a distinctive fluorescent staining pattern based on the formed element's internal composition. Light is scattered depending on the formed element's size. The scattered and fluorescent light signals are detected separately and are transformed into electrical signals.

These signals form the basis for completely automatic, objective classification of all 10 formed element groups by cluster analysis.



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==== Ready ====
1999/07/02 10:10 No.A32575890-1D889
REVIEW (000026-03)

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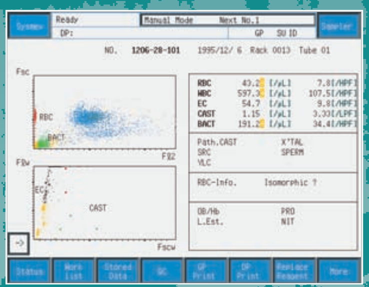
WBC	123. 3	[/µL]	22. 8	[/HPF]
EC	64. 7	[/µL]	12. 0	[/HPF]
CAST	1. 66	[/µL]	4. 83	[/LPF]
BACT	2701	[/µL]	501	[/HPF]

P. CAST +
SRC +

RBC-Info. Microcytic?

Output Delete Mark M. Clear

UF-100



UF-100

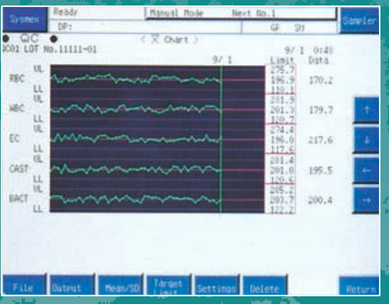
Numerical and graphical results complement each other to achieve maximum standardization in analysis and reporting. Numerical results are displayed and printed. Stored data can be displayed, edited, printed or transmitted to the Host computer at any time.

Standardized, high quality results are available on any specimen and reporting of critical results is accelerated.

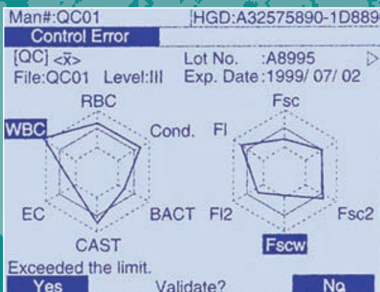
The UF-Series analyzers are designed for efficient unattended operation. SYSMEX builds into every analyzer the systems that enable you to automatically analyze critical patient samples with complete assurance.

Confidence

The integrated quality control program offers reliable system monitoring. UF CHECK, a stable tri-level control from SYSMEX, gives you the assurance of optimal analytical performance. You can verify system performance at a glance using the on-board, advanced graphical QC displays for confidence in analyzer performance.



UF-100



UF-50

UF-100

Reliability

The UF-Series analyzers are designed for low maintenance and high reliability. A few simple, quick maintenance procedures keep the UF running smoothly, reducing interruptions in laboratory service. The UF-Series has one of the highest uptimes of any clinical analyzer on the market. But when you do need them, the SYSMEX team of technology experts is available to provide you with outstanding service and support.

Proficiency

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UF-Series
- Results available in 72 seconds (after sampling)
- Fluorescence and forward scattered light technologies
- Automatic classification of all 10 formed element groups with scattergrams and histograms for reference
- Numerical results automatically by adaptive cluster analysis
- 800 uL sample size

UF-100
- 100 samples per hour
- 2 configurations: Autosampler
  1) with up to five 10-position racks
  2) with up to eight 5-position racks

UF-50
- 50 samples per hour
- Autosampler for a single 10-position rack

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The COULTER® LH 700 Series Hematology Systems

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Bulletin 9223

A New Era In Hematology Testing.

Beckman Coulter is changing the way hematology laboratories operate with the COULTER® LH 700 series—a system platform utilizing the very latest diagnostic technologies. From state-of-the-art hardware and software to highly efficient reagents, calibrators and controls, every aspect of the LH 700 series is designed to simplify and automate the hematology laboratory.

Results, Support And Performance You Can Count On.

The COULTER® LH 700 series was created to help the high-volume hematology laboratory accomplish more with fewer labor resources. Equipped with the most advanced technologies available, the LH 700 series eliminates manual pre-sorting of samples, provides faster results to the ordering clinician and improves overall laboratory efficiency, while maintaining the highest degree of testing accuracy.

The LH 700 series offers a full range of features to optimize workflow efficiency and accuracy. These include:

- Best overall flagging efficiency
- Extended linearity
- Enumeration of NRBCs
- Excellent blast sensitivity
- Accurate low count enumeration
- Trackless slide preparation
- Automated workflow management
- Walkaway calibration
- Body Fluid Analysis
- Innovative RDW-SD Parameter*

The LH 700 series offers AccuCount Technology, our most advanced enumeration technique that delivers the ultimate in precision and accuracy.

And, no matter which LH 700 system you choose, you'll have the peace of mind knowing it's backed by Beckman Coulter, the industry leader in hematology service and support.

*Available on COULTER LH 780 systems.

The COULTER® LH 780 Hematology System with the LH SlideMaker and LH SlideStainer.

The LH 700 series helps laboratories simplify and automate laboratory workflow processes from start to finish—with all the accuracy and quality that has made Beckman Coulter the preferred provider of hematology systems worldwide.

Now, more than ever, the LH 700 series empowers you to create a system that accommodates your unique hematology lab requirements. No matter what configuration you choose, the COULTER® LH 700 series delivers performance and results that will exceed your expectations.

Automate Sample Preparation

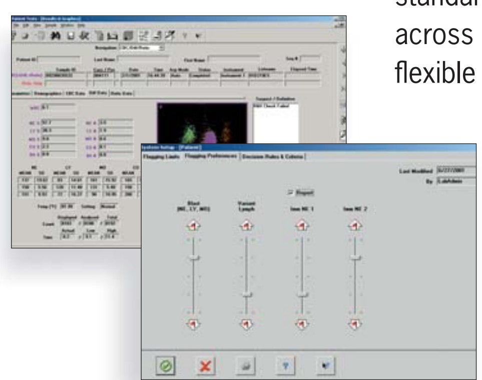
To eliminate labor intensive manual pre-sorting of samples, the LH 700 series delivers true random access by profile type: CBC, CBC/Diff, CBC/Diff/Retic, CBC/Retic or Retic only.

Increase Testing Accuracy and Confidence

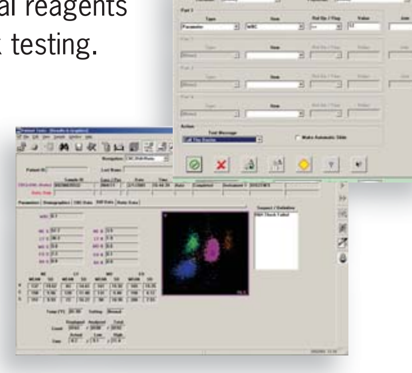
The LH 700 series is powered by AccuCount Technology to deliver expanded linearity and unsurpassed accuracy, particularly at the clinical decision threshold. With LH 700 analyzers, WBC interference correction is automatic. You'll receive an accurate count in the presence of interfering substances, such as NRBCs. You'll also benefit from a reduction in false positives—helping your lab to increase productivity.

The COULTER® LH 700 series offers a number of system configurations and capabilities to fit your demanding workload needs.

AccuCount Technology, combined with AccuFlex, minimizes manual review while improving accuracy, for greater confidence in test results.



The LH 700 series also provides NRBC enumeration to help eliminate manual counting procedures and ensure consistently reliable results. What's more, NRBCs are automatically enumerated with every CBC/Diff without the need for additional reagents or reflex testing.



AccuCount capabilities such as superior flagging efficiency helps improve laboratory efficiency.

Enjoy Flexible Decision Support and Flagging Options

The LH 700 series allows users to easily define decision rules to help optimize and standardize laboratory processes across all shifts. With multiple levels of flexible differential flagging (AccuFlex), a system can also be customized to meet labs' individual needs and specific patient populations. Plus, with the integrated LH SlideMaker and LH SlideStainer, you can set rules to automatically produce blood smears.

Simplify and Automate Body Fluid Analysis

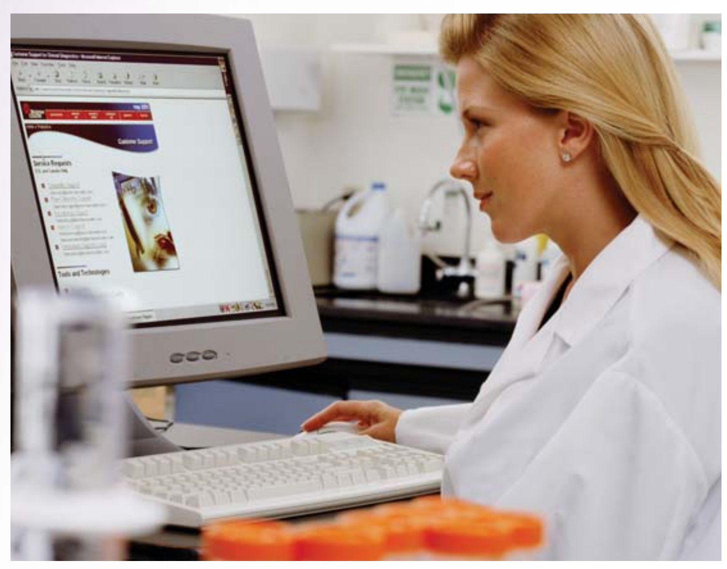
The Body Fluid application allows your lab to efficiently and accurately test three categories of body fluids, such as Cerebrospinal fluid; Serous fluids (pleural, peritoneal, pericardial); and Synovial fluids that have been treated with Hyaluronidase.

Benefit From Superior Reagents, Controls and Calibrators

With a high degree of flexibility in reagent options, the LH 700 series allows laboratories around the world to meet necessary environmental regulations. Laboratories can choose cyanide-free reagents,* or take advantage of reagents that have a reduction in formaldehyde-forming compounds.

*Not available in all countries.

Experience Unparalleled Productivity With Beckman Coulter Service And Support.

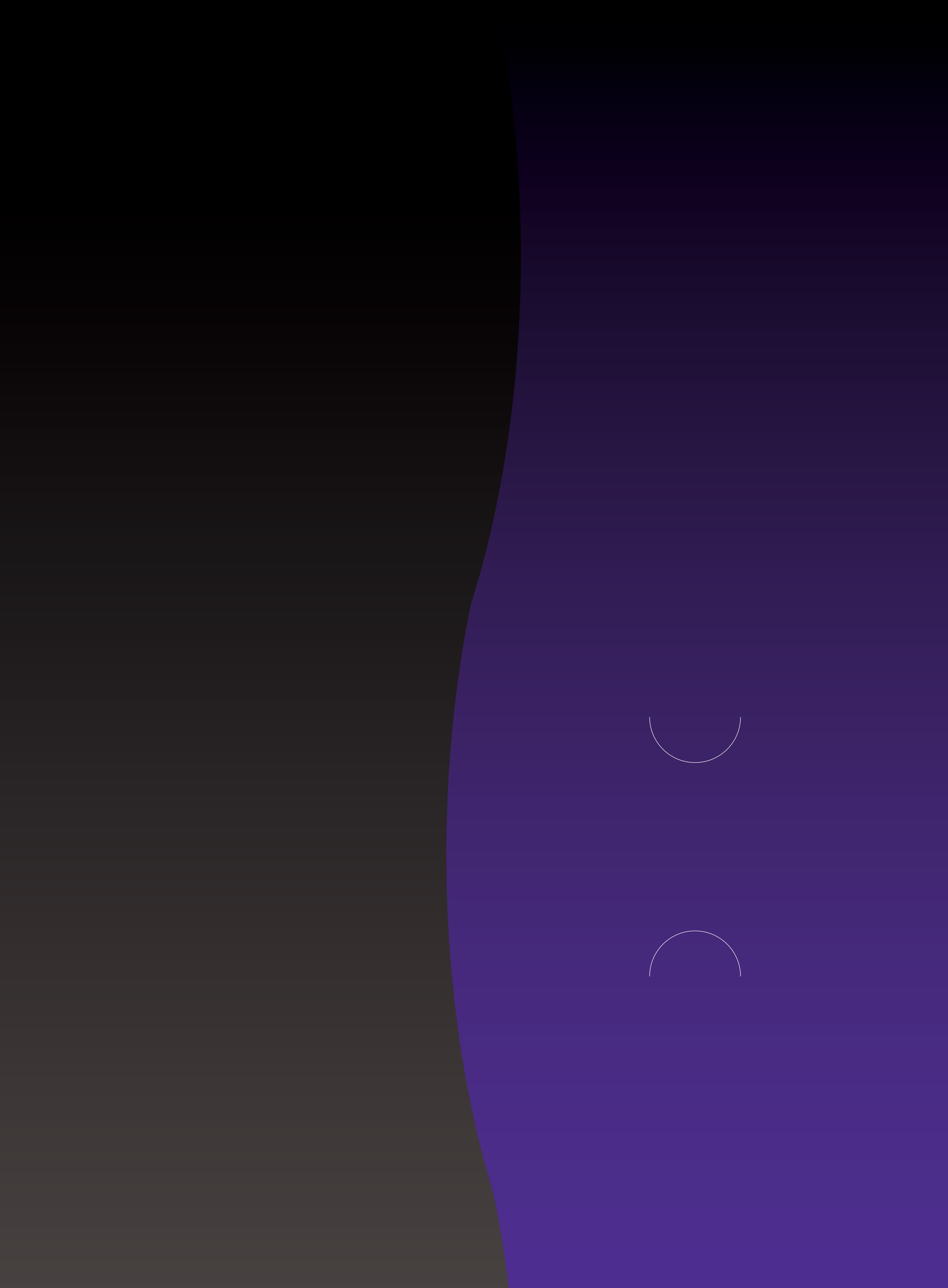


An independent study confirms that Beckman Coulter is the industry leader in customer service and support. The knowledge and expertise of our service organization was not only an essential part of the LH 700 series conceptual design, it helped define the features that let you work better and faster.

With the LH 700 series, your lab will benefit from advanced service features such as:

- Online Intelligent Service which provides troubleshooting for increased productivity and up time
- Zero routine maintenance for improved daily efficiency
- Exceptional customer support through our telephone, on-line and on-site services

Beckman Coulter is proud to offer an innovative line of hematology systems designed to simplify and automate today's high-volume laboratories. For enhanced performance and advanced labor-saving technology, count on the COULTER® LH 700 series to exceed your expectations.



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Higher Performance With You In Mind.

The COULTER® LH 780 Hematology Systems

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Bulletin 9573

The LIS interface of the COULTER® LH 780 series receives STAT information in the Test Order, and communicates directly to the LH SlideMaker, saving time and preventing errors.

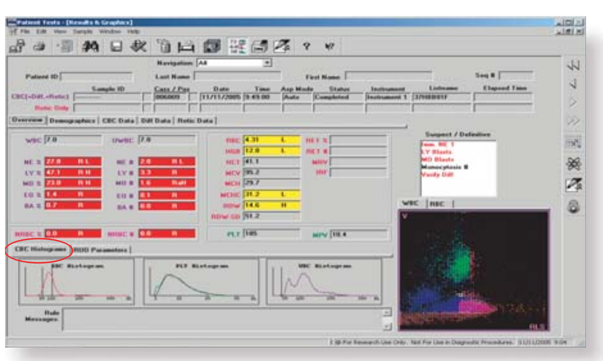
We've tapped the best minds in hematology to design the COULTER® LH 780 hematology systems.

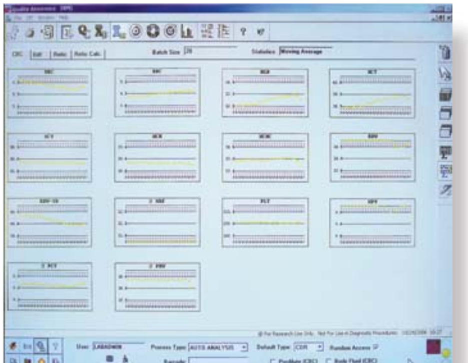
No one understands high-volume hematology analyzers better than our customers. So when we asked what you wanted most in your laboratories, we listened carefully. The result is the ground-breaking COULTER® LH 780 hematology systems, with one of the world's best-in-class combinations of performance, reliability, service and support.

The LH 780 hematology series continues the evolution of advanced technologies, delivering some of the highest sensitivity, specificity and efficiency in WBC differential analysis. To this, we've added a broad spectrum of workflow and functional enhancements in response to your requests.

Advanced Barcode Reading Our customers believe that worry-free barcode reading is essential for smooth, uninterrupted workflow and greater patient safety. Our answer: the innovative, optical-based laser scanning system of the LH 780 analyzers easily reads most barcode labels – even those with lower print quality. What's more, the system has no moving mechanical parts, to ensure the utmost in reliability.

RDW-SD Parameter Expands Your Insights For RBCs Standard deviation of the width of the red cell population (RDW-SD) is an indication of size dispersion within the red cell population. One potential application of the RDW-SD parameter is that it may assist in the differentiation of the cause of anemia. Studies have shown that an elevated RDW-SD may indicate iron deficiency, whereas a normal or decreased RDW-SD may indicate heterozygous thalassemia.



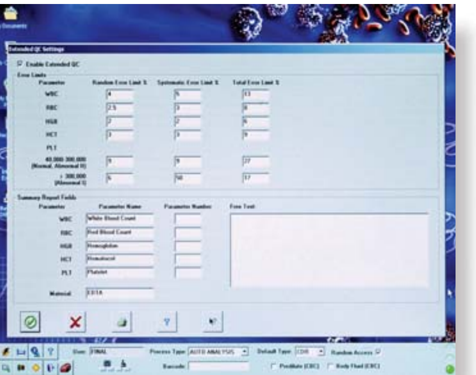


Obtain an exponentially weighted moving average (EWMA) of CBC, 5-Part Diff and NRBC, as well as reticulocyte parameters, with the XM QC package.

Extended Quality Control Packages

Consistency and accuracy are paramount concerns of our customers, so LH 780 analyzers empower them with precision tools for quality control.

The XM quality control package provides an exponentially weighted moving average (EWMA) of CBC, 5-Part Diff and NRBC, as well as reticulocyte parameters. Batch sizes are user-configurable for two to 1,000 runs, for up to 20 batches.



Extended QC Rules provide for the verification of random error or imprecision, systematic error or bias, and total error or inaccuracy.

Extended QC Rules, an additional quality control package, incorporates medically-based performance standards and provides user-enabled rules for the verification of random error or imprecision, systematic error or bias, and total error or inaccuracy. Plus, you can ehance your ability to manage QC data files with designated QC file filters.

Environmentally-Friendly Consumables

Consumables are an all-important consideration in any laboratory because they can help optimize both workflow and performance. The LH 780 analyzers offer two major advancements in this area. COULTER® LH Series Cleaner offers a new, improved formaldehyde-free formulation. COULTER® LIN-C® Linearity Control significantly extends the reportable range for four pivotal LH 780 series parameters: WBC, RBC, HGB and PLT.

Intuitive User Interface The improved user interface of the LH 780 series is carefully designed to be easy to learn and intuitive to use, yet with more features than ever before. To facilitate information access and control, key screens have been redesigned and expanded, providing the most frequently used data and information on one screen.

Productivity is optimized by the easy-to-understand user interface, with its robust reporting and enhanced data handling capabilities.

COULTER® LH SlideMaker and LH SlideStainer The LH 780 configured with LH SlideMaker and LH SlideStainer streamlines one of your lab's most time-consuming manual tasks with automated slide preparation. By eliminating complex tracks for specimen delivery, the LH SlideMaker and LH SlideStainer help decrease errors and increase accuracy with simple, no-prep loading. Operators simply insert sample cassettes into the workcell and return later to remove stained blood films with clear slide labeling, stain-resistant labels and optional bar coding.

COULTER® LH 780 Hematology Analyzer Specifications

Available Parameters

WBC	MO%	Hgb	Retic #	
NE#	EO#	HCT	Retic %	Plt
NE%	EO%	MCV	IRF (Immature Reticulocyte Fraction)	MPV (Mean Platelet Volume)
LY#	BA#	MCH	MRV (Mean Reticulocyte Volume)	
LY%	BA%	MCHC		
MO#	RBC	RDW		
RDW-SD				
NRBC%				
NRBC#				

LH 780 Throughput

Mode	Sample/Hour
CBC Only	110
CBC/Diff	110
Retic Only	45

Linearity/Operating Ranges

Parameters	Linearity Range	Operating Range
WBC	0.00 to 400.00 x 10^3 cells/µL	0.00 to 900.00 x 10^3 cells/µL
RBC	0.00 to 8.00 x 10^6 cells/µL	0.00 to 20.00 x 10^6 cells/µL
Hgb	0.0 to 25.0 g/dL	0.0 to 99.9 g/dL
Plt	0 to 3000 x 10^3 cells/µL	0 to 5000 x 10^3 cells/µL
NE#, LY#, MO#, EO#, BA#		0 to 900 x 10^3 cells/µL
RET%		0.0 to 100.0%
RET #		0.0 to 999.9 x 10^6 cells/µL

Precision

Parameters	Limit	Parameters	Limit
WBC at 9 to 11 x 10^3 cells/µL	≤1.7% CV	NE% at 50 to 60%	2SD ≤3.0
RBC at 4.5 to 5.5 x 10^6 cells/µL	≤0.8% CV	LY% at 25 to 35%	2SD ≤3.0
Hgb at 14 to 16 g/dL	≤0.8% CV	MO% at 5 to 10%	2SD ≤2.0
MCV at 80 to 90 fL	≤0.8% CV	EO% at 2 to 5%	2SD ≤1.0
RDW at 12 to 14%	≤2.2% CV	BA% at 0.5 to 1.5%	2SD ≤1.0
Plt at 280 to 320 x 10^3 cells/µL	≤3.3% CV	RET% at 0.0 to 0.49%	SD ≤0.23 or CV% ≤16.5
Plt at 90 to 110 x 10^3 cells/µL	≤6.6% CV	RET% at 0.5 to 1.49%	SD ≤0.23 or CV% ≤14.5
Plt at 10.0 to 15.0 x 10^3 cells/µL	≤14.0% CV	RET% at 1.50 to 4.00%	SD ≤0.68 or CV% ≤11.0
MPV at 8 to 10 fL	≤2.2% CV	RET% at 4.01 to 15.00%	SD ≤0.68 or CV% ≤5.5
RDW-SD at 33 to 48 fL	≤2.5% CV		

NRBC Accuracy Characteristics

Parameter: NRBC
Units: % (NRBC/100 WBC)

Comparision to Predicate Analyzer

Population Minimum: 0.00
Population Maximum: 24.11
Mean Difference: 0.08

Comparision to Manual Method

Population Minimum: 0.00
Population Maximum: 61.50
Mean Difference: –0.02

Dimensions and Weight

Analyzer/Diluter:

Height: 35 inches (88.9 cm)
Width: 40 inches (101.6 cm)
Depth: 24 inches (61 cm)
Weight: 205 pounds (93.2 kg)

Power Supply:

Height: 23.3 inches (59 cm)
Width: 14 inches (35.5 cm)
Depth: 24 inches (60 cm)
Weight: 125 pounds (56.7 kg)

COULTER® LH 780 with LH SlideMaker and LH SlideStainer

Dimensions and Weight

SlideMaker:

Height: 20.5 inches (52.1 cm)
Width: 29.5 inches (74.9 cm)
Depth: 27 inches (68.6 cm)
Weight: 90 pounds (40.9 kg)

SlideStainer:

Height: 19.75 inches (50.2 cm)
Width: 23.75 inches (60.3 cm)
Depth: 30 inches (76.2 cm)
Weight: 80 pounds (36.4 kg)

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CELL-DYN Sapphire Extended Immunofluorescent Applications

Principles and Practice

Introduction

Progress in haematology instrument reliability, computerised data processing and information handling has undoubtedly advanced laboratory efficiency although the nature of haematological parameters provided by automated analysers has remained largely unchanged for many years. Although the reasons for this are varied, it is generally accepted that impedance and optical measurement principles used by haematology analysers have reached their potential limits.

The Abbott CELL-DYN Sapphire (CD-Sapphire) haematology analyser uses multiple technologies (Table 1) but it is fluorescence that provides the greatest opportunity for the development of new applications. Cellular analysis with fluorochrome-tagged monoclonal antibody (Mab) probes is well established in flow cytometry and there are many procedures with proven clinical utility. An ability to implement similar methodologies in routine practice with a haematology analyser could enhance investigatory options and potentially provide useful supplementary information in areas such as patient lymphocytosis and infection.

Table 1: Overview of CD-Sapphire analytical methods[a]

Measurement Principle	**Red Cell Parameters**	**White Cell parameters**	**Platelet Parameters**
Spectrophotometry	Haemoglobin Concentration		
Impedance	RBC Count (RBCi), MCV, RDW		Platelet Count (Plti), MPV
Optical Scatter	RBC Count (RBCo)	WBC Count, WBC Differential	Platelet Count (Plto)
Fluorescence	Reticulocyte Count, IRF, NRBC Count	WBC Count, WBC Differential, WBC Viability, T-cell Subset Analysis	Immunoplatelet Count

[a] Parallel red cell and platelet counts determined by independent methods. Analysis of some parameters undertaken by a combination of method principles.

This monograph will review the general principles of fluorescence flow cytometry and detail CD-Sapphire procedures for the demonstration of Mab-defined cell antigens. Many of these are straightforward and can be undertaken by a haematology laboratory providing there is a good understanding of instrument strengths and limitations. In addition to providing a focus on extended CD-Sapphire methods for the investigation of leukocyte surface antigens, the illustrative examples of studies undertaken in a number of European laboratories also include details of procedures used for platelet and red cell analyses.

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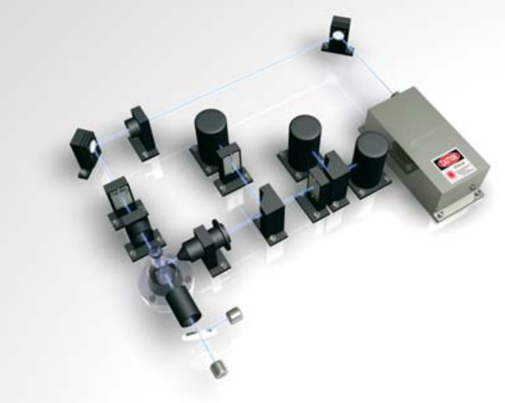
Content



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1 CD-Sapphire Overview

The CD-Sapphire uses a stable 488 nm blue diode laser and three fluorescent detectors and can replicate many of the basic analyses currently provided by flow cytometers. Fluorescence is an integral part of the CD-Sapphire and is used for most central determinations. Reticulocyte analysis uses combined optical scatter and green fluorescence (FL1) [Kim *et al.*, 1997], while for white blood cell (WBC) and nucleated red blood cell (NRBC) differentiation, four angles of light scatter (0°– ALL, 7°– IAS, 90°– DSS and 90°– PSS) and one channel of fluorescence (FL3) are simultaneously integrated in a process known as Multi-Angle-Polarised-Scatter-Separation (MAPSS). FL3 fluorescence (Propidium Iodide staining) is specifically used for WBC viability and NRBC measurements. The CD-Sapphire also has two supplementary processing options for the automated Mab determinations of T-cell subsets [Marshall *et al.*, 2000] and CD61 immuno-platelet counts [Gill *et al.*, 2000]. These assays utilise combinations of optical scatter and fluorescence and form the methodological basis for extended fluorescent applications. For T-cell subsets (CD3/4/8), two optical (0°– ALL and 7°– IAS) and three fluorescent (FL1/FL2/FL3) channels are used while the immuno-platelet mode (CD61) employs two angles (7°– IAS and 90°– PSS) of light scatter and FL1 fluorescence.

2 Principles of Fluorescence Flow Cytometry

Flow cytometry is a general term for the measurement of particle properties in a fluid flow stream. In brief, cells in suspension pass through an optical chamber where they are illuminated by an appropriate light source. Depending on particle structures, and the known properties of added cellular probes, the focussed beam of light is scattered (optical) or modified (fluorescence). Optical and fluorescent signals are filtered, converted to digital values and stored in a numerical format that can be analysed by cytometry software. Particles passing through the flow cell are typically discarded to waste although components that meet specific pre-defined criteria can be collected with some advanced systems (fluorescence cell sorters).

Flow cytometry instruments have light sources that are predominantly lasers or arc-lamps. Laser systems provide milliwatts to watts of light that is typically of a single wavelength (coherent). Common examples include Violet (405 nm excitation), Argon Ion (488 nm), Sapphire (488 nm), and Helium-Neon (633 nm). By comparison, arc-lamps (e.g. xenon and xenon/mercury) are typically of lower energy (milliwatts) and generate mixtures of wavelengths (incoherent light) that need to be filtered to select desired wavelengths. Quantified measurement of fluorescence and scatter is made by detectors, and monitored wavelengths are controlled by the selectivity of optical filters (Figure 1) and mirrors. A complete system for monitoring multiple fluorochromes typically uses a logical order of filters and mirrors in conjunction with independent detectors.

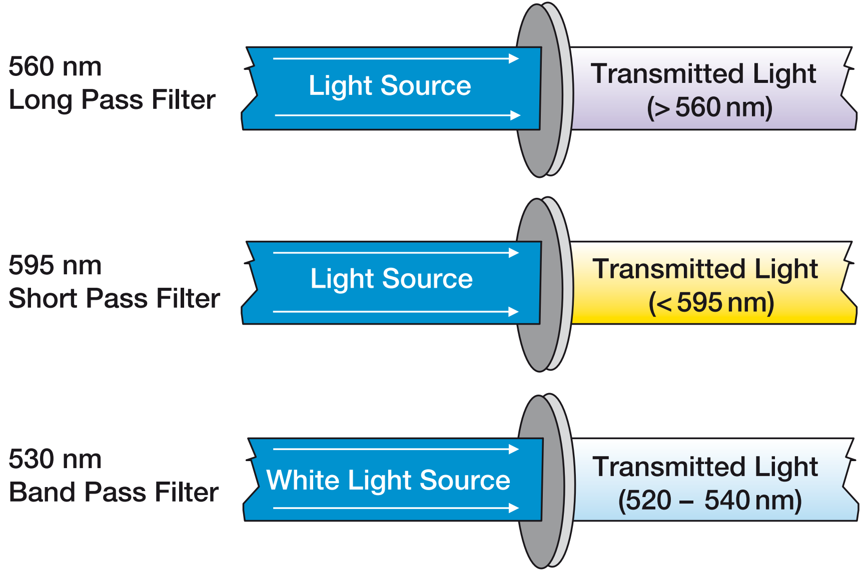


Figure 1: Characteristics of filter types commonly used in flow cytometry. For the filtering of CD-Sapphire excitation and emission wavelengths, FL1, FL2 and FL3 Band Pass filters are used.

3 Fluorescence

Fluorescence occurs as a result of two sequential processes. A light source raises the energy level of a fluorochrome (excitation) and when the light source is removed, the excited molecule returns to its ground state and emits energy in the form of light at a higher wavelength than that used for excitation. The energy difference between the excitation and emission peaks is known as the ‘Stokes Shift’, and it is the excitation wavelength and energy of the light source, together with the characteristics of available fluorochromes, which largely dictate the flexibility and range of potential applications (Table 2).

Table 2: Excitation and emission characteristics of commonly used fluorochromes

Fluorochrome	Excitation	Emission Peak
Fluorescein Isothiocyanate (FITC)	488	525
Phycoerythrin (R-PE)	488	575
R-PE/Texas Red	488	615
R-PE/Alexa Fluor 610	488	628
PerCP	488	680
Cy3	540	570
Propidium Iodide (PI)	536	620
Allophycocyanin (APC)	635	660
Cy5	635	670

4 Spectral Overlap and Compensation

When a fluorochrome molecule is excited, the resulting light emission has a distinct spectrum. Filters are used to ensure that a fluorescent detector collects only emitted rather than excitation light, but it is important to note that the emission spectra of different fluorochromes can overlap. Consequently, if more than one fluorescent probe is combined in a detection system, it may be necessary to make adjustments (compensation) for crossover fluorescence between different detectors. A good example of spectral overlap is seen with Fluorescein Isothiocyanate (FITC) and Phycoerythrin (R-PE) when excited at 488 nm (Figure 2). This shows that while the peak of FITC emission is measured by the FL1 detector, the emission tail also extends into the wavelength bandwidth used to collect R-PE signals (FL2 channel). At the same time, a small amount of emitted light from R-PE extends into the collection region for FL1.

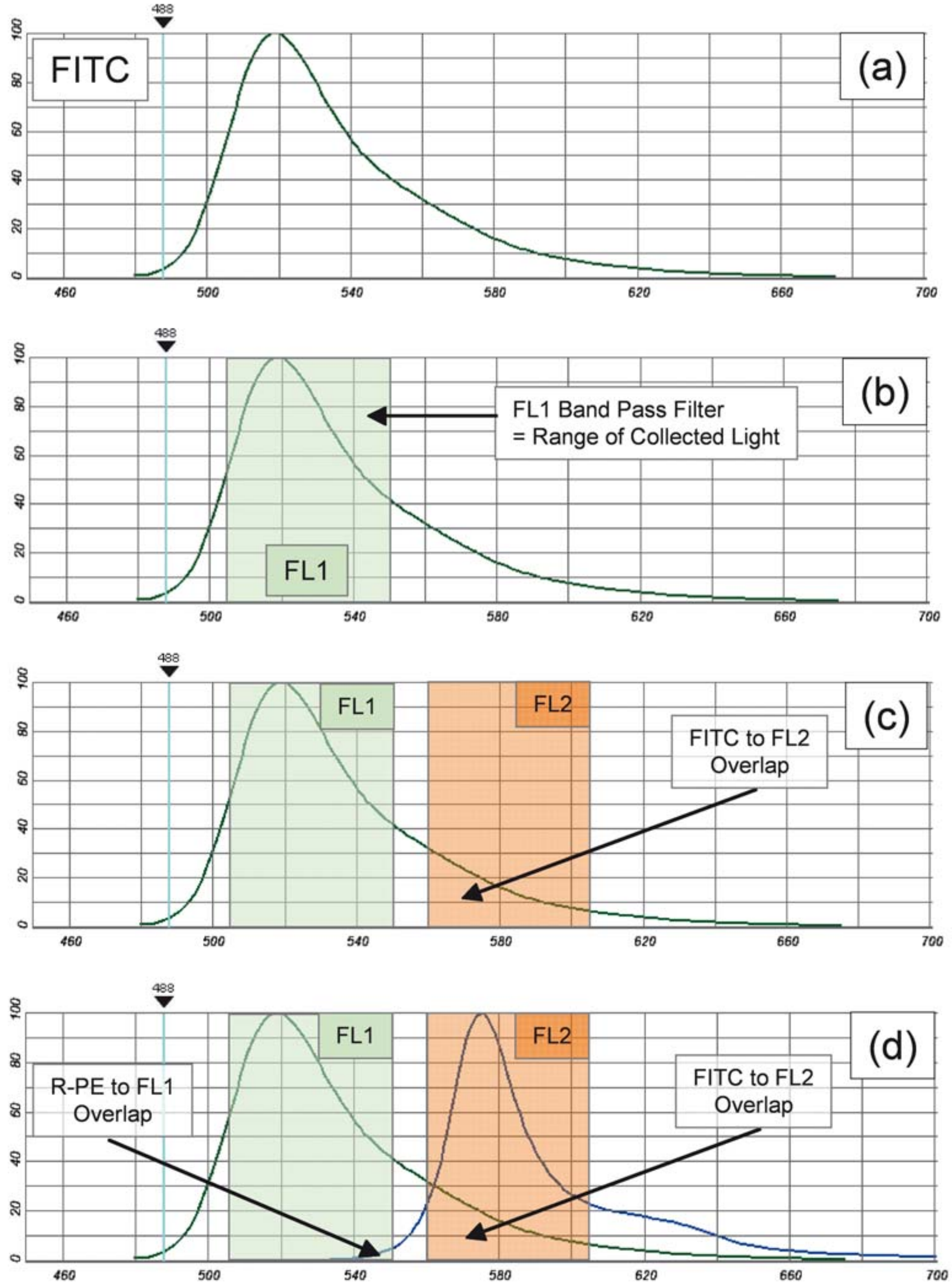


Figure 2: Principles of spectral overlap of fluorochrome emissions. Plot (a) shows the profile of FITC emission when excited at 488 nm. Plot (b) shows a typical range for collected light using an FL1 Band Pass filter and plot (c) shows how the FITC emission spectrum can overlap into the collection range designed for measuring FL2 emission. Plot (d) represents composite overlays for FITC and R-PE emission spectra and indicates significant spectral overlap of FITC into FL2 while R-PE shows only a minor degree of overlap to FL1.

These characteristics are even more applicable to measurement processes involving the monitoring of three fluorescent channels. With the CD-Sapphire 488 nm laser, there is significant FITC (FL1) emission crossover into the FL2 but not the FL3 channel, R-PE (FL2) fluorescence shows minimal overlap into FL1 but is clearly detected in the FL3 channel, while PI fluorescence (FL3) does not contribute to FL1 but has considerable overlap into FL2 (Figure 3). These patterns are similar to those seen with conventional flow cytometers using 488 nm light sources. For two-colour immunocytometry systems, compensation is a relatively simple mathematical process that removes the contribution of R-PE fluorescence in FL1 and FITC fluorescence in FL2. When three colours are used, the same concept is extended to provide cross-compensation for all three fluorescent channels (Figure 4). An awareness of this is necessary for interpreting data from multiple fluorescence procedures although it is also important to state that fluorescence channel compensation is not always required. General guidelines on whether or not compensation is required are given in Section 7e of this monograph.

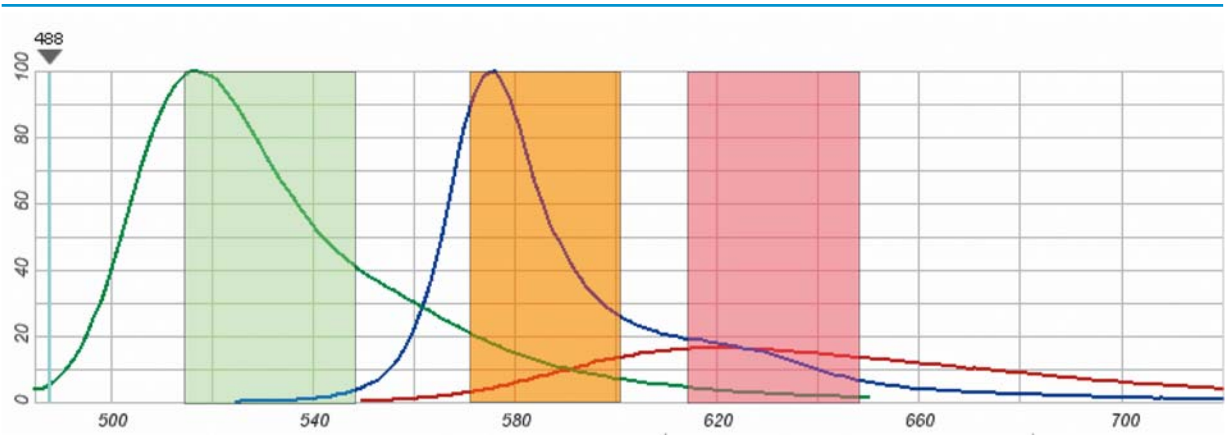


Figure 3: Emission profiles of Fluorescein Isothiocyanate (FITC, green), R-Phycoerythrin (R-PE, blue) and Propidium Iodide (PI, red) fluorochromes excited by 488 nm blue CD-Sapphire laser light. The green, orange and red blocks, respectively, show the band pass filter ranges (with more than 5% transmission) for the FL1 (515 to 548 nm), FL2 (571 to 601 nm) and FL3 (615 to 648 nm) detectors. Note the significant spectral overlap of FITC to the FL2 detector, R-PE to FL3 and PI to FL2. In contrast, there is insignificant detection of FITC emission by FL3, R-PE emission by FL1 or PI emission by FL1.

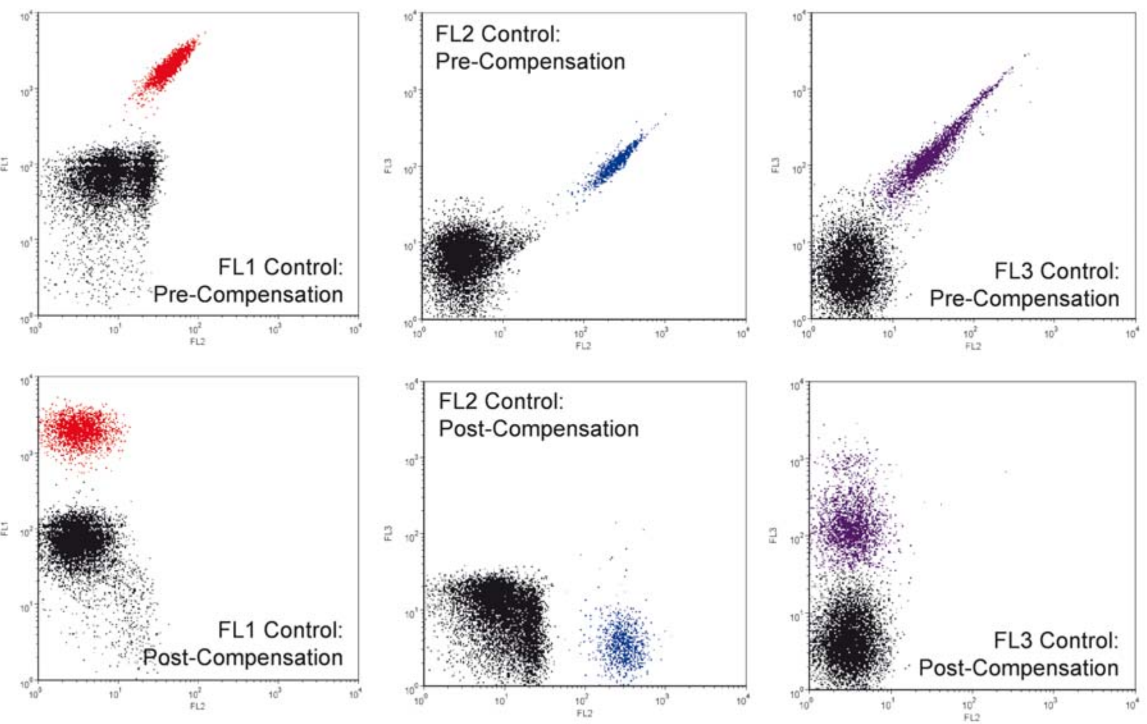


Figure 4: Pre- and post-compensation population clusters for control samples stained with a FITC-antibody (FL1 control), R-PE-antibody (FL2 control), and Propidium Iodide (FL3 control). Compensation was applied post-acquisition with FCS Express software.

5 Monoclonal Antibody Reagents

To detect cellular antigens, antibodies with appropriate specificities are chemically linked to fluorochrome molecules by a process known as conjugation. Purification procedures are then used to ensure maximal retention of antibody avidity, minimal 'contamination' with free fluorochrome, and removal of unlabelled antibody and immunoglobulin aggregates/complexes. Although there are some exceptions, most currently used immunological reagents are derived from mouse hybridomas. Monoclonal antibodies were initially referred to by clone name, but the rapid exponential increase in reagents and specificities created problems when comparing phenotyping results from different laboratories. To resolve this, the 'Cluster of Differentiation' (CD) nomenclature was established in the 1st International Workshop and Conference on Human Leukocyte Differentiation Antigens (Paris, 1982). This was an important advance and there have now been eight Workshops with 339 CD types being designated. With regards to routine haematology, only a small proportion of these are required to deal with most applications (Table 3).

Table 3: CD designations and primary specificities of monoclonal antibodies for typical applications in routine haematology

CD Number	Primary Specificities
CD2	Thymocytes, mature T-cells and NK-cells
CD3	Thymocytes and mature T-cells
CD4	T-Helper cells and monocytes (weak expression)
CD8	T-Suppressor cells and some NK cells (weak expression)
CD13	Mature and immature myeloid cells (neutrophils and monocytes)
CD14	Monocytes (strong expression) and neutrophils (weak expression)
CD16	Neutrophils and NK-cells (subset)
CD19	B-cells
CD22	B-cells
CD33	Mature and immature myeloid cells (neutrophils and monocytes)
CD34	Haemopoietic progenitor cells
CD45	All haemopoietic cells
CD56	NK-cells (most)
CD61	Platelet Glycoprotein IIIa
CD64	Monocytes (high expression) and neutrophils (variable expression)
CD235a	Red blood cell Glycophorin A
HLA-DR (Ia)	Monocytes, immature neutrophils, B-cells and activated T-cells

6 Practical Procedures

a) Sample Preparation

The Mab detection of cell surface antigens is well established and can be transposed to the CD-Sapphire with little modification. Heparinised and citrated blood may be processed with the CD-Sapphire but it is likely that in most cases a haematology laboratory will use samples that are anticoagulated with EDTA. Whether or not sample manipulation is required will depend on the cell type of interest and the nature of the analysis. For example, when leukocyte antigens are investigated it is necessary to remove erythrocytes by lysis prior to passage through the flow cell. In flow cytometry, this is generally carried out with lysing agents such as ammonium chloride after Mab staining. With CD-Sapphire Mab leukocyte analyses, whole blood is stained with antibodies, and erythrocytes are subsequently haemolysed by the instrument reagent system before data acquisition. One further point relevant to method design is that if the antigen to be

analysed is also present in plasma then it may be necessary to remove the plasma by washing prior to antibody staining in order to avoid possible blocking. This is particularly relevant to the analysis of B-cell surface immunoglobulin although most other leukocyte markers can be used in whole blood methods without the need for an initial sample washing step.

b) Antibody Preparation

Antibodies from reputable commercial sources are quality validated (often designated for IVD use), and if they have a Cluster Designation then their specificity requires no additional investigation. These antibodies are produced as liquid fluorochrome conjugates (direct staining) or without a fluorochrome (indirect staining). Most common applications in routine haematology can be undertaken with conjugated antibodies. For fluorochrome selection, detection of high density antigens can be detected by FITC and R-PE conjugates whereas detection of low density antigens is best assessed with R-PE.

Prior to use, commercial reagents need to be titrated to determine the required ratio of antibody to blood in the staining mixture. This initial step is needed to optimise sample staining and should be assessed once for each antibody batch (Figure 5). Increasing the amount of antibody relative to a fixed sample volume will result in increased staining levels until all available antigen sites are occupied. However, at this point it may well be that the amount of antibody is prohibitively expensive. For simple determinations of antigen expression, antigen saturation is actually less important than optimising the level of discrimination between positive and negative cell populations. Providing this is consistent, it is quite acceptable to use sub-saturating amounts of Mab. Moreover, reducing the relative amount of antibody can additionally decrease the level of background cell staining and improve the signal (positive events) to noise (negative cells) ratio.

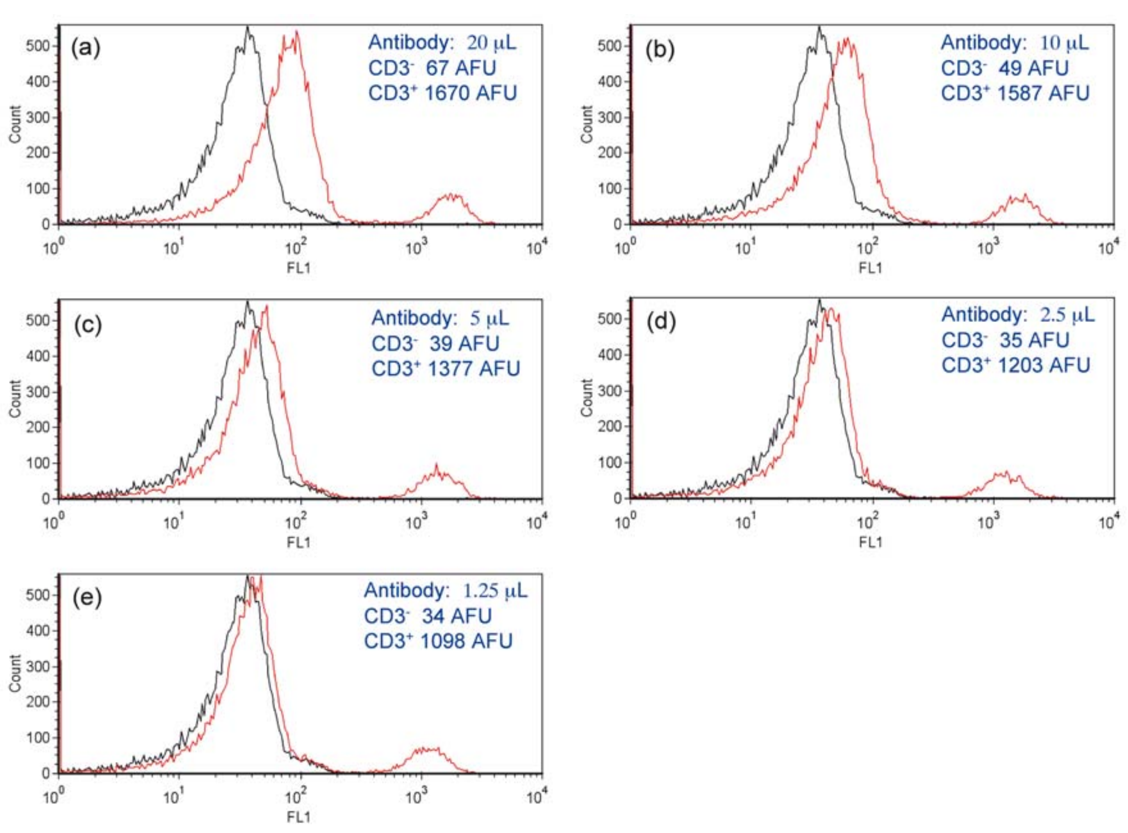


Figure 5: Representative titration of an FITC-conjugated antibody against T-cells (CD3). Antibody fluorescence is indicated by the continuous red lines while the background fluorescence (black) is obtained by testing the same sample with no antibody present. Each plot shows the calculated amount of liquid antibody reagent per 100 µL of EDTA-anticoagulated whole blood, and the geometric means of the $CD3^{+}$ and $CD3^{-}$ fractions. For comparison, the geometric mean of the unstained control sample was 28 Arbitrary Fluorescent Units (AFU).

c) Sample Staining

Staining of samples simply involves pipetting antibody and sample into a plain tube and mixing. The sample volume is generally fixed (100 µL with automated CD-Sapphire processes), and the amount of antibody will have been defined by preliminary titration. The sample itself will correspond to either whole blood (when RBC lysis is undertaken following staining) or a modified cell suspension (when sample manipulation or population enrichment is undertaken prior to staining). As CD-Sapphire automated Mab processes incorporate an incubation step, and because Mab staining equilibrium usually occurs rapidly, the pre-instrument incubation time for staining can be standardised at two minutes.

d) Control Procedures

An understanding of the mechanisms that lead to the attachment of antibodies to cells is important. Specific binding occurs when the antibody Fab region binds to antigen and is thus a reflection of antibody specificity. However, antibody molecule binding can also occur when the antibody Fc region binds to leukocyte Fc receptors. This is undesirable as it can result in non-specific staining when samples contain leukocytes with high-affinity Fc receptors (e.g. monocytes). This phenomenon was first recognised when polyclonal antibodies from animal species such as rabbit, sheep and goats were the predominant source of immunological reagents. Rabbit IgG in particular shows a high binding affinity with human leukocyte Fc receptors [Scott, 1979; Scott *et al.*, 1983], and the use of control (non-antibody) immunoglobulins of the same species/subclass is therefore necessary. In contrast, the affinity between human leukocyte Fc receptors and mouse Mab immunoglobulin is considerably lower than rabbit immunoglobulin. Despite this, the practice of using Mab control reagents has persisted even though there are considerable doubts as to its validity. Mab controls essentially comprise isotype-matched mouse immunoglobulins, with no antibody specificity against human antigens, which are conjugated with the same fluorochrome as the test antibody. The perceived efficacy of this practice is somewhat illusory however because isotype controls often show considerable differences with regards to the protein concentration and F : P (fluorochrome : protein) ratio and rarely have the same characteristics as test antibodies. Further bearing in mind the inherently low affinity between mouse immunoglobulins and human Fc receptors, it seems that the benefits of using costly control materials may be more apparent than real. Indeed, more effective control of Mab test systems may be obtained by examining inter-sample staining consistency of different leukocyte populations (i.e. are the staining levels of defined populations broadly similar for different samples), intra-sample discrimination of negative and positive leukocyte fractions (i.e. do the staining profiles of the main leukocyte populations conform to what might be expected) and the use of multi-reagent panels which essentially serve to control themselves.

Another form of non-specific binding occurs through protein-protein interactions and can be a particular problem if cells are non-viable or have been fixed as part of staining protocols for the demonstration of intracellular or nuclear antigens. The use of isotype controls to confirm or exclude this type of non-specific binding may therefore have more relevance when analysing fixed cells, tissue cells or cultured cell lines.

7 CD-Sapphire Processing

a) Sample Requirements

Samples that are analysed with the CD-Sapphire should not contain clumped cells or aggregated proteins. Although there are various procedures which can be used to remove these, the resulting suspension may not be entirely representative of the original sample. In addition, leukocyte components should ideally have high WBC viability because PI contained in the reagent system will stain non-viable leukocytes. If significant PI^+ components are present, the resulting emission spectrum will also be measured by the FL2 detector (in addition to FL3) and 'contaminate' specific fluorescence associated with R-PE-conjugated antibodies (Figure 3). In such situations, fluorescence compensation may be required in order to avoid difficulties in data interpretation (Section 7e). Samples should also not contain significant numbers of lysis-resistant red blood cells (RRBC). If this is anticipated (e.g. samples from patients with thalassaemia or haemoglobinopathy) then the sample may require treatment with erythrocyte lysing reagents such as BD PharmLyse (BD Biosciences, San Jose, CA, USA) after staining and prior to analyser processing.

b) CD-Sapphire Analysis of Leukocyte Surface Antigens

The CD-Sapphire analyser determination of T-cell subsets uses simultaneous measurements of optical scatter (0°– ALL and 7°– IAS) and fluorescence (FL1/FL2/FL3). In brief, the procedure incorporates three sequential rack positions where the first is occupied by an EDTA-anticoagulated patient sample and the second and third by reaction tubes containing CD3/CD4 and CD3/CD8 antibody mixtures. In this operational mode, the analyser takes fixed volumes of blood from the sample tube and injects them into the two reagent tubes. After mixing and a timed incubation period, aliquots of the blood antibody mixtures are aspirated and diluted in a WBC reagent (containing PI) to facilitate erythrocyte lysis prior to passage through the optical flow cell.

Adaptation of this procedure for user-defined Mab combinations against leukocyte antigens [Molero *et al.*, 2005; Johannessen *et al.*, 2006] (Appendix Abstracts 1 and 2) can be summarised as follows. The patient sample is replaced by an empty Vacutainer tube (with or without a patient barcode identifier) and the two reaction vials (CD3/CD4 and CD3/CD8) are replaced by two bar-coded non-anticoagulated Vacutainer tubes. Into these two tubes is pipetted 100 µL whole blood and a pre-titrated volume of either a single FITC or R-PE-conjugated antibody or a FITC/R-PE antibody mixture. Optionally, the two tubes can be configured for different blood samples with the same two-colour antibody combination, or the same blood sample with different two-colour antibody mixtures. Using the CD-Sapphire operator menu, the CD3/4/8 process is selected, List-Mode data collection enabled and automated analyser processing commenced after preliminary sample/antibody incubation at room temperature for two minutes. Total analyser processing time to data acquisition (up to 20k individual events) and availability for file downloading is six minutes. No sample washing is required, and red cell lysis is incorporated by the analyser into the automated procedure.

As this automated analysis collects data from three fluorescent channels, the question arises as to whether or not a three-colour fluorochrome system can be facilitated. In general terms, three antibodies can be used providing the cell population of interest is viable and does not stain with PI in the reagent system. However, it is also important to note that the FL3 detector used by the CD-Sapphire has a functional bandwidth between 615 and 648 nm and is thus unsuitable for FL3 fluorochromes such as R-PE/Cy5 tandem conjugate or PerCP which have emission peaks of 670 nm and 680 nm, respectively. It is, however, possible to use a R-PE/Texas Red tandem conjugate (emission max 615 nm), although care needs to be taken because of its significant spectral overlap with R-PE, or R-PE/Alexa Fluor 610 (emission max 628 nm). The availability of these conjugates for some antibodies may be limited.

c) CD-Sapphire Analysis of Platelet and RBC Antigens

In addition to the supplementary CD3/4/8 processing mode, the CD-Sapphire has a dedicated CD61 immunoplatelet procedure which can also be used for the qualitative demonstration of other Mab-defined platelet antigens. This primary process uses two sequential rack positions where the first is occupied by an EDTA-anticoagulated patient sample tube and the second by a reaction tube containing CD61/FITC antibody conjugate. In contrast to the CD3/4/8 mode, the sample is passed through the CD-Sapphire flow cell without erythrocyte lysis, and hardware thresholds are applied to raw data as it is acquired. This ‘live gating’ process facilitates the collection of events in the optical region associated with platelets and enables up to 50,000 events to be analysed by 7°– IAS, 90°– PSS and FL1. For single colour analyses of platelet antigens with FITC conjugates [Johannessen *et al.*, 2006], the patient sample is replaced by an empty Vacutainer tube and the single reaction vial by a bar-coded non-anticoagulated Vacutainer tube. Into this tube is pipetted 100 µL whole blood and a pre-titrated volume of FITC-conjugated antibody. The CD61 mode is selected, List-Mode data collection enabled, and automated analyser processing started after sample/antibody incubation at room temperature for two minutes. Total analyser processing time to data acquisition and availability for file downloading is less than four minutes.

Compared to leukocyte and platelet antigen methods, the analysis of red cell antigens is less straightforward. This because the automated erythrocyte lysis incorporated by the CD3/4/8 mode will obviously remove the cells of interest, while the gating protocol utilised by the CD61 immunoplatelet method is primarily optimised for collecting platelet rather than red cell events. As both of these automated procedures

are unsuitable for red cell antigen studies, an alternative means of analysis is used. This is based on a modification of the Standard Reference Particle (SRP) mode that is normally utilised for optimising channel settings and instrument calibration. For this, there are two procedures that can be used; SRP-Reticulocyte and SRP-CD4. The SRP-Ret procedure has been applied for the determination of foeto-maternal haemorrhage (Section 8e) and is the method of choice when a high number of events (50k) is required and when the analysis can be undertaken with a single fluorochrome (FITC). When using the SRP-Ret process, a standard 50 µl volume of whole blood is mixed in a plain tube with a pre-determined amount of FITC-conjugated antibody. After mixing and incubation, 50 to 100 µL of diluent is added, the SRP-Ret mode selected and the sample aspirated using the Open Mode process. The SRP-Ret procedure aspirates 18.75 µl of sample which is then mixed with 3.0 mL of Diluent-Sheath and streamed through the optical flow cell illuminated with laser light. During event acquisition, cellular information is stored for 7°– IAS and FL1 fluorescence. There is no RBC lysis and PI is excluded from the reagent system. For comparison, the SRP-CD4 mode is used in a similar way and allows the simultaneous analysis of two optical (0°– ALL and 7°– IAS) and three fluorescent (FL1, FL2 and FL3) channels although fewer events (20k) are collected. This particular procedure is unsuitable for leukocyte antigen analysis because of a high instrument dilution after sample aspiration but can be used for multi-colour analysis of RBC antigens.

d) CD-Sapphire File Downloading

Analysis of CD-Sapphire raw data requires the sample file to be initially downloaded from the instrument and transferred to a computer with installed cytometry software. The download procedure to DVD+R discs uses a simple instrument command routine as follows:

I With the CD-Sapphire in ‘Ready State’, press F3 to enter the Service and Support Console.

II Selecting ‘FCS’ in the menu launches a window which asks for the sequence numbers of the files for downloading. Irrespective of the operational mode used for the analysis, a sequence number is assigned to all tests and can be used for subsequent file identification.

III Insert a blank DVD+R and click on ‘Copy’ and the selected records will be copied. These steps can be repeated until all required files have been transferred.

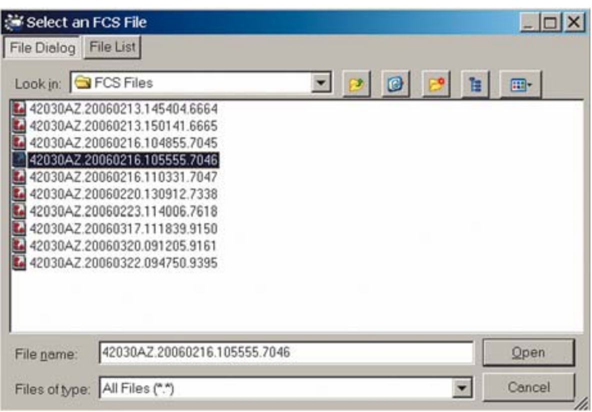
IV When completed, pressing F2 takes the instrument back to its normal operational mode.

e) Data Processing

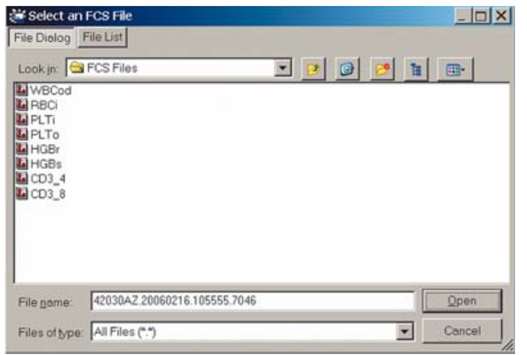
Raw (List-Mode) files downloaded from CD-Sapphire are identified according to the following convention; Instrument number.yyyymmdd.hhmmss.Seq ID (year/month/day.hour/min/sec.Seq ID). These files are actually groupings of individual subfiles representing measurements of different parameters. As these ‘bundled’ files are different from international flow cytometry standard (FCS) structures, analytical processing can only be achieved if they are either separated into individual files prior to software analysis, or specific software is used to enable viewing of individual subfiles. Although there are many cytometry software programmes (e.g. CellQuest, WinList, WinMDI, Summit and EXPO32), none of these are able to open CD-Sapphire files directly. While it is possible to create initial data conversion routines to create individual subfiles for subsequent processing, these are not formally validated and should not be used. Currently, the only software that can directly open individual CD-Sapphire files is FCS Express Version 3 (De Novo Software, Thornhill, Ontario, Canada). This is a fully comprehensive software package that provides multiple plot options and post-acquisition compensation routines that are well-suited for CD-Sapphire file analysis. All graphical examples of immunofluorescent plots shown in this monograph were obtained using FCS Express.

With the CD61, CD3/4/8 and SRP processing modes, the instrument files associated with Mab reagent tubes are clearly identifiable (Figure 6). Processing of individual files is achieved in a step-wise process that allows user-selectable configurations of plot type and desired parameters. Once these have been chosen and the appropriate plot displayed, it is only necessary to decide if compensation is required and how the positioning of population regions (gates) should be applied. With regards to fluorescence compensation, some general principles can be used. For example, compensation is not necessary for single colour measurements and is only rarely required when the analysis is designed to detect discrete cell populations. Fluorescent compensation may, however, be appropriate when samples are (a) analysed for two markers co-

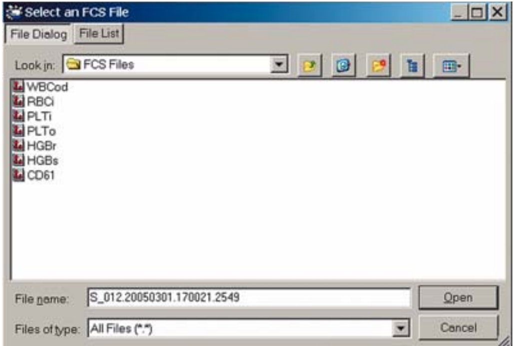
expressed by single cells, if (b) CD-Sapphire WBC antigen analysis indicates high WBC non-viability, or (c) when a multi-colour analysis is designed for antigen quantitation. In considering the approach to population region setting, there are two main types of leukocyte antigen expression; lineage-specific and differentiation-associated. Lineage-specific markers, which by definition characterise distinct cell populations, are usually expressed with moderate to high density and software discrimination of these is not usually problematic. In contrast, differentiation markers are associated with cell populations that show significant differences in staining at different levels of maturation (i.e. transitional expression). A good example of a differentiation antigen is HLA-DR (Ia) which is expressed by immature granulocytes but is lost with maturation to neutrophils. There are no specific guidelines for region setting of differentiation antigens as they typically require the arbitrary setting of a single discriminator between negative and positive. The only requirements are that the discriminator should be logical, consistent and informative.



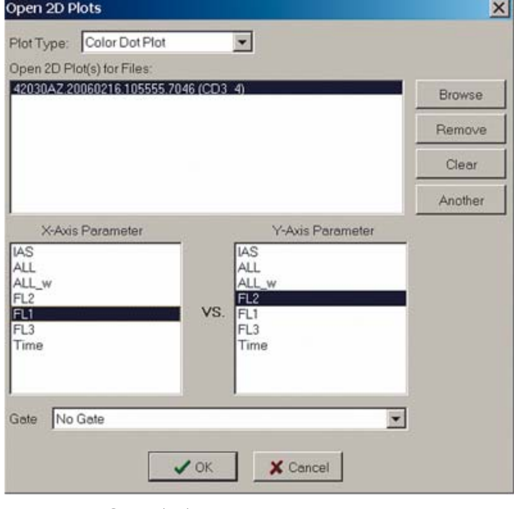
CD-Sapphire File Identification



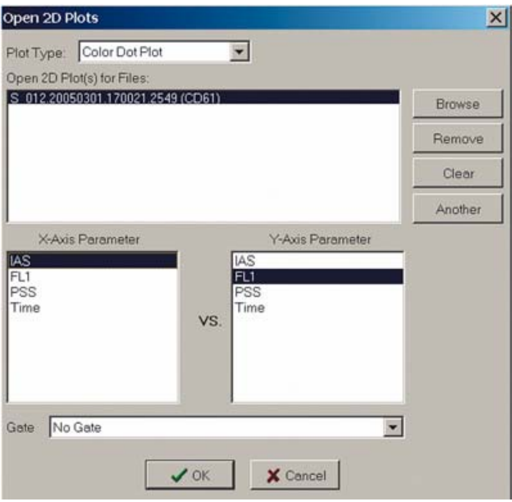
CD3/4/8 Mode Subfiles



CD61 Mode Subfiles



CD3/4/8 Mode Parameters



CD61 Mode Parameters

Figure 6: Sequential FCS Express software dialogs when opening CD-Sapphire raw data files for samples processed with the automated CD3/4/8 and CD61 processing modes.

8 Evaluations of CD-Sapphire Extended Applications

a) Lymphocyte Population Analysis in Routine Haematology

Changes in leukocyte counts and population distributions provide important insights into a wide range of disorders although morphological assessments of stained blood smears may not always allow definitive conclusions to be made without further follow up testing. Most haematology laboratories process patient samples with morphologically atypical lymphoid cells and/or significant numerical changes in the lymphocyte count (lymphopenia or lymphocytosis). In such situations, the primary question for the laboratory haematologist is whether the observed lymphocyte abnormality is reactive or malignant. Any decision regarding the need for further investigations will clearly be influenced by clinical details (e.g. symptoms and patient age), but the immediate availability of extended supplementary analysis with the CD-Sapphire could be useful in some situations.

In a preliminary feasibility study [Molero *et al.*, 2005] (Appendix Abstract 1), Cell-Dyn fluorescence technology was shown to provide T, B and NK-cell counts that were equivalent to those obtained by flow cytometry. To further explore the supplementary utility of lymphocyte population analysis in a routine haematology setting, a second study [Molero *et al.*, 2006] systematically examined over a four-month period a series of samples from patients with lymphocytosis of unknown aetiology. In practical terms, preliminary sample 'screening' comprised a single tube containing 100 µL of EDTA-anticoagulated whole blood plus aliquots of three different Mab reagents (CD3/FITC, CD19/R-PE and HLA-DR(Ia)/R-PE). This single analysis was designed to provide an initial indication of the primary abnormality (i.e. T- or B-cell lymphocytosis) and a supplementary assessment of whether or not the T-cells were activated (HLA-DR^{+}). For samples with non-activated T-cell lymphocytosis and normal B-cell numbers, secondary procedures comprising (a) CD4/FITC plus CD8/R-PE, and (b) CD16/FITC plus CD56/R-PE were carried out. Software analysis of downloaded CD-Sapphire files with FCS Express further confirmed the potential utility of automated analytical algorithms in batch processes, and an ability to categorise samples into a number of diagnostic groups (Appendix Abstract 3). The differentiation of reactive and potentially malignant disorders was an important focus of this study, and the results substantiated the view that preliminary analysis of EDTA-anticoagulated blood sample lymphocyte subpopulations with restricted reagent panels (Figure 7) could considerably advance the capability of haematology laboratories. In particular, CD-Sapphire Mab information could aid the immediate interpretation of lymphocytosis and allow the identification of patients for more detailed immunophenotypic analysis. Implementation of such a strategy would also increase laboratory efficiency by creating a synergistic link between haematology and flow cytometry.

b) Analysis of Myeloid Antigens for the Assessment of Patient Sepsis

The routine full blood count (FBC) has traditionally been used as part of the clinical assessment of infection and sepsis. Haematological parameters of particular interest include neutrophil leucocytosis, neutrophil toxic changes (e.g. hypergranulation and Dohle bodies), and increased proportions of non-segmented band neutrophils or immature granulocytes. The sensitivity and specificity of these indices are, however, limited in young children and elderly patients [Crocker *et al.*, 1985; Shapiro *et al.*, 1987], and are largely uninformative when patients have disturbances in neutrophil counts associated with haematological malignancies or myelosuppressive therapies. It is also recognised that the morphological recognition of band cells is problematic, both for comparisons between observers and for the consistency and reproducibility of individual observers. Because of these limitations, clinicians commonly request additional diagnostic tests such as C-Reactive Protein (CRP) and Procalcitonin (PCT). CRP is, however, of little use in detecting infection/sepsis in patients with other disease processes. Other tests such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-6 (IL-6) and IL-10 have also been used to indicate disease severity but they are less widely available.

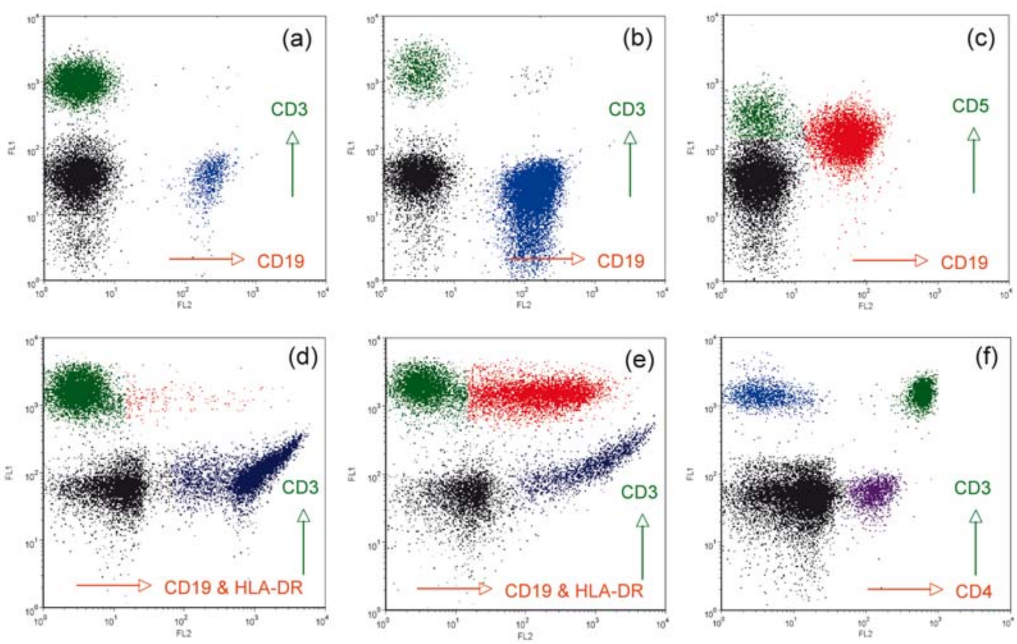


Figure 7: Representative Mab staining combinations used for lymphocyte subpopulation analysis. Plots (a) and (b) show the analysis of T-cell (green) and B-cell (blue) fractions stained with CD3/FITC and CD19/R-PE; the first sample is relatively normal while the second corresponds to a B-cell proliferative disorder. Plot (c) combines CD5/FITC and CD19/R-PE staining for the demonstration of abnormal CD5/CD19 co-expression (red) in a case of B-CLL. Plots (d) and (e) use a mixture of CD3/FITC, CD19/R-PE and HLA-DR/R-PE [Molero *et al.*, 2006] to define T-cells (green), activated T-cells (red) and B-cells/monocytes (blue); the second example differs from the first in having a high proportion of CD3+ T-cells with HLA-DR co-expression. Plot (f) shows the combined staining of CD3/FITC and CD4/R-PE. In this example, separation between T-Helper cells (green), presumptive T-Suppressor cells (blue) and monocytes (purple) is seen.

There are many reports regarding the measurement of neutrophil membrane CD64 (PMN-CD64) for the diagnostic assessment of sepsis [Guyre *et al*., 1990; Davis *et al*., 1995; Hirsh *et al*., 2001; Naccasha *et al*., 2001; Layseca-Espinosa *et al*., 2002; Ng *et al*., 2004]. The membrane molecule defined by monoclonal antibody CD64 is a high-affinity receptor (FcγRI) found on normal monocytes and only weakly expressed by normal neutrophils. Upregulated expression of PMN-CD64 appears to be a sensitive marker for early-onset clinical infection in newborn children [Fjaertoft *et al*., 1999; Ng *et al*., 2004], and in adults its use has been variously suggested for differentiating systemic infection from active inflammatory disease [Allen *et al*., 2002], monitoring γ-interferon therapy [Davis *et al*., 1995] and as an indicator for initiating or discontinuing antibiotic treatment [Ng *et al*., 2004]. The potential utility of PMN-CD64 quantitation is further substantiated by in vitro observations showing mediated upregulation by lipopolysaccharides [Wagner *et al*., 2003], γ-interferon [Schiff *et al*., 1997] and G-CSF [Kakinoki *et al*., 2004]. PMN-CD64 also has a number of potential practical advantages in that it is expressed at very low levels by normal neutrophils, it is relatively insensitive to sample manipulation, and it does not appear to be aberrantly expressed in myeloproliferative or dysmyelopoietic disorders [Davis *et al*., 1995; Davis, 1996].

To date, PMN-CD64 analysis has been achieved by flow cytometry with fluorochrome-monoclonal antibody conjugates. A commercial kit (Leuko64™ Assay; Trillium Diagnostics LLC, Maine, USA) allowing the quantitation of PMN-CD64 is now available and has been validated for use with the CD-Sapphire. A recently published study also evaluated a combined PMN-CD64 and monocyte HLA-DR method using individual Mab reagents with the CD-Sapphire [van der Meer *et al.,* 2006] (Appendix Abstract 4). The HLA-DR marker was incorporated because it aids the separation of monocytes and granulocytes in the software gating process and, in contrast to CD64, its expression by monocytes is decreased in patients with sepsis and transient immunosuppression [Muller-Kobold *et al.,* 2000; Le Tulzo *et al.,* 2004]. The method

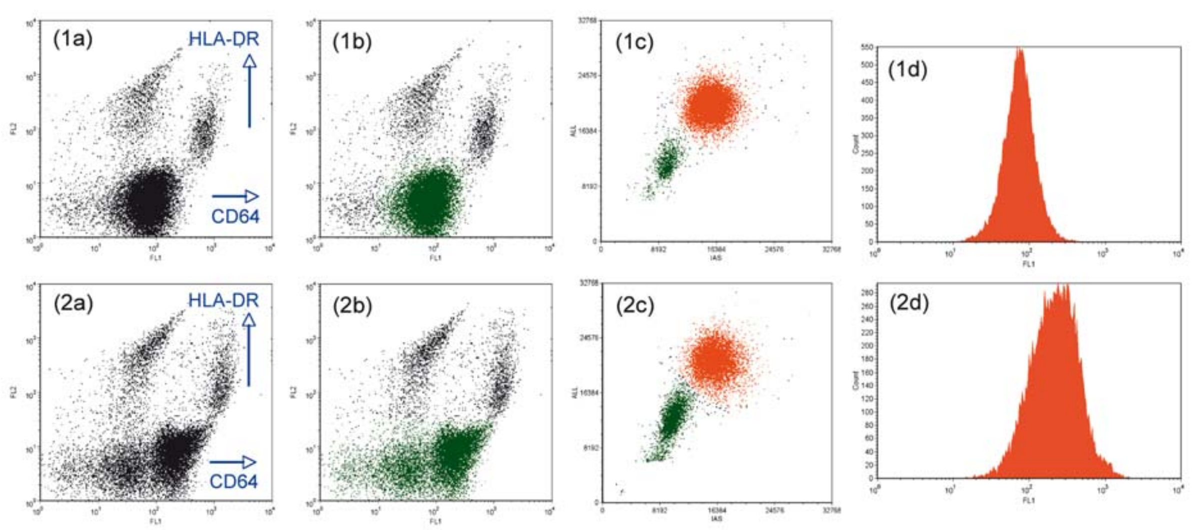


Figure 8: Semi-quantitative determination of neutrophil membrane CD64 (PMN-CD64) expression as part of a two-colour (CD64/FITC and HLA-DR/R-PE) analysis of myeloid cell antigens [van der Meer *et al.,* 2006]. Samples 1 and 2 illustrate the gating procedure of patients with different levels of PMN-CD64. Plots (a) show the FL1/FL2 profiles of the CD-Sapphire raw data files and plots (b) show the preliminary region setting (gating) for all HLA-DR negative events (green). Display of these HLA-DR negative events in the CD-Sapphire 7°–IAS versus 0°–ALL optical plot allowed the setting of a second gate for the neutrophil cluster. This step essentially removed all remaining lymphocytes and 'debris' from the analysis. The final stage was to show the gated neutrophil population in plots (c) as histograms (d) for the determination of PMN-CD64 expression as Arbitrary Fluorescent Units (AFU). Using this procedure, the two samples showed PMN-CD64 levels of 75 (normal) and 221 (increased) AFU.

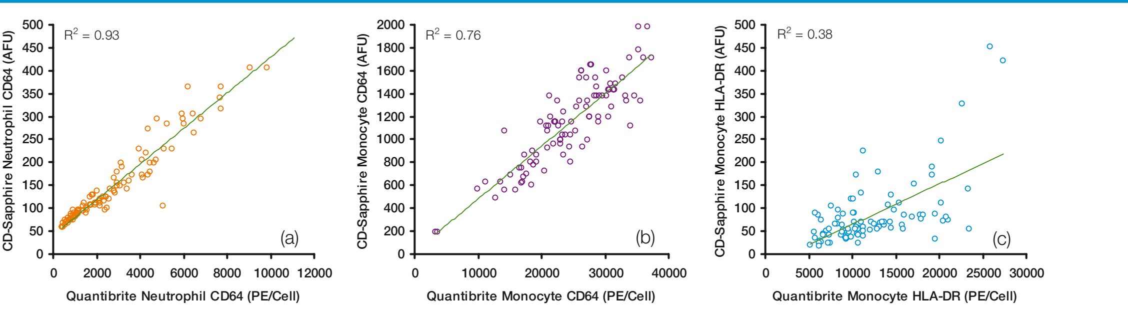


Figure 9: Comparative quantitative determinations of neutrophil CD64 (plot a, n=104), monocyte CD64 (plot b, n=93) and monocyte HLA-DR (plot c, n=93) expression obtained with the CD-Sapphire (Arbitrary Fluorescent Units, AFU) compared to flow cytometry reference methods (Becton Dickinson Quantibrite, PE molecules/cell). The plots show individual data points, the linear regression line (green) and the Coefficient of Determination (R^2).

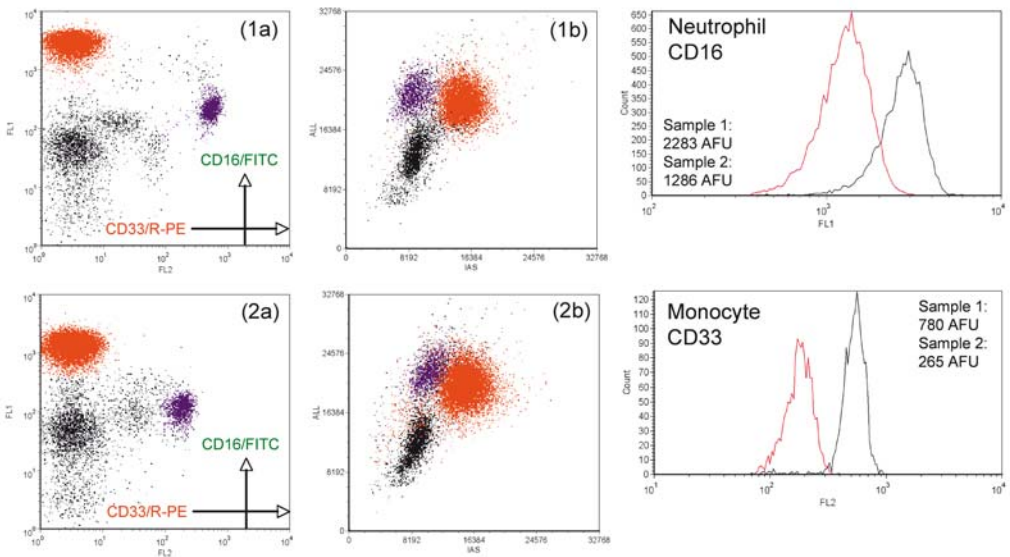


Figure 10: Combined CD-Sapphire analysis of neutrophil CD16 and monocyte CD33 expression. Initial compensated FL1 (CD16/FITC) versus FL2 (CD33/R-PE) plots for the two illustrated samples (1a and 2a) show the primary region setting of the neutrophil (orange) and monocyte (purple) clusters. Plots 1b and 2b confirm the locations of the two gated populations in the MAPSS 7°–IAS versus 0°–ALL optical plot, and the two histograms show fluorescent overlays and quantitative results for neutrophil CD16 (upper) and monocyte CD33 (lower) staining.

essentially involved the staining of whole blood with CD64/FITC and HLA-DR/R-PE antibodies, automated instrument processing with the CD3/4/8 mode and subsequent software analysis to determine the antigen staining levels specifically for the neutrophil and monocyte fractions (Figure 8).

A further evaluation undertaken in Aachen, Germany confirmed good agreement between an in-house procedure using a combination of liquid reagents (CD64/FITC and HLA-DR/R-PE) versus the Becton Dickinson Quantibrite assay for neutrophil ($R^2 = 0.93$) and monocyte CD64 ($R^2 = 0.76$) expression (Figure 9). Lower agreement ($R^2 = 0.38$) was seen for monocyte HLA-DR expression although the quantitative trends were similar. These ongoing studies in Aachen are also exploring the relative utilities of other myeloid antigens (CD16 and CD33; Figure 10) as well as determining the relationships between immunophenotypic markers and biochemical parameters (CRP, PCT, IL-6 and TNF-α) in patients with sepsis.

c) Analysis of Lymphocyte HLA-B27 Status

The human leukocyte B27 antigen (HLA-B27) is strongly associated with ankylosing spondylitis, a chronic systemic inflammatory disease that primarily affects the axial skeleton and the sacroiliac joints. The prevalence of ankylosing spondylitis tends to vary with the population frequency of HLA-B27, and its main application as a diagnostic marker is as a 'rule-out' test. Analysis of HLA-B27 was initially undertaken by the microlymphocytotoxicity test [Terasaki *et al.,* 1964], but this has been largely superseded by flow cytometry analysis using fluorochrome-labelled monoclonal HLA-B27 antibodies [Levering *et al.,* 2003].

This evaluation of CD-Sapphire HLA-B27 analysis (Appendix Abstract 5) examined two different commercial reagent systems with methods that essentially replicated those used in flow cytometry. The first incorporated HLA-B27/FITC IgG2b antibody clone FD705 (One Lambda Inc, Canoga Park, California, USA) while the second reagent system comprised a mixture of HLA-B27/FITC (Clone HLA-ABC-m3, IgG2a) and HLA-B7/R-PE (clone BB7.1, IgG1) antibodies (Immunotech, Beckman Coulter Ltd, High Wycombe, UK). The two reaction tubes normally used for the automated CD3/4/8 assay were replaced by non-anticoagulated Vacutainer tubes containing 100 µL whole blood plus 10 µL of the One Lambda reagent (HLA-B27/FITC) or 20 µL of the Immunotech antibody mixture (HLA-B27/FITC plus HLA-B7/R-PE). CD-Sapphire fluorescence files were compensated and a sequential gating procedure applied as shown in Figure 11. With the One Lambda antibody, agreement between the CD-Sapphire and comparative flow cytometry procedures was 100%. For the Immunotech HLA-B27/B7 reagent combination, HLA-B7$^-$ samples showed clear discrimination between low (negative) and high (positive) expression of HLA-B27, but the interpretation of samples with HLA-B7$^+$ lymphocytes was less straightforward because of HLA-B27 reagent cross-reactivity. Consequently, when analysing HLA-B27 with antibodies that have non-specific reactivities, a cut-off (discriminant)

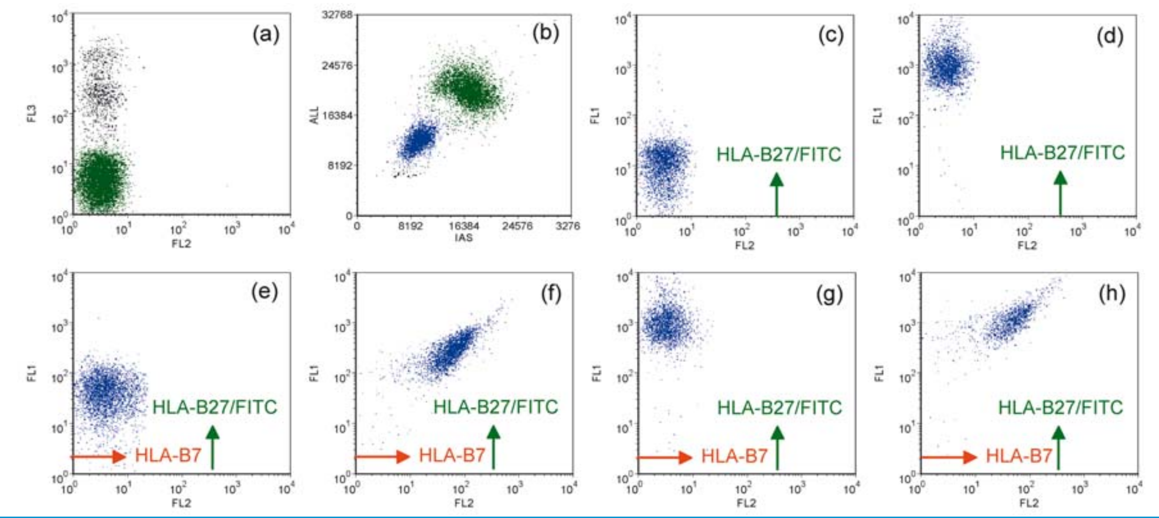


Figure 11: Data processing sequence for the CD-Sapphire determination of lymphocyte HLA-B27 expression. Plot (a) shows the raw data FL2/FL3 file for all leukocytes following fluorescence channel compensation, where the green events correspond to viable leucocytes. Plot (b) shows the viable fraction displayed in a 0°–ALL versus 7°–IAS optical plot, where a second gate is applied to the lymphocyte population (blue events). Plot (c) shows the viable lymphocyte fraction in a FL1 versus FL2 plot which is used for the creation of a histogram for the determination of median HLA-B27 expression. Plots (c) and (d) correspond, respectively, to B27$^-$ and B27$^+$ samples as determined with antibody clone FD705 (One Lambda). Plots (e) to (h) are four different samples analysed with the Immunotech HLA-B27/FITC plus HLA-B7/R-PE antibody combination. These are consistent with B27$^-$B7$^-$, B27$^-$B7$^+$, B27$^+$B7$^-$, and B27$^+$B7$^+$ samples. Note the intermediate level of apparent HLA-B27 staining due to cross-reactivity of this antibody clone (ABC-m3) with the HLA-B7 allele (plot f).

staining level with high specificity should be chosen. Moreover, when using monoclonal antibodies for HLA-B27 analysis, a clear protocol needs to be established which specifically addresses indeterminate staining patterns and defines follow-up procedures.

These studies confirmed that HLA-B27 analysis with the CD-Sapphire was comparable to conventional flow cytometry and easy to carry out even without specific flow cytometry training. The CD-Sapphire HLA-B27 method was straightforward and required little additional technical training for biotechnologists who were familiar with the instrument. HLA-B27 is a diagnostic investigation that could be of interest for smaller laboratories, where the use of a dedicated multiparameter flow cytometer would not be practically or economically feasible.

d) Platelet Antigen Analyses

Platelet Glycoproteins

Although fluorescent measurements are only acquired for one colour (FITC/FL1) with the CD-Sapphire CD61 processing mode, it is still possible to undertake extended platelet analysis for the investigation of patients with suspected platelet glycoprotein deficiencies. Antibody conjugates to CD36 (GpIV or GpIIIb), GpIIb (CD41), GpIX (CD42a), GpIb (CD42b and CD42c), GpV (CD42d) and GpIIb/IIIa (CD61) are commercially available for use in flow cytometry. As part of an initial feasibility study of extended CD-Sapphire fluorescent applications [Johannessen *et al.,* 2006], FITC-conjugated antibodies to CD41, CD42b and CD61 were used with the automated immunoplatelet mode and gave clear discrimination of platelet populations (Figure 12). An illustrative example of how these analyses can be applied is provided by a comparative study of platelet CD41 and CD61 expression in a family with a history of Glanzmann's Disease (Figure 13). By standardising the method of antigen quantitation, it has been suggested that such methods could aid the diagnostic differentiation between normal individuals and heterozygote carriers [Sharp *et al.,* 1998].

Platelet VASP Analysis

Clopidodrel is widely used as an antiplatelet agent in the treatment of ischaemic cerebrovascular, cardiac and peripheral arterial disease. It is also used (often in combination with aspirin) to reduce the risk of coronary stent thrombosis. However, there is considerable evidence that in some patients Clopidodrel is less effective and that platelet function is not sufficiently inhibited. In order to identify this particular patient subgroup, a number of laboratory 'screening' tests such as platelet aggregometry and flow cytometry have been evaluated. Of the prospective flow methods, the quantitative determination of platelet intracellular VASP (Vasodilator Stimulated Phosphoprotein) is considered the most informative predictor for Clopidodrel response [Aleil *et al.,* 2005; Geiger *et al.,* 2005]. A commercial assay developed for measuring VASP (Biocytex, Marseille, France) uses a procedure where the platelets being tested are exposed to either Prostaglandin E1 (PGE1) alone or PGE1 plus ADP. After incubation and fixation, platelets are permeabilised and stained with a two-colour antibody procedure comprising anti-VASP/FITC and anti-CD61/R-PE. The purpose of the CD61 antibody is to facilitate gating of the platelet population and, after correction of the mean level (MFI) of VASP staining (by deducting the FL1 fluorescence obtained with an isotype control), the ratio of staining between the PGE1 and PGE1 plus ADP tubes is determined. The resulting Platelet Reactivity Index (PRI = $MFI_{PGE1} - MFI_{PGE1+ADP}/MFI_{PGE1} * 100$) is considered to be high in bad responders to Clopidodrel and low in good responders. For bad responders, alternative therapeutic strategies to Clopidodrel for the prevention of thrombosis are recommended [Gurbel *et al.,* 2003; Muller *et al.,* 2003].

To establish the feasibility of using a modified VASP assay with the CD-Sapphire immunoplatelet mode, a series of patients who were referred for flow cytometry testing with the Biocytex kit were analysed in parallel using the same method after excluding the CD61/R-PE antibody (because the immunoplatelet mode only measures FL1 fluorochromes) and omitting the RBC lysis step which is not required because of the CD-Sapphire 'live gating' process. The platelet population used for determining MFI values was defined by 7°–IAS and 90°–PSS optical scatter (Figure 14), and the resulting comparisons of patients analysed in parallel by flow cytometry and with the CD-Sapphire revealed good overall agreement between the two procedures (Figure 15).

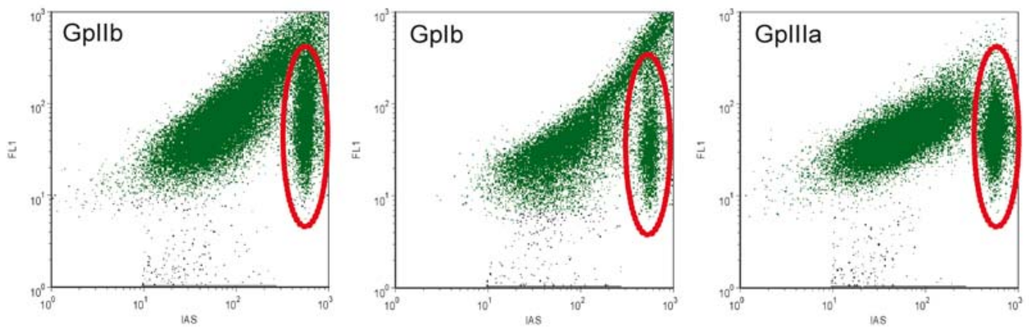


Figure 12: CD-Sapphire analysis of platelet glycoprotein expression. The three plots show FL1 versus 7°– IAS optical scatter profiles for normal platelets stained with antibodies against platelet glycoprotein IIb (CD41), Ib (CD42b) and IIIa (CD61). The green events are antigen-positive platelets, with the green events within the red ellipse corresponding to red cell/platelet coincident events.

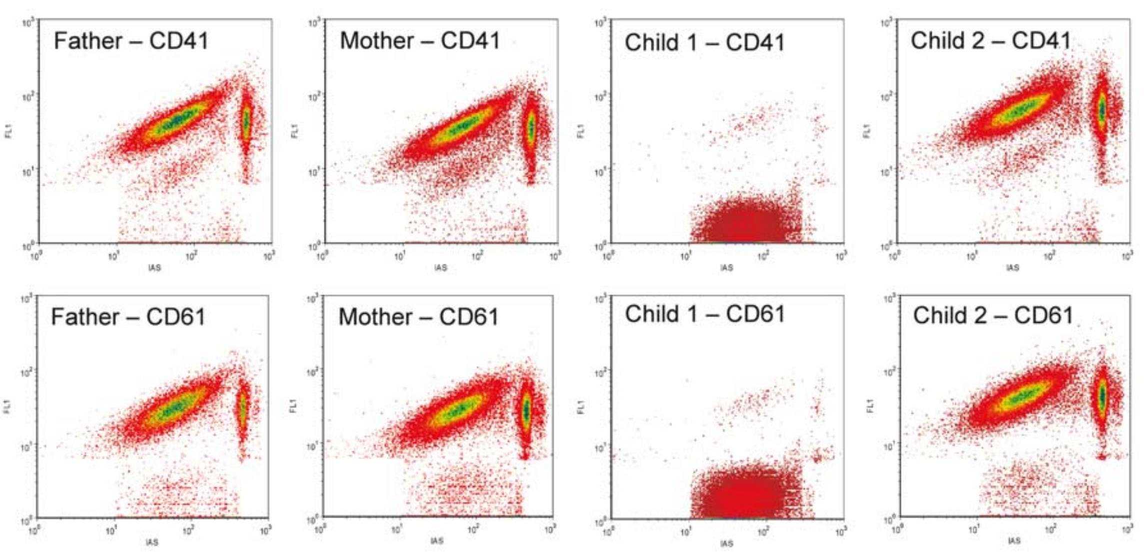


Figure 13: Glanzmann's Thrombasthenia family study of platelet CD41 (GpIIb) and CD61 (GpIIIa) platelet glycoprotein expression determined with the automated immunoplatelet mode. The mother and father were both heterozygotes with CD41:CD61 ratios (after correction for platelet MPV) of 1.09 (95% confidence limits determined for 25 normal subjects = 0.993 to 1.009). Their first child was an affected homozygote with virtually absent platelet CD41 and CD61 expression. The second child was not homozygotic but the CD41:CD61 ratio was again abnormally increased (1.11) suggesting the possibility of heterozygote status. Density plots were produced with FCS Express for FITC/FL1 versus IAS (7° optical scatter).

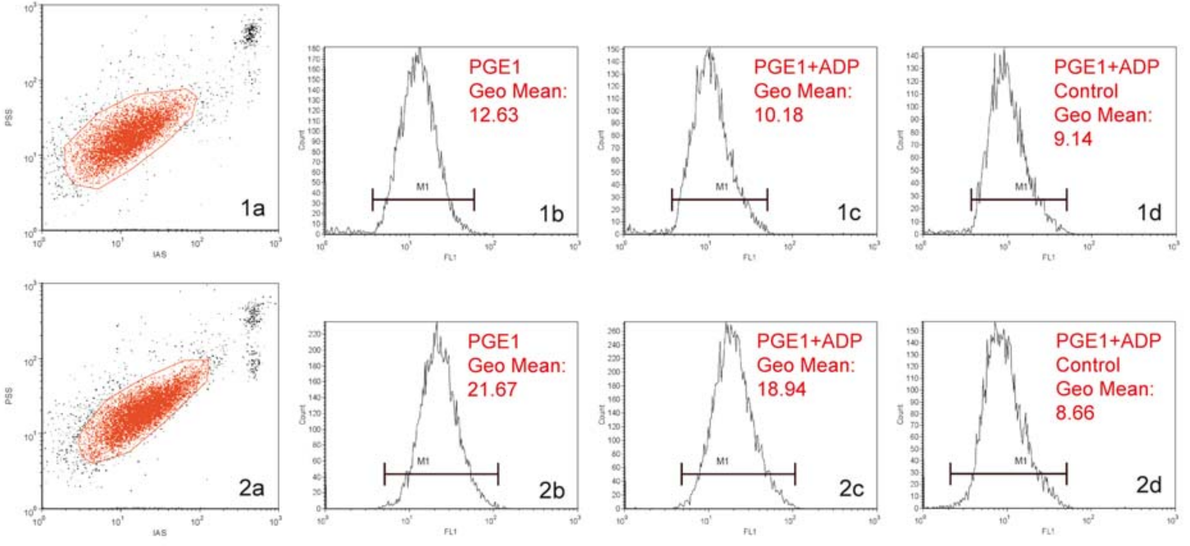


Figure 14: Platelet VASP measurement (Biocytex kit) with the CD-Sapphire. Plots 1a and 2a show the primary gating of platelet populations for two different samples using 7°– IAS versus 90°– PSS optical scatter. Plots (b) and (c) show the fluorescent histograms of antibody staining after sample incubation with PGE1 or PGE1 plus ADP, and plot (d) shows the control antibody staining. The Platelet Reactivity Index (PRI) of these patients were 70.2% (1) and 21.0% (2) and are, respectively, suggestive of poor and good responders to Clopidodrel therapy.

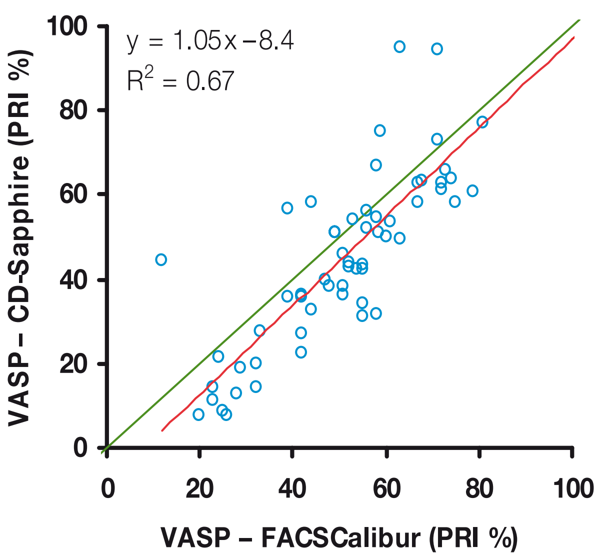


Figure 15: Agreement between VASP Platelet Reactivity Index (PRI) determined with a flow cytometry reference procedure (Biocytex Assay kit) and the CD-Sapphire. The green line represents the line of equality while the red line corresponds to the linear regression equation. The Platelet Reactivity Index (PRI) is considered to be high in bad responders to Clopidodrel and low in good responders.

e) Detection of Red Cell Rhesus D Antigen for Assessment of FMH

When a foetal RBC leak or foeto-maternal haemorrhage (FMH) occurs in Rhesus-D negative women carrying an RhD^+ foetus, there is the potential for alloimmunisation and subsequent Haemolytic Disease of the Newborn (HDNB). To minimise these clinical risks it is necessary to mediate the therapeutic clearance of RhD^+ foetal erythrocytes from the maternal circulation within 72 hours of delivery through the administration of a single dose of anti-D gamma globulin. Quantitation of FMH is necessary to confirm that this is sufficient, and to identify mothers where additional gamma globulin may be required if the volume of FMH is unusually high. The presence (qualitative) and extent (quantitative) of RhD^+ FMH is widely assessed with the Kleihauer-Betke acid elution test (KBT) but it is limited by its laborious nature, high observer subjectivity and unacceptable inter-institutional variations exceeding 500% [Mollison *et al.,* 1993; Duckett & Constantine, 1997; Duckett & Constantine, 1997; Raafat *et al.,* 1997]. Flow cytometric methods using anti-RhD or anti-HbF (gamma chain) antibodies represent a major improvement on KBT procedures [Garratty & Arndt 1995; Nance *et al.,* 1989; Patton *et al.,* 1990; Lloyd-Evans *et al.,* 1996] but are of limited use in routine haematology laboratories because of the need for a dedicated flow cytometer and associated technical expertise.

During the pre-launch phase of the CD-Sapphire, a study was initiated in Scotland to examine the potential use of the Cell-Dyn SRP-Ret mode (Section 7c) for single colour (FITC) fluorescence of red cell antigens (Figure 16). The evaluation was stimulated by the well-recognised technical limitations and poor performance characteristics of the KBT procedure and the clear advantages of flow cytometry. The method design was inherently simple and involved the quantitation of RhD-positive red cells stained with a FITC-conjugated monoclonal anti-D (BRAD-3; International Blood Group Reference Laboratory, Bristol, UK). Constructed standards and comparisons with the KBT method were used to determine the performance of the Cell-Dyn fluorescence method for measurement of FMH, and the results were subsequently published [Little *et al.,* 2005] (Appendix Abstract 6). Interestingly, in this preliminary feasibility study the instrument FCS file sizes were restricted to 50k individual events. However, since then a modification of the FCS Express software has been introduced which allows the merging of multiple aspirations into a single file. If the blood and dilution volumes in Section 7c are used, then it is possible to sequentially aspirate up to five aliquots, create a single analytical file for up to 250k events and further improve the statistical accuracy of low FMH measurements.

f) Monoclonal Antibody Analysis of Cerebrospinal Fluid (CSF)

Most of the previously discussed areas of extended CD-Sapphire analysis in this monograph have direct associations with routine haematology. However, it is also possible to consider applications where the role of the haematology laboratory is less obvious. One such example is the analysis of body fluids where automated analysers are widely used to obtain cell counts. While these numerical estimates have some utility, it

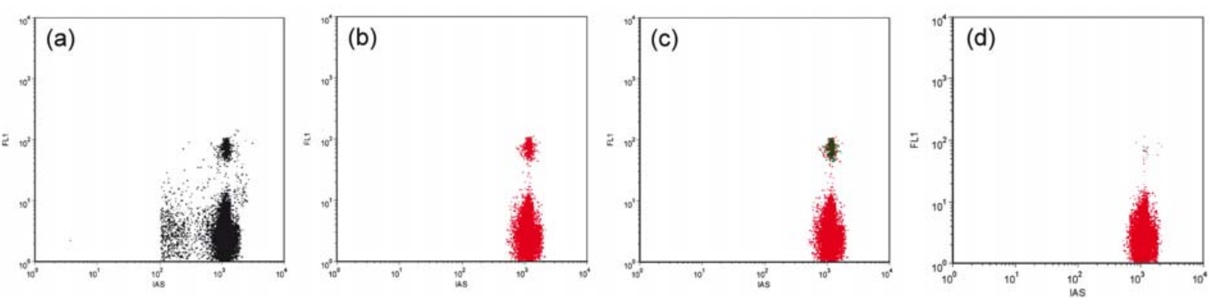


Figure 16: Representative plots of the gating procedure used for the quantitation of Rhesus-D positive red blood cells with BRAD-3/FITC monoclonal antibody. In the CD-Sapphire SRP-Ret mode used for this analysis, 7°– IAS optical scatter and FL1 fluorescence measurements are recorded. Plot (a) shows all collected events from a stained sample containing approximately 1% RhD+ red cells according to IAS versus FL1. A gate setting for the region associated with red cells is applied and when these gated events are displayed (b), two clear populations corresponding to RhD– and RhD+ cells are seen. Following gating of the positive population (c), numerical estimates of the relative population frequencies can be determined. Plot (d) shows a profile for a negative control sample for comparison.

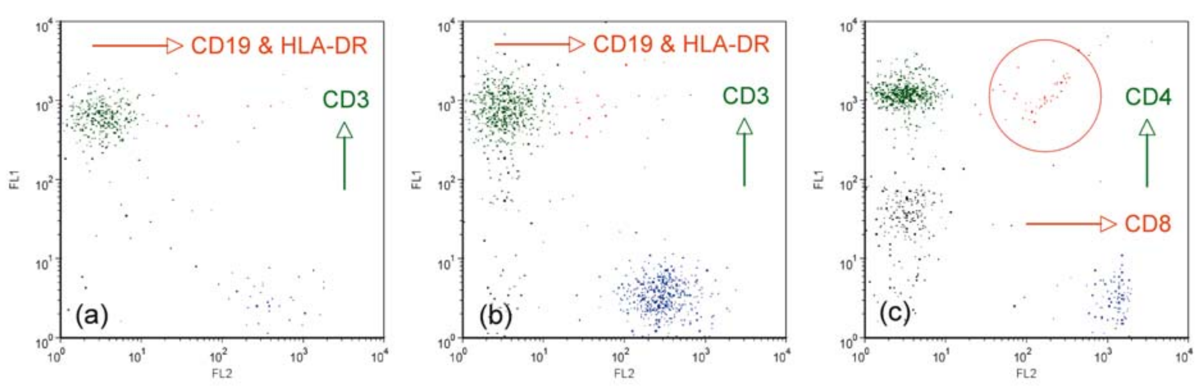


Figure 17: Representative Mab analyses of CSF cells. Plots (a) and (b) show the same combinations of reagents (CD3/FL1 and CD19/R-PE plus HLA-DR/R-PE). Both show the presence of distinct CD3+ T-cell fractions (green events) but plot (b) differs in that there is a more predominant CD19/HLA-DR+ fraction which, when taken into account with cytological examination, corresponds to B-cells. Plot (c) shows a CD4/FITC versus CD8/R-PE analysis. The CD4+ T-helper cell and CD8+ T-Suppressor cell components are seen as discrete green and blue-coded populations respectively but in addition there is a third CD4+CD8+ population. These almost certainly correspond to degenerate cellular particles with non-specific staining.

is probably an understanding of the cellular components that are of greater diagnostic importance [Johnson *et al.,* 1987; Rathmell *et al.,* 1988]. For example, cytological preparations of CSF are a mandatory requirement for the differential diagnosis of diseases that impair the central and peripheral nervous system. Routine investigatory protocols therefore incorporate both classical and specialised staining procedures to assist the recognition of cellular components. This is relatively straightforward when the disease process is characterised by increased neutrophilic cells but may be more problematic when mononuclear cells are present. Cytochemical preparations can aid the identification of monocytes/macrophages but lymphoid cells are essentially uninformative unless plasmacytic forms are present. An ability to determine the lineage of lymphoid cells would be potentially useful for the differentiation of various CNS diseases and the feasibility of Mab analysis has been demonstrated in a number of reports [French *et al.,* 2000; Okuda *et al.,* 2005; Nuckel *et al.,* 2006] that have focussed on changes in specific cell populations, the detection of lymphomatous CNS involvement and the assessment of activation markers. The CSF and Neuroimmunology Laboratory of Homolka Hospital, Prague examines a large number of CSF samples and is often faced with the need to diagnostically differentiate disorders such as neuroborreliosis (Lyme Disease), viral encephalitis, multiple sclerosis, and malignancy. In order to further evaluate the application of extended cellular investigations, a study exploring the utility of a restricted antibody panel is currently in progress with the aim being to devise Mab methods for CSF analysis. Initial methodological approaches are based on the CD-Sapphire CD3/4/8 procedure and although CSF cell numbers are often low, it has been possible to obtain some insights into cellular patterns by using a preliminary concentration step followed by Mab staining (Figure 17). Although these studies are ongoing, the initial indications are promising and it is hoped that further studies will define consistent patterns of change in defined neurological conditions (disease related patterns).

9 Concluding Comments

The CD-Sapphire is a haematology analyser with a unique capability for extended fluorescent analysis using methods that are analogous to conventional flow cytometry. EDTA-anticoagulated blood samples are pre-incubated with FITC/R-PE-labelled monoclonal antibodies, analysed in a laser-illuminated optical system with fluorescent detectors, and the resulting raw-file information processed with cytometry software to determine the nature of specific cell populations. Compared to flow cytometry, CD-Sapphire analysis is technically simpler in that instrument calibration and gain setting are continuously maintained for the purposes of routine blood count analysis. Processing of pre-stained samples, subsequent data acquisition and red cell lysis are also part of the automated procedures. There is no need for sample washing, and the method has the additional advantage of simultaneous leukocyte viability measurements which can be used to minimise potential inconsistencies of antigen measurements associated with leukocyte population deterioration. For these reasons, the implementation of basic and well-established immunofluorescent procedures into routine haematology practice would appear to be quite feasible.

The contents of this monograph have provided a focus on areas where the potential of fluorescent cellular measurements could be of benefit to routine laboratories. Some of the included applications may be of less immediate relevance to routine patient investigations but they do serve to demonstrate the range of CD-Sapphire analytical options. In providing these examples, it is important to emphasise that method development requires an understanding of fluorescence measurement principles and the patterns of antigen expression by normal and abnormal cells. It is also necessary to acknowledge that while the CD-Sapphire processing configurations can be utilised for many basic applications, more sophisticated multicolour diagnostic methods and research procedures are better suited to dedicated flow cytometers.

National and international regulations governing the use of clinical laboratory instruments such as haematology analysers do not permit the direct processing and reporting of non-approved parameters or applications. There is, however, no specific restriction on using instrument mechanisms to acquire raw data for subsequent ‘off-line’ software analysis providing there is no change to (or impact on) the instrument’s approved functions. As the procedures described in this monograph do not involve changes to instrument settings, this important requirement is essentially met. Additionally, however, it is important to stress that extended Mab procedures require the implementation of appropriate local safeguards to ensure competency, adequate training and adherence to defined operational guidelines. Extended processing of monoclonal antibody applications with the CD-Sapphire is neither validated nor approved by Abbott Laboratories and, as with most flow cytometry immunophenotyping protocols, the interpretation and communication of analytical outcomes (which may or may not have clinical implications) are subject to adherence to good laboratory practice. As an additional and specific caveat, while the screening of samples for possible malignancy can be simply achieved with the CD-Sapphire and could be useful if there is no immediate access to a flow cytometer, the authors of this monograph wish to emphasise that the definitive diagnosis of leukaemia should only be reached following appropriate and comprehensive investigation by laboratories with appropriate experience in phenotypic interpretation.

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11 Appendix

Abstract 1

Molero TL, Roemer B, Del Mar Perera Alvarez M, Lemes A, De La Iglesia Inigo S, Palacios G, & Scott CS. (2005) Analysis and enumeration of T cells, B cells and NK cells using the monoclonal antibody fluorescence capability of a routine haematology analyser (Cell-Dyn CD4000). Clinical and Laboratory Haematology **27**, 224.

This communication details a method for the quantitative and qualitative analysis of blood T-, B- and NK-cell populations using the Abbott Cell-Dyn CD4000 haematology analyser. A series of 66 ethylenediaminetetraacetic acid (EDTA)-anticoagulated samples with lymphocyte counts between 0.2 and 33.3 x 10^9/l were selected and analysed with CD3, CD19, Ia and CD56 monoclonal reagents. The flow cytometry reference method utilized a lymphocyte gate defined by optical scatter, with phenotypic analyses referencing to this gate and the absolute lymphocyte count. The CD4000 method analysed all leukocyte events, set primary gates for specific immunophenotypic fractions, and then determined population counts by reference to the white blood cell (WBC) count. Comparisons of $CD3^+$ T-cell and $CD19^+$ B-cell numbers showed high coefficients of correlation ($R^2 > 0.95$) and agreement ($y = 1.01x$) between the CD4000 and flow cytometry reference methods. Lower coefficients of correlation were obtained for $CD3^-CD56^+$ (R2 = 0.52) and $CD3^+CD56^+$ (R2 = 0.83) components. No major discrepancies were observed, and the CD4000 procedures additionally provided qualitative insights into the possibility of T-cell activation. The potential to undertake immediate analysis of EDTA-anticoagulated blood samples to determine the nature of abnormal lymphocyte morphology or numbers represents a considerable advance in the capability of haematology laboratories.

Abstract 2

Johannessen B, Roemer B, Flatmoen L, Just T, Aarsand AK, & Scott CS. (2006) Implementation of monoclonal antibody fluorescence on the Abbott CELL-DYN Sapphire haematology analyser: evaluation of lymphoid, myeloid and platelet markers. Clinical and Laboratory Haematology **28**, 84.

Apart from qualitative flags, that are typically inefficient and uninformative, haematology instruments provide little meaningful information about lymphocyte populations or the lineage of atypical or immature elements, The CELL-DYN Sapphire haematology analyser uses integrated optical and fluorescence (488 nm) measurements, with FL1 (FITC) and FL2 (PE) detectors being configured for fluorescent analysis. As monoclonal antibodies (Mab) are widely used as cellular probes, and are likely to constitute the future basis for immunodifferentials, we explored the feasibility of implementing immunofluorescence on this routine haematology analyser. An extensive series of Mab (CD2, CD3, CD4, CD8, CD11b, CD13, CD14, CD16, CD19, CD22, CD33, CD34, CD41, CD42b, CD45, CD56, CD61, CD64, CD235a and HLA-DR) were tested singly or in FITC/PE combinations. Analyser processing and data acquisition was achieved using CD-Sapphire automated CD61 immunoplatelet or CD3/4/8 assay procedures and, apart from mixing EDTA-blood and antibody, no further sample manipulation was required. Downloaded raw files were processed with cytometry software, and all evaluated reagents showed population discrimination analogous to flow cytometry. Practical procedures were straightforward and required minimal operator training. Extended information that can be obtained from monoclonal antibodies with a routine haematology analyser has the potential to extend haematology laboratory practices and positively impact laboratory and clinical efficiency.

Abstract 3

Molero T, Lemes A, de la Iglesia S, & Scott CS (2006) Monoclonal antibody fluorescence for routine lymphocyte subpopulation analysis with the Abbott CELL-DYN Sapphire haematology analyser, International Journal of Laboratory Hematology (in press).

Using previously described procedures, this study quantified T-cell, T-cell subset, B-cell and NK-cell populations with the CD-Sapphire haematology analyser in a series of patients with mild to moderate lymphocytosis. Lymphocyte counts ranged from 6.0 to 14.9 x 10^9/L, with 86/97 being <10.0 x 10^9/L. Immunophenotyping (CD3/CD19/HLA-DR, CD4/CD8 and CD16/CD56 combinations) was performed using EDTA-anticoagulated blood, automated CD-Sapphire analysis and subsequent software processing. Of 35 samples from younger (<12 years) patients, 22 (63%) had non-specific lymphocyte changes, 4 (11%) showed specific increases in non-reactive T-Helper or T-Suppressor cells, and 5 showed a reactive T-cell lymphocytosis. The remaining 4 were classified as 'Transient/Persistent NK-associated (NKa) Expansion' (n = 3) and specific B-cell lymphocytosis (n = 1). For older patients (n = 59), 15 (25 %) had an increase (>1.5 x 10^9/L) in B-cells, and seven investigated for surface immunoglobulin expression were all found to be clonal. The remaining samples were categorised as 'Transient/Persistent NK-associated (NKa) Expansion' (13/59), Reactive Lymphocytosis (5/59), 'Reactive Lymphocytosis or Transient/Persistent NKa Expansion' (8/59), specific T-Helper cell (n = 8) or T-Suppressor cell (n = 3) lymphocytosis, and 'Lymphocytosis of Undetermined Significance' (n = 7). This study has demonstrated the feasibility of applying limited immunophenotyping protocols to the investigation of patients with abnormal lymphocyte counts in routine haematology. By using commercially purchased liquid monoclonal reagents to determine lymphocyte subpopulation profiles, haematology laboratories can provide more definitive information of potential clinical importance.

Abstract 4

Van der Meer W, van Dun L, Gunnewiek JK, Roemer B, & Scott CS. (2006) Simultaneous determination of membrane CD64 and HLA-DR expression by blood neutrophils and monocytes using the monoclonal antibody fluorescence capability of a routine haematology analyser. Journal of Immunological Methods **311**, 207.

This study reports the design of an immunofluorescent method for the co-determination of neutrophil CD64 (PMN-CD64), monocyte CD64 (MON-CD64) and monocyte HLA-DR (MON-Ia) expression with the Cell-Dyn CD4000 haematology analyser. Normal PMN-CD64, MON-CD64 and MON-Ia expression, defined as the mean ±2SD of 25 healthy adults after correction for isotype control staining, corresponded to 17–67, 515–1045 and 170–670 AFU, respectively. Analytical reproducibility determined by duplicate analysis of 12 random samples revealed good assay consistency for all three analysed antigens, with day to day variation in normal subjects being relatively minor in significance. CD4000 PMN-CD64 and HLA-DR values showed good inter-method correlation with flow cytometry although short term (12 hours) stability studies suggested an in vitro trend for increasing PMN-CD64 and variable HLA-DR antigen expression with progressive storage. Observed ranges of PMN-CD64, MON-CD64 and MON-Ia for 109 randomly-selected clinical samples were 31–1058, 307–2843 and 10–876 AFU. Abnormal PMN-CD64 and MON-CD64 shared the same trend (upregulation) while abnormal monocyte MON-Ia was characterised by declining expression. Normal PMN-CD64 was only seen with normal (45/52) or intermediate (7/52) MON-CD64, while high PMN-CD64 was mostly associated with intermediate (18/22) or high (3/22) MON-CD64. MON-Ia expression was largely independent ($p = 0.04$) of PMN-CD64 although marked decreases in MON-Ia were invariably associated with intermediate or high PMN-CD64. MON-Ia expression was inversely related ($p < 0.0001$) to absolute granulocyte counts, and patients with high PMN-CD64 were more likely (8/25) to have in excess of 10% Band Cells compared to samples with normal/intermediate PMN-CD64 (0/84). When compared to C-Reactive Protein (CRP), high PMN-CD64 and MON-CD64 were always associated with an increased CRP concentration, but minor proportions of samples with normal PMN-CD64 (11/52) or normal MON-CD64 (11/65) could also have an increased CRP. The procedures described in this communication overcome a number of limitations associated with flow cytometry, and co-determination of CD64 and HLA-DR antigen expression may provide complimentary insights into patient heterogeneity in the assessment of suspected sepsis compared to CD64 analysis alone.

Abstract 5

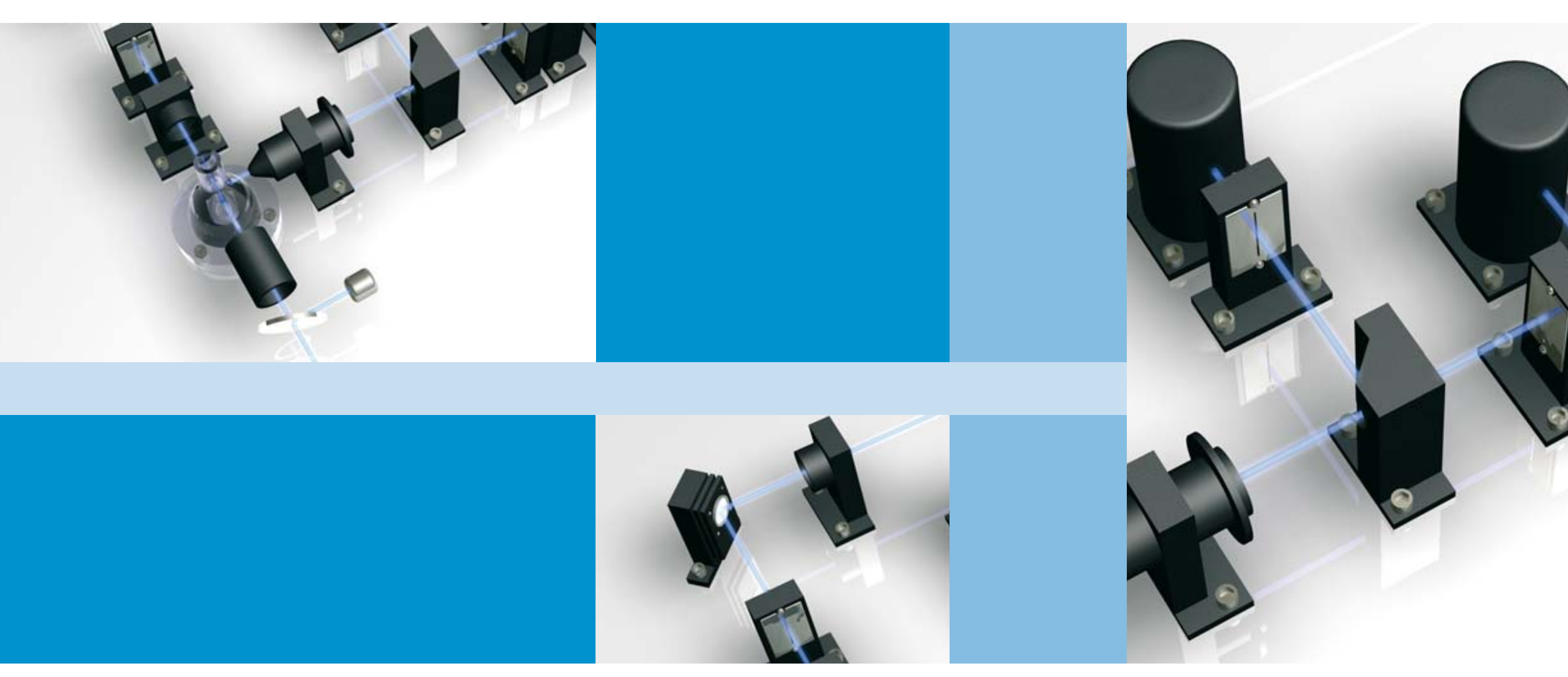
Aarsand AK, Johannessen HB, & Scott CS. (2006) Evaluation of a method for monoclonal antibody HLA-B27 analysis with the Cell-Dyn Sapphire haematology analyzer, International Journal of Laboratory Hematology (in press).

Analysis of HLA-B27 is usually performed by flow cytometry using commercial single or two colour fluorescence reagents. The CELL-DYN (CD) Sapphire is a high-volume routine haematology analyzer that allows cell population analysis by monoclonal antibody fluorochromes analogous to flow cytometry. In this study, in-house flow cytometry analysis (n=96, HLA-B27, One Lambda) performed on routine patient samples was used as the comparison method for analysis of HLA-B27, One Lambda (n=40) and HLA-B27/HLA-B7, Immunotech (n=96) reagents on the CD Sapphire. The One Lambda results agreed 100% with the comparison method and offered clear population discrimination. The Immunotech combination also had a high level of agreement, but interpretation was more complex because of the wider cross-reactivity of the ABC-m3 antibody with B7 and other HLA-B alleles. When analysing HLA-B27 with antibodies showing non-specific reactivity, a cut-off staining level yielding high specificity should be chosen, as the primary diagnostic value of HLA-B27 is as a 'rule-out' test for ankylosing spondylitis. The CD Sapphire incorporates automated sampling and lysis, and medical scientists familiar with the instrument would require little additional technical training to perform the analysis. The reduced pre-analytical work and total turnaround time constitute an important step towards automation of HLA-B27 and similar simple high-volume flow cytometry analysis.

Abstract 6

Little B, Robson R, Roemer B, & Scott CS. (2005) Immunocytometric quantitation of foetomaternal haemorrhage with the Abbott Cell-Dyn CD4000 haematology analyser. Clinical and Laboratory Haematology **27**, 21.

*This study evaluated the extended use of a haematology analyser (Abbott Cell-Dyn CD4000) for the immunofluorescent enumeration of foeto-maternal haemorrhage (FMH) with fluorescein isothiocyanate-labelled monoclonal anti-RhD. Method performance was assessed with artificial FMH standards, and a series of 44 clinical samples. Within run precision was <15% (coefficient of variation, CV) for FMH volumes of 3 mL and above, 18.8% at an FMH volume of 2 mL and 31.7% at an FMH volume of 1 mL. Linearity analysis showed excellent agreement (observed FMH% = 0.98 * expected FMH% + 0.02), and a close relationship (R^2 = 0.99) between observed and expected FMH percentages. The lower limit of quantification of the CD4000 (SRP-Ret) method with a maximum CV of 15% was 1.6 mL, and the limit of detection was <1 mL. Parallel Kleihauer–Betke test (KBT) assessments of FMH standards showed an overall trend for higher KBT values (observed = 1.25 * expected – 0.38). At an FMH level of 4 mL, KBT observer estimates ranged from 0.57 to 11.94 mL with a mean inter-observer CV of 63%. For 44 clinical samples, there was decision point agreement between KBT and SRP-Ret results for 42 samples with an FMH of <2 mL. Analysis in the low FMH range (< 1 mL) showed that small volume foetal leaks could be detected with the SRP-Ret method in most of 23 samples with negative KBT results. CD4000 SRP-Ret method performance for FMH determination was similar to that reported for flow cytometry.*



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KNOW YOUR IMAGE ▶ CELLAVISION® DM96

CellaVision DM96 enables laboratories to analyze blood and body fluids, including cerebro-spinal, synovial and pleural fluids, in a safe, efficient and comfortable way.

Automatic cell location and pre-classification improves resource utilization, the quality of results and employee satisfaction. One particular labor benefit is that highly skilled medical technologists are able to spend more time on difficult cases that require careful analysis and assessment. The ability to archive images enables hospitals to look at previous cell images of patients in case of relapse.

An additional service is the ability to create digital slides.
Although primarily designed for hematology smears, the digitization function is also applicable to other types of samples, for educational purposes.

Break the cycle—find the perfect workflow

The CellaVision® DM96 is a digital morphology system, for performing automatic identification of the various types of white blood cells in peripheral blood and body fluids smears. The system also pre-characterizes parts of the red blood cell morphology and provides functionality for platelet estimation. In addition, functionality for digitizing an entire slide is offered.

Automatic cell-location and suggested classification, along with unique cell views on the PC screen, reduce the time spent performing differentials and make real-time collaboration between colleagues a natural part of the classification process. Time saved may reach 50 percent [1]. Moreover, an ergonomically correct and relaxed working environment is also provided.

Remotely share images via Tele-Hematology

The CellaVision DM96 can share database with other CellaVision DM analyzers, installed at the same or a remote location, allowing centralized database management. By using the CellaVision® Remote Review Software it is possible to transfer digital images and results within and between laboratories. The software allows for strengthened competence, qualified review and shorter turn around times for complicated patient cases. Furthermore, it allows for digital archiving of samples together with patient records, as well as sharing of digital images with experts outside the laboratory.

The CellaVision DM96 will fill one of the remaining missing pieces in the Hematology Laboratory's quest for total automation in routine testing and will endow us with a very useful tool for teaching staff and students in hematological morphology.

Dr. Brian Sheridan, Head, Division of Hematological Pathology, St. Michael's Hospital, Toronto

Advantages of automated image analysis as compared to manual analysis

- Increased productivity—speed and cost-efficiency
- Quality assurance—competence assurance and standardized results
- Ability to track information—digital archiving of samples together with patient journals
- Improved conditions for sample assessment—enhanced communication with other information systems and instruments in the laboratory, giving the user more access to information
- Improved cooperation within and between laboratories—transfer of digital images to experts outside the laboratories
- Improved ergonomics—eyes, neck, and back

[1] *J Clin Pathol. 2006 May 12; : 16698955*
Examination of peripheral blood films using automated microscopy; evaluation of Diffmaster Octavia and CellaVision DM96
Huib Ceelie, Rob D Dinkelaar and Warry van Gelder

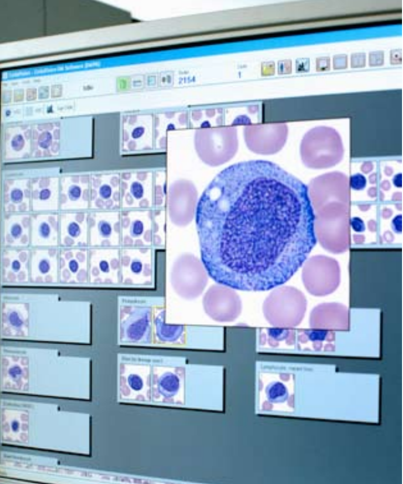
NEW CellaVision® *Body Fluid Application*

Now body fluids and blood smears can be analyzed on the CellaVision DM96 at the same time, either batched in blue or orange magazines or in a random access mode when interfaced to an LIS.

Key features & benefits

- Designed to utilize standard cytocentrifuged slides.
- Pre-classification of seven classes.
- View cell classes side by side or all cells in a full screen view.
  Digital scan of entire sample area:
  - navigation in a digital sample overview
  - available in 10x or 50x magnification
- Regions Of Interest (ROI):
  - Tag interesting areas for Pathology review and for educational purposes
  - Export your ROI into presentations and educational material.
- Add pre-coded or free text comments to any slide, cell class or specific cell.
- Permanently store images —access a patient's image history.

Pending 510(k), not available for sale in the US.

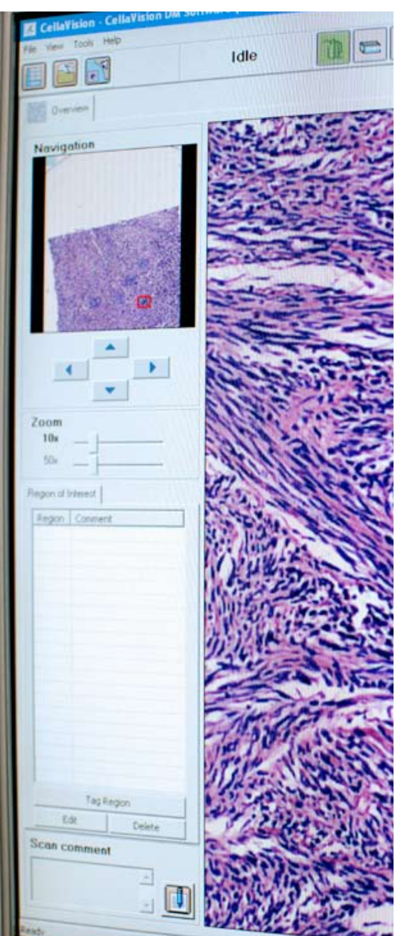


CellaVision® *Peripheral Blood Application*

Enhance blood film review and make more efficient use of experienced morphologists' time. Screening for abnormalities allows fast confirmation of the CBC analyzer's results.

Key features & benefits

- Cells are pre-classified into 18 classes.
- RBC Morphology is automatically pre-characterized for polychromasia, hypochromasia, aniso-, micro-, macrocytosis and poikilocytosis as set by the lab, providing you with percentages of each type.
- Allows confirmation of cell counter results in seconds.
- View cell classes side by side or all cells in a full screen view.
- Complete traceability of results at the individual cell level.
- Access customized reference cell library.
- Add pre-coded or free text comments to any slide, cell class or specific cell.
- Permanently store images —access a patient's image history
- Handling leukopenic samples is more efficient due to the ability to merge cells from multiple slides on a single patient.
- Multiple RBC fields can be scanned as if on a virtual microscope.
- A dynamic micrometer facilitates additional RBC measurements.
- Provides functionality for performing platelet estimates.



NEW FEATURE *Digital Slide Creation*

Digitize desired areas of interesting specimens within hematology, pathology and cytology. Get new possibilities such as networking and annotating.

Key features & benefits

- Enhance, annotate and share digital slides with your students or colleagues.
- Digital scan of desired sample area:
  - navigation in a digital sample overview
  - available in 10x or 50x magnification.
- Regions Of Interest (ROI):
  - Tag interesting areas for review or for educational purposes.
  - Export your ROI into presentations and educational material.
  - Long term storage of regions of interest.

For research and education only

Part of CellaVision® Peripheral Blood Application, software v. 2.0.

Technical Specifications

General

System components
- PC with Windows XP
- Slide Scanning Unit
- CellaVision® DM Software (U.S. Patent No. 6268611 and 6341180)

Electronical Specification *all system components*
- Voltage input: 230 VAC (115 VAC for US)
- Current input: 4A (8A for US)

Size (W x D x H)
- 530 x 600 x 630 mm
- 20,9 x 23,6 x 24,8 inches

Slide handling
- Requires barcode labeled slides with clipped/round corners
- Slides are loaded into magazines with the capacity of 12 slides each.
- Continuous feed, walk away; up to 96 slides in eight magazines

Oil dispensing Automatic

Archiving of results and images
- Supported media: CD-R/CD-RW and LAN

Storage capacity
- Primary storage: On local hard drive 20 GB
- Secondary storage: Unlimited when transferred to external storage media via LAN

Printer support
- Laser/inkjet printers supported by Windows XP

Communications
- Bi-directional LIS support, ASTM
- Ethernet 10/100 Mbps
- E-mail

Applications

Peripheral Blood

Slide methods (wedged)
- Automated slide maker and stainers
- HemaPrep®/MiniPrep® automated blood smearing device
- Manual smears

Stains
Romanowsky stains (May Grünwald/Wright/Giemsa)

Number of cells counted User definable

Quality control
- Built-in QC module for verification of the cell location accuracy.

Throughput
Peripheral Blood
- Up to 35 slides/h for complete differential (100 WBC+RBC+PLT)
- Up to 60 slides/h for RBC and/or PLT only.

Digital Slides
- Up to 30 slides/h for 10x10 mm in 10x
- Up to 2 slides/h for 10x10 mm in 10x + 50x

Slide image size
Peripheral Blood

100 WBCs:	~5 MB
100 WBCs + RBC:	~6 MB
100 WBCs + RBC + PLT:	~6 MB

Digital Slides

10x10 mm in 10x:	~45 MB
10x10 mm in 10x + 50x:	~500 MB

Result parameters
- ***WBC pre-classification:*** Segmented and Band Neutrophils, Eosinophils, Basophils, Lymphocytes, Monocytes, Blast Cells, Promyelocytes, Myelocytes, Metamyelocytes, Variant Lymphocytes, Plasma Cells and Unidentified
- ***Non WBC pre-classification:*** Smudge, Artefacts, Giant Platelets, Platelet Clumps, Erythoblasts (NRBC)
- ***RBC pre-characterization:*** Automated pre-characterization of aniso-, micro- and macrocytosis, polychromasia, hypochromasia and poikilocytosis is performed in an overview image corresponding to eight high power fields (100x)
- ***PLT estimate:*** The graphical user interface allows manual estimation of the PLT concentration, based on eight high power fields (100x)

Body Fluids

Slide preparation methods
- Standard cytocentrifuge preparation
- Default settings for Shandon™/Wescor™/Statspin™

Stains
Romanowsky stains (May Grünwald/Wright/Giemsa)

Number of cells counted User definable

Quality control
- Built-in QC module for verification of the cell location accuracy

Throughput
Based on 6 mm sample area
- Up to 25 slides/h for differential (100 WBCs + 10x)
- Up to 7 slides/h for differential (100 WBCs + 10x + 50x)

Slide image size
Based on 6 mm sample area

100 WBCs:	~5 MB
100 WBCs + 10x:	~10 MB
100 WBCs + 10x + 50x:	~150 MB

Result parameters
- ***WBC pre-classification:*** Neutrophils, Eosinophils, Lymphocytes, Macrophages (including Monocytes), Other (Basophils, Lymphoma cells, Atypical lymphocytes, Blasts and Tumor cells) and Unidentified.
- ***Non WBC pre-classification:*** Smudge cells and Artefacts

Optional Software

CellaVision® Body Fluid Application

CellaVision® Remote Review Software
Recommended PC specifications
- 64 MB graphics RAM with Open GL 1.2 support
- Ethernet adapter 10/100 Mbps • 512 MB RAM
- 100 MB free disk space • CPU Pentium IV

CellaVision® Competency Software

Accessories

- Label printer kit
- Barcode labeled slide magazines
- Immersion oil
- QC barcode labels

MM-033-2008-08-18

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KNOW YOUR IMAGE ▸ CELLAVISION™ DM96

Efficiency in your daily work

Automated Digital Cell Morphology is the process by which cells are automatically located on a stained peripheral blood smear, pre-classified, stored and transmitted for confirmation by a technologist. This technology improves the efficiency and proficiency of the historically subjective differential review.

Additionally, the advances in image analysis and network integration allow the cell image to finally become an integral part of the patient's record.

The CellaVision DM96 is an Automated Digital Cell Morphology System for the location, pre-classification, display, storage and transmission of red and white blood cell images.

Using an Artificial Neural Network (ANN), WBCs are pre-classified and RBC morphology is pre-characterized, grouped, and stored to await technologist confirmation.

ANNs are exceptionally good in pattern recognition and classification due to their ability to generalize and make decisions about imprecise data. The CellaVision DM96 analyzes over 250 specific characteristics for each cell during the classification process.

Quality assurance. CellaVision DM96 allows different technologists to perform differentials at the same high level of consistency. The system provides traceability of results at the individual cell level.

Connectivity. Multiple Remote Review Stations enable supervisory review, inter/intra-lab comparison and the opportunity for real-time collaboration with Pathology.

Efficiency. Automatic cell-location and pre-classification, along with unique cell views, reduce the time spent performing differentials, training new technologists and monitoring proficiency.

Value to clinicians. As a result of network integration, the CellaVision DM96 will provide new value to clinicians, saving them time with remote, real-time access to a patient's image history.

Laboratory benefits

Utilizing NCCLS guidelines in conjunction with a neural network trained on thousands of cells identified by a group of global experts, the CellaVision DM96 offers an opportunity to improve efficiency and consistency.

With continued cost constraints and the shortage of skilled technologists, especially in differential analysis, the CellaVision DM96 permits technologists to use their skills to the best benefit of the laboratory.

Maintain high quality

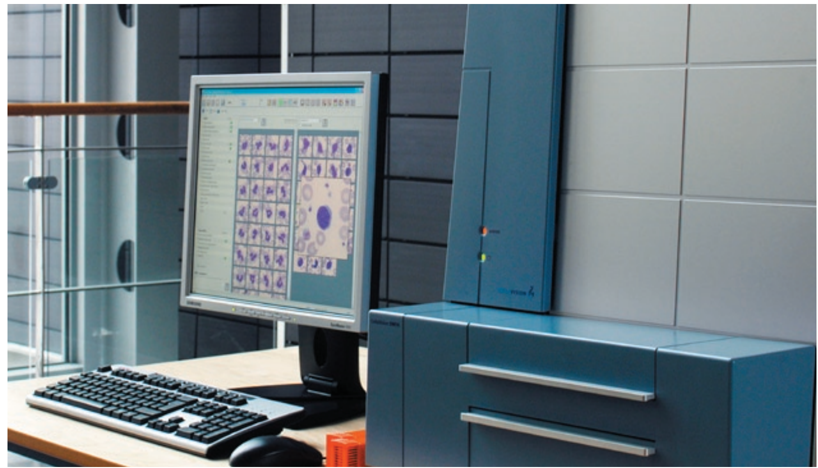
- Use of NCCLS guidelines standardizes performance across all technologists, shifts and locations.
- Viewing cell classes side by side.
- Access to on-board, customized, reference cell library.
- Access to a patient's image history, months or years back in time.
- Complete traceability of results at the individual cell level.
- Standardizes training and proficiency testing.

Improve resource utilization

- Automatic cell location and pre-classification performed at 35-60 slides per hour, including leukopenic samples.
- Allows confirmation of cell counter results in seconds.
- Networking allows centralized review and/or remote operation of multiple locations.
- Improved ergonomics increases employee satisfaction.

Extensive database

- Quick access to results and images for consultations, collaboration and education.
- Trace and compare patient progress over time.
- Permanently store images without degradation over long time periods.
- Unlimited number of results and images can be stored on the LAN.
- Export individual interesting slides to a separate database.
- Ability to search the database by 15 criteria including; Doctor, Technologist and Comments.



Automatic cell location and pre-classification performed at 35-60 slides per hour, including leukopenic samples.

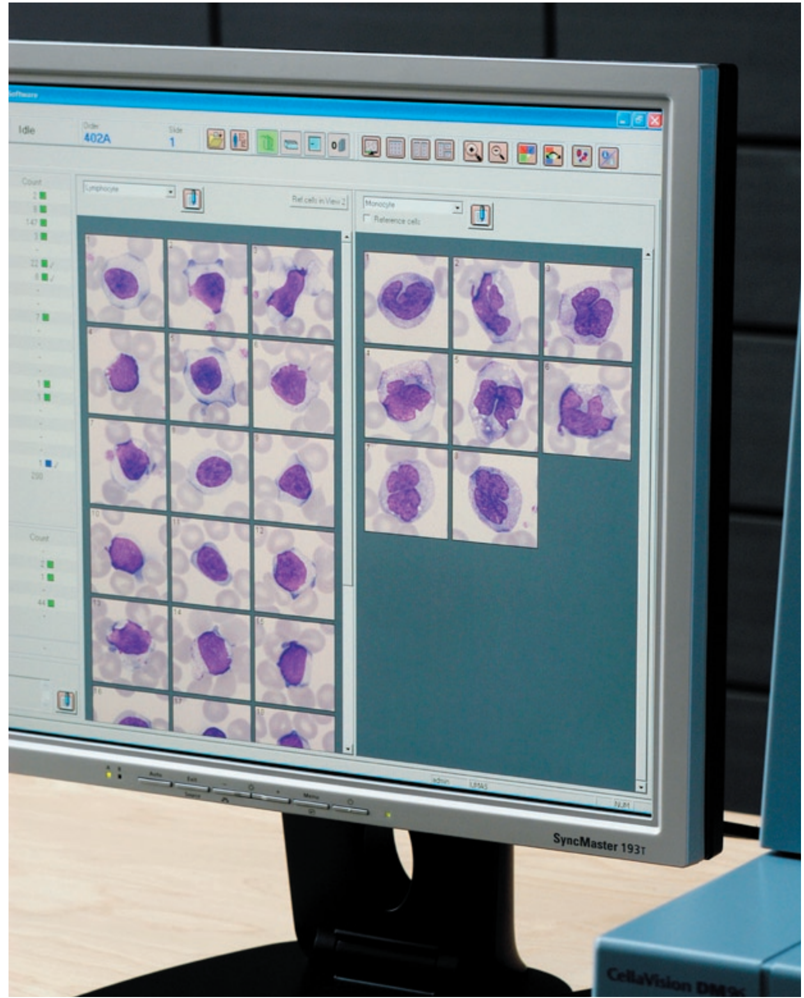
Remotely share images via tele-hematology

- Review images with experts regardless of location.
- Users anywhere on the network can open the same slide simultaneously for collaboration.
- Multiple Remote Review Stations enable supervisory review, inter/intra-lab comparison and the opportunity for real-time collaboration with pathology.

	+	LIS					Order ID	Patient ID	First Name	Last Name	Analyzed	Signed by
							25315665 4	1234232	Pete	Dow	2004-05-18 12:54	demo
	+						25315665 2	12345456	Sahra	Jones	2004-05-18 12:52	admin
							25315665 1	12345678	John	Smith	2004-05-18 12:51	demo
							25315665 3	1236547	Linda	Swanson	2004-05-18 12:53	pt
							25315665 7				2004-05-18 12:56	
							25315665 8				2004-05-18 12:57	

Ability to search the database by 15 different criteria. Order status, such as signed slide, sent to LIS, STAT sample, comments attached is clearly indicated by icons.

Know your image



View cell classes side-by-side or compare one cell class with reference cells of the same cell class.

It is possible to quickly screen for abnormalities, allowing fast confirmation of the CBC analyzer's results. When classifications need to be changed, cells can be dragged and dropped into the appropriate cell class.

The system analyzes between 35-60 slides per hour depending on the tests requested.
Slides are transported into the system and:
10X dry objective finds monolayer and coordinates of nucleated events. Oil is automatically added. 50X objective takes 35 HPF images in order to pre-characterize RBC morphology. 100X objective returns to each WBC, and captures a high quality image. An Artificial Neural Network evaluates 250 cellular characteristics and pre-classifies each cell. Results with images are stored and displayed for the technologist's verification.

Proficiency. The CellaVision DM96 provides the technologist with the ability to perform a-side-by-side comparison within different cell classes, by viewing similar cells together. Additionally, there is an on-board reference cell library for use by the technologist. This library is customized from the individual hospital's own patient population. This process not only streamlines training but it also ensures consistency across technologists and shifts.

Easy access to powerful features: Add pre-coded or free text comments to any slide, cell class or specific cell. Attach any number of cells and send them via email. Customize the coloration and brightness of cell images in your personal profile.

Slides are placed into a 12-position magazine; up to 8 magazines, containing a total of 96 slides, can be loaded at once.

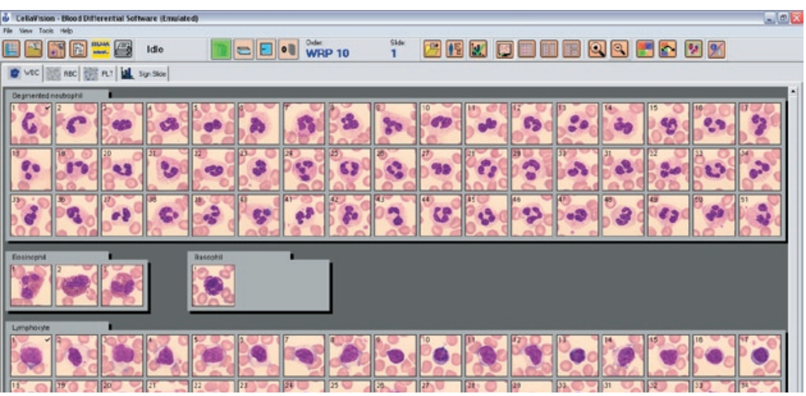
WBC:

- Cells are grouped with like cells to facilitate verification.
- Cells are pre-classified into 18 classes:

  LEUKOCYTES: Segmented neutrophils, band neutrophils, eosinophils, basophils, lymphocytes, monocytes, blast cells, promyelocytes, myelocytes, metamyelocytes, variant lymphocytes, plasma cells and unidentified.
  NON-LEUKOCYTES: smudge cell, artifact, giant platelet, platelet clumps, erythoblasts (NRBC).
- Handling leukopenic samples is more efficient due to the ability to merge cells from multiple slides on a single patient.

RBC:

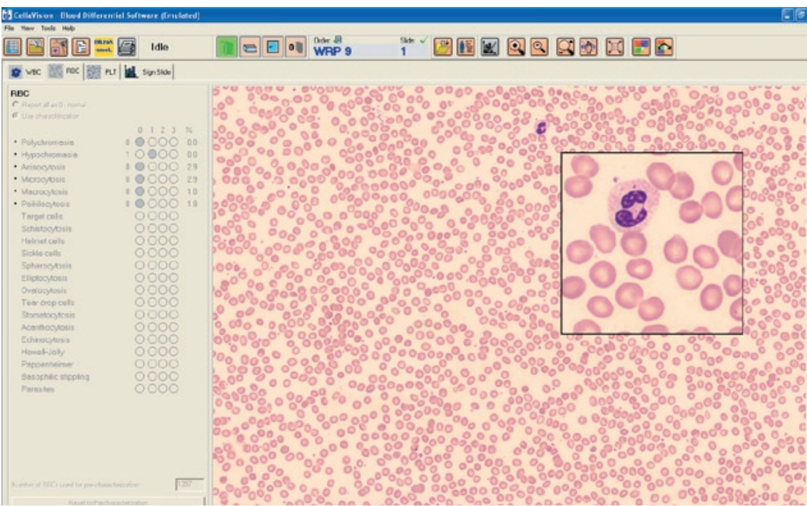
- RBC Morphology is automatically pre-characterized for polychromasia, hypochromasia, aniso-, micro-, macrocytosis and poikilocytosis as set by the lab, providing you with percentages of each type.
- Multiple fields can be scanned as if on a virtual microscope.
- A dynamic micrometer facilitates additional cell measurements.



100 WBCs can be displayed on one single screen for a quick confirmation of the CBC analyzer result.

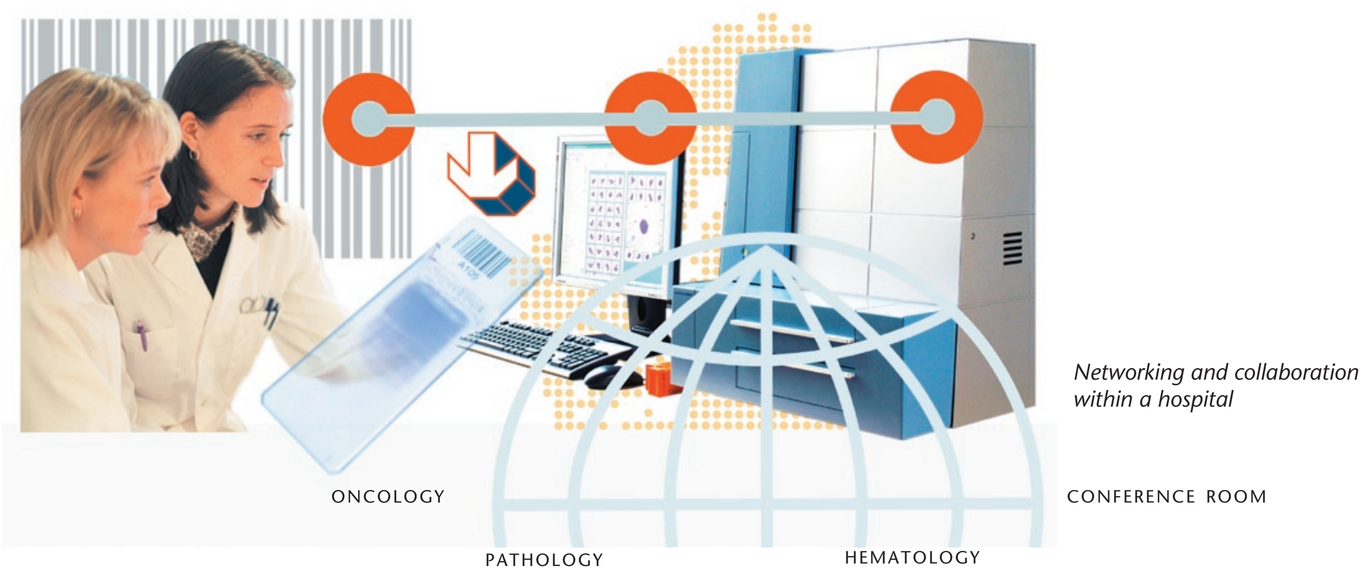
Steve DeVine, UTMB, Galveston, Texas:
"We early on identified a number of key points with the DM96, like reduction in labor costs and increased quality of produced results. The software's numerous options and high image quality make it a very user-friendly and efficient system to use with a minimum of maintenance.

The networking capability, like remote review and email functionality, adds new values to the laboratory and clinicians which in the end should provide better patient care. In addition to this, it is also a great training tool."



Activate the dynamic mouse-over zoom and move the cursor over the area of interest to display a detailed magnified image. Magnification level is easily controlled by the mouse-wheel.

Networking, collaboration and education



Networking and collaboration within a hospital

Using the Remote Review software in conjunction with the CellaVision DM96 opens up exciting new possibilities for networking, collaboration and education. CellaVision Remote Review Software is an optional software product enabling remote access to a DM96 database.

Connectivity. *Clinicians and pathologists save time by accessing their patients' images remotely in real time. Technologists save time searching for the requested smear.*

Collaboration. *Should there be lack of personnel; slide review can be performed remotely from another hospital. Smears needing a second opinion can easily be reviewed remotely by a pathologist or clinician from within or outside the hospital.*

Rational archiving. *Archive images and results to CD-R/RW or LAN gives access to a patient's image history, months or years back.*

Education and training. *Exporting individual interesting slides to a separate database and thereby create a "digital slide box" accessable from anywhere by anyone, making training and education of staff easier and more efficient.*

Shared database. *Multiple systems can be run from a central location, eliminating transportation problems and reducing turn-aroundtime (TAT). Morphology expertise can be centralized, resulting in staffing flexibility at remote locations.*

Samples with a STAT flag are clearly indicated in the database view giving high attention to these slides for a quick TAT.

Quality of results

Extract from a customer testimonial

"We quickly identified a number of values to our laboratory which met or exceeded our expectations including better quality of delivered results, increased service to our clinicians, significantly improved ergonomics, excellent educational tool, higher interest in morphological diagnosis in the laboratory and improved and easier quality assurance.

The Remote Review Station allows us to work parallell while verifying analyzed slides and to access stored information remotely."

Linda Hilstedt, Dept. Clinical Biochemistry, Rigshospitalet , Copenhagen

Pre-classification performance

During the evaluations of CellaVision™ DM96 the following results were collected at two of the hospitals, one in the US and one in Europe.

As shown by the results for these specific hospitals, the pre-classification agreement for the group, making up for the majority of the cells in a sample, segmented neutrophils, lymphocytes and monocytes is above 90% leaving few cells to be reclassified by the operator.

Study data

	US STUDY	EUROPEAN STUDY
NO OF SAMPLES	246	100
COUNTED CELLS/SAMPLE	100	200
STAINING PROTOCOL	WRIGHT	MGG

Agreement of preclassification for different groups of cells

	US STUDY	EUROPEAN STUDY
ALL CELL CLASSES	87%	84%
ALL CELL CLASSES (SN=BN)	92%	92%
SEG, LYMF, MONO	93%	97%
SEG, LYMF, MONO EOS, BASO	91%	87%

Technical Specifications

Supported smear methods (wedged)
- Automated slide makers and/or stainers
- HemaPrep™/MiniPrep™ automated blood smearing device
- Manual smears

Supported stains
Romanowsky stains (MGG/W/WG)

Slide handling
- Requires barcode labelled slides with clipped/round corners.
- Slides are loaded into magazines with the capacity of 12 slides each.
- Continuous feeding, with an initial load of 96 slides using eight magazines.
- Magazines are ideal for storage and retrieval of slides.

Throughput
- Approximately 35 slides/hour for complete differential (WBC+RBC+PLT).
- Approximately 60 slides/hour for RBC and/or PLT only.

Archiving of results and images
- Supported media: CD-R / CD-RW and LAN.

Results storage capacity:
- On hard drive: Approximately 20.000 slides, 100 cells/slide.
- On CD: Approximately 200 slides, 100 cells/slide.
- On LAN: unlimited

Oil dispensing Automatic

Number of cells counted User definable

Quality control
- Built-in QC module for verification of the cell location accuracy.

Communication support
- Bi-directional LIS support, ASTM
- Ethernet 10/100 Mbps
- E-mail

Printer support
- Laser/inkjet printers supported by Windows XP.

Specific performance characteristics
A clinical evaluation based on NCCLS standard H-20A showed equivalence to DiffMaster™ Octavia regarding accuracy, imprecision and clinical sensitivity/specificity.

CellaVision DM96 automatically preclassifies the following cell types. **WBCs**: Segmented and band neutrophils, eosinophils, basophils, lymphocytes, monocytes, blast cells, promyelocytes, myelocytes, metamyelocytes, variant lymphocytes, and plasma cells. **Non WBCs:** Smudge, artefacts, giant platelets, platelet clumps, erythoblasts (NRBC), unidentified.

RBC precharacterization: Automated precharacterization of aniso-, micro- and macrocytosis, polychromasia, hypochromasia and poikilocytosis is performed in an overview image corresponding to eight high power fields (100x).

PLT estimate: The graphical user interface allows manual estimation of the PLT concentration, based on eight high power fields (100x).

System components
- PC with Windows XP
- Slide scanning unit
- CellaVision Blood Differential Software

Electronical Specification *all system components*
- Voltage input: 230 VAC (115 VAC for US)
- Current input: 4A (8A for US)

Size (W x D x H)
- 530 x 600 x 630 mm
- 20,9 x 23,6 x 24,8 inches

Accessories
- Label printer kit
- Barcode labelled slide magazines
- Immersion oil
- QC barcode labels

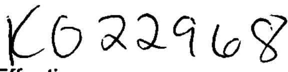
System options
CellaVision Remote Review Software
Recommended PC specifications

- 64 MB graphics RAM with Open GL 1.2 support
- Ethernet adapter 10/100 Mbps • 512 MB RAM
- 100 MB free disk space • CPU Pentium IV

PM-10068 2004-06-07

World Headquarters • CellaVision AB, Ideon Research Park, SE-223 70, Lund, Sweden • tel +46 46-286 44 00 • fax +46 46-286 44 70 • info@cellavision.com
U.S. Office • CellaVision Inc • 1555 Jupiter Park Drive, Suite 6, Jupiter, FL 33458, USA • tel 800 390 1374 • fax 561 7413823 • www.cellavision.com

Competitors
FDA files



510(k) Summary of Safety and Effectiveness

SEP 24 2002

The following information provides data supporting a substantially equivalent determination for the ADVIA TESTpoint CSF Controls.

Predicate Device
Cell-Chex body fluid controls manufactured by Streck Laboratories, Inc (K000076).

Intended Use
ADVIA TESTpoint CSF Controls are hematology reference materials for monitoring the precision and accuracy of the ADVIA 120 Hematology System when analyzing CSF samples.

Device Description
ADVIA TESTpoint CSF Controls is an assay control mixture used for quality control of the ADVIA 120 CSF (cerebral spinal fluid) method. The control materials are composed of red blood cells and white blood cells derived from human sources stored in a stabilizing medium. The control materials consist of two levels that simulate a low cell count (Level 1) and a higher abnormal count (Level 2).

The following RBC and WBC parameters are reportable with the control materials:

Level 1
WBC - white blood cell count
RBC – red blood cell count

Level 2
WBC - white blood cell count
RBC – red blood cell count
% Neut – percent neutrophil count
% Lymph – percent lymphocyte count
% Mono – percent monocyte count
% Eos – percent eosinophil count
% MN – percent mononuclear cell count
% PMN – percent polymorphonuclear cell count

Comparison with Predicate Device
The following table provides similarities and differences between ADVIA TESTpoint CSF Controls and the predicate device (K000076).

Similarities/Differences	Characteristic	Predicate Device	ADVIA TESTpoint CSF Controls
Similarities	Intended Use	Quality control material for control of cerebrospinal fluid cell counts.	Same as predicate device.
	Control Composition	Human red blood cells and human white blood cells in a preservative medium.	Similar to predicate device.
	Targeted Control Ranges	Level 1 – Low cell count Level 2 – High abnormal cell count	Similar to predicate device.
Differences	Intended Use	Intended to control manual cell counts.	Intended to control ADVIA 120 automated cell counts.
	Level 1 WBC Differential	Two part WBC Differential	No WBC Differential on Level 1
	Level 2 WBC Differential	Two part WBC Differential	Five part WBC Differential

Conclusion

The test results included in this submission demonstrate that the ADVIA TESTpoint CSF Controls are substantially equivalent to the predicate device. The control materials have demonstrated acceptable precision as observed by the minimal variability (SD and %CV) found in stability testing, and meets the manufacturer's intended specifications for both shelf life and open vial stability.

[signature] Date 9/05/02

Kenneth T. Edds, Ph.D.
Manager, Regulatory Affairs
Bayer Corporation
511Benedict Avenue
Tarrytown, New York 10591-5097

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

SEP 24 2002

Kenneth T. Edds, Ph.D.
Manager, Regulatory Affairs
Bayer Diagnostics
511 Benedict Avenue
Tarrytown, NY 10591-5097

Re: k022968
Trade/Device Name: ADVIA TESTpoint CSF Controls
Regulation Number: 21 CFR 864.8625
Regulation Name: Hematology Quality Control Mixture
Regulatory Class: Class II
Product Code: JPK
Dated: September 6, 2002
Received: September 6, 2002

Dear Dr. Edds:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

This letter will allow you to begin marketing your device as described in your 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and additionally 809.10 for in vitro diagnostic devices), please contact the Office of Compliance at (301) 594-4588. Additionally, for questions on the promotion and advertising of your device, please contact the Office of Compliance at (301) 594-4639. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR 807.97). Other general information on your responsibilities under the Act may be obtained from the Division of Small Manufacturers International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its internet address "http://www.fda.gov/cdrh/dsma/dsmamain.html".

Sincerely yours,

Steven Gutman

Steven I. Gutman, M.D., M.B.A.
Director
Division of Clinical Laboratory Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

510(k) Number: K022968

Device Name: ADVIA TESTpoint CSF Controls

Indications for Use: ADVIA TESTpoint CSF Controls are hematology reference materials for monitoring the precision and accuracy of the ADVIA 120 Hematology System when analyzing CSF samples.

(PLEASE DO NOT WRITE BELOW THIS LINE - CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Prescription Use √ OR Over-The-CounterUse________
(Per 21 CFR 801.109)

(Optional Format 1-2-96)

(Division Sign-Off)
Division of Clinical Laboratory Devices
510(k) Number K022968

NOV 2 0 2007

5. 510(k) SUMMARY of the XE-5000

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.
The assigned 510(k) number is: K071967.

1. Submitted by:	Sysmex America, Inc. One Nelson C. White Parkway Mundelein, IL 60060 Phone: (847) 996-4675; FAX: (847) 996-4655 Contact person: Nina Gamperling Date prepared: July 13, 2007
2. Name of Device:	Trade or proprietary name: Sysmex® XE-5000 Common name: Automated Hematology Analyzer. Classification name: Sysmex® XE-Series, Automated Hematology, an Automated Differential Cell Counter (21 CFR 864.5220) is a Class II device. Related Items: CELLPACK™ (Diluent) — Product Code: 81GIF CELLSHEATH™ (Diluent) — Product Code: 81GIF STROMATOLYSER-FB™ (Lyse) — Product Code: 81GGK STROMATOLYSER-4DL™ (Lyse) — Product Code: 81GGK STROMATOLYSER-4DS™ (Stain) — Product Code: 81KJK STROMATOLYSER-NR™ (Dilent) — Product Code: 81GGK STROMATOLYSER- NR™ (Stain) — Product Code: 81KJK STROMATOLYSER-IM™ (Lyse) — Product Code: 81GGK SULFOLYSER (Lyse) — Product Code: 81GGK RET-SEARCH II (Diluent) — Product Code: 81GIF RET-SEARCH II (Stain) — Product Code: 81KJK XE Calibrators — Product Code: 81KSA e-Check (XE) (Control) — Product Code: 81JPK Option: Graphic printer Bar code Reader
3. Predicate Method:	Sysmex® XE-2100 Body Fluid (K040073-Cleared March 18, 2004)
4. Device Description:	The Sysmex® XE-5000 is part of the XE-Series instrument line. It is a multi-parameter hematology analyzer intended to perform tests in anti-coagulated blood and body fluids. The instrument consists of three principal units: (1) Main Unit which aspirates, dilutes, mixes and analyzes blood and body fluid samples; (2) Auto Sampler Unit supplies samples to the Main Unit automatically; (3) IPU (Information Processing Unit) which processes data from the Main Unit and provides the operator interface with the system. The XE-5000 is equipped with a Sampler that provides continuous automated sampling for up to 100 tubes. The XE-5000 performs analyses using the following methods: RF/DC Detection Method, Sheath Flow DC Detection Method, and Flow Cytometry Methods using a Semiconductor Laser. Particle characterization and identification is based on detection of forward

scatter, fluorescence and adaptive cluster analysis. Using the same reagents as the XE-2100, the XE-5000 automatically classifies cells from blood and body fluids and carries out all processes automatically from aspiration of the sample to outputting the results.

The body fluid analysis mode of the XE-5000 uses the 4DIFF scattergram & the RBC distribution obtained from a specialized analysis sequence to calculate & display the WBC (WBC-BF) counts, mononuclear cell (MN) / polymorphonuclear cell (PMN) counts & percentages, TC-BF (Total Count) & RBC (RBC-BF) counts found in the body fluid.

Analysis results and graphics are displayed on the IPU screen. They can be printed on any of the available printers or transmitted to a Host computer.

5. Intended Use:

Sysmex® XE-5000 is an automated hematology analyzer for *in vitro* diagnostic use in screening patient populations found in clinical laboratories. The XE-5000 classifies and enumerates the same parameters as the XE-2100 using whole blood as described below, cord blood for HPC and has a body fluid mode for body fluids. The Body Fluid mode analyzes WBC-BF, RBC-BF, MN%/#, PMN%/# and TC-BF in body fluids (cerebrospinal fluids (CSF), serous fluids, and synovial fluids with EDTA, as needed).

WBC	White Blood Cell Count
RBC	Red Blood Cell Count
HGB	Hemoglobin
HCT	Hematocrit
MCV	Mean Cell Volume
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
PLT	Platelet Count
NEUT% / #	Neutrophil Percent and Count
LYMPH% / #	Lymphocyte Percent and Count
MONO% / #	Monocyte Percent and Count
EO% / #	Eosinophil Percent and Count
BASO% / #	Basophil Percent and Count
NRBC% / #	Nucleated RBC Percent and Count
RDW-SD	RBC Distribution Width-SD
RDW-CV	RBC Distribution Width-CV
MPV	Mean Platelet Volume
RET% / #	Reticulocyte Percent and Count
IRF	Immature Reticulocyte
IG% / #	Immature Granulocyte Percent and Count
HPC#	Hematopoietic Progenitor Cells
RET-He	Reticulocyte Hemoglobin
IPF	Immature Platelet Fraction
WBC-BF	WBC count in the body fluid mode analysis.
RBC-BF	RBC count in the body fluid mode analysis.
MN% / #	Percent and number of mononuclear cells within WBC-BF.
PMN%/ #	Percent and number of polymorphonuclear cells in WBC-BF
TC-BF#	The total count including WBC-BF and HF-BF# (the number of particles which appear in a stronger fluorescence area in DIFF scattergram)

6. Substantial equivalence- Similarities and Differences:	Table 1 shows substantial equivalence of the XE-5000 to the XE-2100.
7. Conclusion	The XE-5000 demonstrates substantial equivalence to the XE-2100 Body Fluid application.

Table 1: Substantial Equivalence—Similarities and Difference to XE-2100

	Sysmex XE-2100 Predicate	Sysmex XE-5000 Modification of Predicate	Similarity/ Difference
Intended Use	The Sysmex® XE-2100 Series Hematology Analyzer is a quantitative, automated hematology analyzer and leukocyte differential counter for *in vitro* diagnostic use in clinical laboratories. The body fluid application adds a quantitative, automated procedure for analyzing cerebrospinal fluid, serous fluid and synovial fluid. Body Fluid Parameters: WBC RBC Capillary Mode Parameters: WBC RBC HGB HCT MCV MCH MCHC PLT RET% / #	Sysmex® XE-5000 is an automated hematology analyzer for *in vitro* diagnostic use in screening patient populations found in clinical laboratories. The XE-5000 classifies and enumerates the same parameters as the XE-2100 using whole blood as described below, cord blood for HPC and has a body fluid mode for body fluids. The Body Fluid mode analyzes WBC-BF, RBC-BF, MN%/#, PMN%/# and TC-BF in body fluids (cerebrospinal fluids (CSF), serous fluids, and synovial fluids with EDTA, as needed). Body Fluid Parameters: WBC-BF RBC-BF MN%/ # PMN% / # TC-BF# Capillary Mode Parameters: WBC RBC HGB HCT MCV MCH MCHC PLT RET% / # NEUT% / # LYMPH% / # MONO% / # EO% / # BASO% / # NRBC% / # IG% / #	Both systems have the same intended use but the XE-5000 has additional capillary and body fluid parameters. 1) Body Fluid Mode has new differential parameters (MN%/# and PMN %/#) and detects WBC and RBC cells at a lower level than the XE-2100. The body fluid mode is used on body fluid samples with RBC counts greater than 0.003 x 10^6/ul, WBC counts greater than 0.01 x 10^3/ul for CSF and 0.030 x 10^3/ul for other body fluids and a WBC differential (MN%/# and PMN %/#) for samples with WBC counts. 2) Capillary mode on whole blood includes differential with NRBC#/% & IG#/%. 3) Addition of WBC-D parameter on whole blood.
Methodology	The XE-2100 performs analyses using the following methods: RF/DC Detection Method, Sheath Flow DC Detection Method, and Flow Cytometry Methods using a Semiconductor Laser. The RF/DC detection method	The XE-5000 performs analyses using the following methods: RF/DC Detection Method, Sheath Flow DC Detection Method, and Flow Cytometry Methods using a Semiconductor Laser. The RF/DC detection method detects the size of the	Both systems use the same methodology.

	detects the size of the cells by changes in direct-current resistance & the density of the cell interior by changes in radio-frequency resistance. Cells pass through the aperture of the detector surrounded by sheath fluid using the sheath flow method. The principle of flow cytometry is also used. A semiconductor laser beam is emitted to the cells passing through the flow cell. The forward scattered light is received by the photodiode, & the lateral scattered light & lateral fluorescent light are received by the photo multiplier tube. This light is converted into electrical pulses, thus making it possible to obtain cell information.	cells by changes in direct-current resistance & the density of the cell interior by changes in radio-frequency resistance. Cells pass through the aperture of the detector surrounded by sheath fluid using the sheath flow method. The principle of flow cytometry is also used. A semiconductor laser beam is emitted to the cells passing through the flow cell. The forward scattered light is received by the photodiode, & the lateral scattered light & lateral fluorescent light are received by the photo multiplier tube. This light is converted into electrical pulses, thus making it possible to obtain cell information.	
Reagents	CELLPACK™ (Diluent) CELLSHEATH™ (Diluent) STROMATOLYSER-FB™ (Lyse) STROMATOLYSER-4DL™ (Lyse) STROMATOLYSER-4DS™ (Stain) STROMATOLYSER-NR™ (Diluent) STROMATOLYSER-NR™ (Stain) STROMATOLYSER-IM™ (Lyse) SULFOLYSER (Lyse) RET-SEARCH II (Diluent) RET-SEARCH II (Stain)	CELLPACK™ (Diluent) CELLSHEATH™ (Diluent) STROMATOLYSER-FB™ (Lyse) STROMATOLYSER-4DL™ (Lyse) STROMATOLYSER-4DS™ (Stain) STROMATOLYSER-NR™ (Diluent) STROMATOLYSER-NR™ (Stain) STROMATOLYSER-IM™ (Lyse) SULFOLYSER (Lyse) RET-SEARCH II (Diluent) RET-SEARCH II (Stain)	The XE-2100 and the XE-5000 use the same reagents.
Quality Control/ Calibrator	*e*-Check—3 levels XE Calibrator (X Cal)	*e* -Check (XE) —3 levels XE Calibrator (X Cal)	The XE-2100 and the XE-5000 use the same calibrator, but the XE-5000 uses a specific quality control material, *e* -Check (XE), that has been previously cleared.
Software/ Hardware Differences	The XE-pro software was added to the original XE-2100 in order to include additional master programs (HPC, IG, RET, IPF) and perform Body Fluid analysis.	The XE-5000 uses the same XE-pro software and includes additional masters (HPC, IG, RET, IPF) along with a Body Fluid mode in the initial standard software model.	The XE-5000 performs the same as the XE-2100 with the HPC, IG, RET and IPF masters along with a Body Fluid mode that has additional parameters (TC-BF, MN%/# and PMN %/#). The XE-5000 capillary mode includes the differential, NRBC and IG parameters.
Specimen Type	Random whole blood and body fluid samples	Random whole blood and body fluid samples.	Both systems use the same specimen types.
Throughput	Approximately 113-150 samples/hour depending on the mode used.	Approximately 113-150 samples/hour depending on the mode used.	Both systems have the same throughput.
Equivalency Data:	Performance was initially established in XE-2100 510(k) submission (K992875) & then additional masters/parameters were submitted in subsequent submissions:	Performance of the XE-5000 whole blood mode is the same as the XE-2100 with additional masters. The Body Fluid mode of the XE-5000 has additional parameters. Comparison of the	Data consisting of carryover, linearity, accuracy and reproducibility was collected to show performance to the manufacturer's specification for the Body Fluid mode. This

	XE-2100/HPC (K020496), XE-2100/IG (K032039), XE-Body Fluid (K040073), XE-2100 RET/He (K050589), XE-2100/IPF (K051199).	XE-5000 body fluid mode to the XE-2100 demonstrated excellent correlation.	analysis supports the claim that the XE-5000 Body Fluid mode is substantially equivalent to the XE-2100 Body Fluid.

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

NOV 20 2007

Sysmex America, Inc.
C/O Nina M. Gamperling
One Nelson C. White Parkway
Mundelein, Illinois 60060

Re: k071967
Trade/Device Name: Sysmex XE-5000
Regulation Number: 21 CFR 864.5220
Regulation Name: Automated Hematology Analyzer
Regulatory Class: Class II
Product Code: GKZ
Dated: July 13, 2007
Received: July 17, 2007

Dear Ms. Gamperling:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket

notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at (240) 276-0450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding postmarket surveillance, please contact CDRH's Office of Surveillance and Biometric's (OSB's) Division of Postmarket Surveillance at (240) 276-3474. For questions regarding the reporting of device adverse events (Medical Device Reporting (MDR)), please contact the Division of Surveillance Systems at (240) 276-3464. You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (240) 276-3150 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Robert L. Becker, Jr., M.D., Ph.D.
Director
Division of Immunology and Hematology
Office of In Vitro Diagnostic Device Evaluation
and Safety
Center for Devices and Radiological Health

Enclosure

4. INDICATIONS FOR USE STATEMENT

510(k) Number (if known): K071967

Device Name: Sysmex® XE-5000, Automated Hematology Analyzer

Indications For Use:

Sysmex® XE-5000 is an automated hematology analyzer for *in vitro* diagnostic use in screening patient populations found in clinical laboratories. The XE-5000 classifies and enumerates the same parameters as the XE-2100 using whole blood as described below, cord blood for HPC and has a body fluid mode for body fluids. The Body Fluid mode analyzes WBC-BF, RBC-BF, MN%/#, PMN%/# and TC-BF in body fluids (cerebrospinal fluids (CSF), serous fluids, and synovial fluids with EDTA, as needed).

WBC	White Blood Cell Count
RBC	Red Blood Cell Count
HGB	Hemoglobin
HCT	Hematocrit
MCV	Mean Cell Volume
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
PLT	Platelet Count
NEUT% / #	Neutrophil Percent and Count
LYMPH% / #	Lymphocyte Percent and Count
MONO% / #	Monocyte Percent and Count
EO% / #	Eosinophil Percent and Count
BASO% / #	Basophil Percent and Count
NRBC% / #	Nucleated RBC Percent and Count
RDW-SD	RBC Distribution Width-SD
RDW-CV	RBC Distribution Width-CV
MPV	Mean Platelet Volume
RET% / #	Reticulocyte Percent and Count
IRF	Immature Reticulocyte
IG% / #	Immature Granulocyte Percent and Count
HPC#	Hematopoietic Progenitor Cells
RET-He	Reticulocyte Hemoglobin
IPF	Immature Platelet Fraction
WBC-BF	WBC count in the body fluid mode analysis.
RBC-BF	RBC count in the body fluid mode analysis.
MN% / #	Percent and number of mononuclear cells within WBC-BF.
PMN%/ #	Percent and number of polymorphonuclear cells within WBC-BF.
TC-BF#	The total count including WBC-BF and HF-BF# (the number of particles which appear in a stronger fluorescence area in DIFF scattergram)

Division Sign-Off

Office of In Vitro Diagnostic Device Evaluation and Safety

510(k) K071967

Prescription Use X **(Part 21 CFR 801 Subpart D)** **AND/OR** **Over-The-Counter Use** ____ **(21 CFR 801 Subpart C)**

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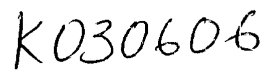
Concurrence of CDRH, Office of Device Evaluation (ODE)



APR 21 2003

Section 1 D: 510(k) Summary of Safety and Effectiveness for COULTER® LH 750 Body Fluids Application

1.0 General Information

Applicant Name and Address: Beckman Coulter, Inc.
Cellular Analysis Division
11800 SW 147 Avenue
Miami, FL 33196-2500

Primary Contact: Stan Sugrue, Ph.D.
Senior Regulatory Affairs Specialist
Telephone: (305) 380-4552
FAX: (305) 380-3618
E-mail: stan.sugrue@coulter.com

Date: February 24, 2003

Device Trade Name(s): COULTER® LH 750 Hematology Analyzer

Device Generic Name(s): Automated differential cell counter

Device Classification: The COULTER® LH 750 Hematology Analyzer is a Class II medical device.

2.0 Pre-amendment Predicate Method

The COULTER® LH 750 Body Fluids Application claims substantial equivalence to the pre-amendment predicate method for enumeration of WBCs and RBCs via manual cell count method in a counting chamber by a skilled competent technician.

FDA 510(k) Number(s): Not applicable

3.0 Device Description

The COULTER® LH 750 Body Fluids Application is an automated method for enumeration of RBCs and WBCs in body fluids on the COULTER LH 750 Hematology Analyzer, an automated hematology analyzer capable of supplying a complete blood cell analysis and includes a differential leukocyte cell count. The LH 750 also provides automated reticulocyte analysis and enumeration of nucleated red blood cells (NRBCs).

4.0 Principle of Method:

The COULTER LH 750 Body Fluids Application is an automated method for enumeration of RBCs and WBCs in body fluids on the COULTER LH 750 Hematology Analyzer. The LH 750 utilizes the Coulter Principle for automatically enumerating and sizing blood cells. The analyzer uses a reagent system consisting of an isotonic diluent, lytic reagents to lyse the red cells without significantly affecting the white cells and an instrument cleaner.

5.0 Comparison to Predicate

Similarities/ Differences	Characteristic	Manual method (Predicate)	LH 750 Body Fluids Application
Similarities	Intended Use	To provide a quantitative determination of blood cells in cerebrospinal fluid, serous fluid, and synovial fluid	Same as manual method
	Specimen Analyzed	Body Fluids collected in a container with or without anti-coagulant	Same as manual method
Differences	WBC Count (cells/µL)	Manual cell count performed in a counting chamber by a skilled competent technician	Automated count
	RBC count (cells/µL)	Manual cell count performed in a counting chamber by a skilled competent technician	Automated count

6.0 Indications for Use:

The COULTER LH 750 Hematology Analyzer is a quantitative, automated hematology analyzer and leukocyte differential counter For In Vitro Diagnostic Use in clinical laboratories.The LH 750 Body Fluids Application adds a quantitative, automated procedure for analyzing cerebrospinal fluid, serous fluid, and synovial fluid to the LH 750, providing enumeration of the WBCs and the RBCs.

7.0 Conclusion:

The COULTER LH 750 Body Fluids Application is substantially equivalent to the manual microscopic predicate method for enumeration of RBCs and WBCs in body fluids.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

APR 2 1 2003

Stan Sugrue, Ph.D.
Senior Regulatory Affairs Specialist
Beckman Coulter, Inc.
11800 S.W. 147 Avenue
P.O. Box 169015
Miami, FL 33116-9015

Re: k030606
Trade/Device Name: COULTER® LH 750 Hematology Analyzer
Regulation Number: 21 CFR 864.5220
Regulation Name: Automated Differential Cell Counter
Regulatory Class: Class II
Product Code: GKZ
Dated: April 2, 2003
Received: April 3, 2003

Dear Dr. Sugrue:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific information about the application of labeling requirements to your device, or questions on the promotion and advertising of your device, please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at (301) 594-3084. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). Other general information on your responsibilities under the Act may be obtained from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its Internet address http://www.fda.gov/cdrh/dsma/dsmamain.html.

Sincerely yours,

Steven Gutman

Steven I. Gutman, M.D., M.B.A.
Director
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

Section 1C: **INDICATIONS FOR USE**

510(k) Number (if known): ~~Not assigned~~ K030606

Device: COULTER® LH 750 Hematology Analyzer

Indications For Use:

The COULTER® LH 750 Hematology Analyzer is a quantitative, automated hematology Analyzer and leukocyte differential counter For In Vitro Diagnostic Use in clinical laboratories. The COULTER® LH 750 Hematology Analyzer also provides automated Reticulocyte analysis and enumeration of nucleated red blood cells (NRBCs). The system also provides an automated method for enumeration of RBCs and WBCs in body fluids.

Future commercialization will add ISOTON® 4 diluent /Lyse S® 4 Lytic reagent to the indications for use.

21 CFR 864.5220 Automated differential cell counter

An automated differential cell counter is a device used to identify and classify one or more of the formed elements of blood.

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Prescription Use ✓ **Use (Per 21 CFR 801.109)** **OR** **Over-The-Counter** ____

(Division Sign-Off)
Division of Clinical Laboratory Devices
510(k) Number K030606

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

A. 510(k) Number: K081930 Abbreviated

B. Purpose for Submission: New hematology analyzer

C. Measurand: CBC, 5-part differential, NRBC, Reticulocyte, RBCs and TNCs in body fluids.

D. Type of Test: Quantitative

E. Applicant: Beckman Coulter, Inc.

F. Proprietary and Established Names:

- Proprietary Name: UniCel® DxH 800 Coulter® Cellular Analysis System
- Established Name: Automated Differential Cell Counter

G. Regulatory Information:

1. Regulation section: 21 CFR 864.5220

2. Classification: Class II

3. Product code: GKZ

4. Panel: Hematology (81)

H. Intended Use:

1. Intended use(s):

   The UniCel® DxH 800 Analyzer is a quantitative, automated hematology analyzer for *in vitro* diagnostic use in screening patient populations found in clinical laboratories. The UniCel® DxH 800 Analyzer provides:

   - A Complete Blood Count (CBC), Leukocyte 5 Part Differential (Diff), Reticulocyte (Retic) and Nucleated Red Blood Cell (NRBC) on whole blood
   - A Total Nucleated Count (TNC) and Red Cell Count (RBC) on Body Fluids (cerebrospinal, serous, and synovial) (BF)

2. Indication(s) for use: same as the Intended use

3. Special conditions for use statement(s): N/A

4. Special instrument requirements: N/A

I. Device Description:

The UniCel® DxH 800 Coulter® Cellular Analysis System is comprised of the analyzer and a suit of analytical reagents that allow for simultaneous quantitative determination of hematological parameters. The system provides automated CBC, leukocyte 5-part differential, reticulocyte analysis, NRBC enumeration, and RBCs and TNCs enumeration in body fluid. The purpose of the DxH 800 analyzer is to separate the normal patient, with all normal system-generated parameters, from patient who needs additional studies of any of these parameters.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   - Coulter® LH 750 Hematology Analyzer
   - Coulter® LH 780 Hematology Analyzer

2. Predicate 510(k) number(s):
   - K011342
   - K061616

3. Comparison with predicate:

Similarities			
Item	Device UniCel® DxH 800	Predicate Coulter® LH 780	Predicate Coulter® LH 750
Intended Use	The UniCel® DxH 800 Analyzer is a quantitative, automated hematology analyzer for *in vitro* diagnostic use in screening patient populations found in clinical laboratories. The UniCel® DxH 800 Analyzer provides: - a CBC, Leukocyte 5 Part Diff, Retic, and NRBC on whole blood - a TNC and RBC on Body Fluids	The Coulter LH 780 Hematology Analyzer is a quantitative, automated hematology analyzer and leukocyte differential counter for *in vitro* diagnostic use in clinical laboratories. The Coulter LH 780 Hematology Analyzer provides automated Retic analysis and enumeration of NRBCs as well as an automated method for enumeration of RBCs and WBCs in body fluids	The Coulter LH 750 Hematology Analyzer is a quantitative, automated hematology analyzer and leukocyte differential counter for *in vitro* diagnostic use in clinical laboratories. The Coulter LH 750 Hematology Analyzer provides automated Retic analysis and enumeration of NRBCs as well as an automated method for enumeration of RBCs and WBCs in body fluids
Principle of Measurement	- WBC, RBC, MCV, PLT, and TNC: Aperture impedance - HGB: Spectrophotometric	Same as DxH 800	Same as DxH 800
Sample identification	- Automated barcode reading of cassette and sample tube identifier - Manual keyboard entry of sample identifier	Same as DxH 800	Same as DxH 800
Calibrator	Coulter® S-CAL Calibrator kit	Same as DxH 800	Same as DxH 800
Sample types	- Whole blood	- Whole blood	-Whole blood

Similarities			
Item	Device UniCel® DxH 800	Predicate Coulter® LH 780	Predicate Coulter® LH 750
	- BF	- BF	-BF

Differences			
Item	Device UniCel® DxH 800	Predicate Coulter® LH 780	Predicate Coulter® LH 750
Quality Control Techniques	- Daily Instruments Check - Commercial Controls - Delta Checks - Patient Controls - XB Analysis - Inter-laboratory Quality Assurance Program - Extended QC & XM Analysis	Same as DxH 800	Same as LH 780 without Extended QC & XM Analysis
Principle of Measurement	VCSn technology: VCS technology with additional Light Scatter measurements - Diff, Retic: VCSn technology - NRBC: Direct measurement in dedicated channel using VCSn technology	- Diff, Retic: VCS technology using Aperture impedance (DC), Conductivity (RF), Laser Light Scatter - NRBC: Combined used of aperture impedance and VCS technology	Same as LH 780
IVD Parameters	WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, RDW-SD, PLT, MPV, NE%, LY%, MO%, EO%, BA%, NE#, LY#, MO#, EO#, BA#, NRBC%, NRBC#, RET%, RET#, IRF, MRV, TNC in BF	Same as DxH , except TNC is reported as WBC	Same as LH 780 without RDW-SD
Sampling mechanism	- Manual: open and closed - Automated: closed, 5 position cassette, maximum load capacity 20 cassettes	- Manual: open - Automated: closed, 12 position cassette, maximum load 12 cassette	Same as LH 780
Sample aspiration volume	- Automatic, manual, and predilute: 165 µL - Fixed dilution: 1:5	- Automatic: 300 µL - Manual: 200 µL - Predilute: 200 µL – customer defined dilution factor in the range of 1:1 to 1:5	Same as LH 780
Sample identification	Manual barcode scanning of sample tube identifier (single tube station or handheld scanner)	Manual barcode scanning of sample tube identifier (handheld scanner)	Same as LH 780
Quality controls and calibrators	- Coulter® 6C Cell Control - Coulter® Latron CP-X Control - Coulter® Retic-X Cell Control - Coulter® LIN-X Linearity Control - Coulter® Body Fluid Control	- Coulter® 5C Cell Control - Coulter® 5C Latron Primer and Latron Control - Coulter® Retic-C Cell Control - Coulter® LIN-C Linearity Control	Same as LH 780
Service	ProService Remote Diagostics	ProService Remote	Same as LH780

Differences			
Item	Device UniCel® DxH 800	Predicate Coulter® LH 780	Predicate Coulter® LH 750
diagnostics	plus enhanced On-board System diagnostics and system monitoring	Diagnostics	
Throughput	- CBC, CBC/Diff: ≥100 samples per hour - CBC/Diff/NRBC: ≥90 samples per hour - Any cycle with Retic: ≥45 samples per hour	- CBC, CBC/Diff: >110 samples per hour - CBC/Diff/Retic: >45 samples per hour	Same as LH 780
Reagents	- Coulter® DH Diluent - Coulter® DH Diff Pak - Coulter® DH Retic Pak - Coulter® DH Cell Lyse	- Coulter® Isoton Diluent - Coulter® LH Series Pak - Coulter® LH Series Retic Pak - Coulter® Lyse S III Lytic Agent - Coulter® Lyse S 4 Lytic Agent - Coulter® LH Series Diluent	Same as LH 780

K. Standard/Guidance Document Referenced (if applicable):

Class II Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells; Final Guidance for Industry and FDA

L. Test Principle:

CBC analysis is based on the established Coulter Principle and Hemoglobinometry. The Coulter method counts and sizes cells by detecting and measuring changes in electrical resistance, when a cell suspended in a conductive liquid passes through a small aperture. The system counts the individual cells and provides cell size distribution. Hemoglobin is measured photometrically at 525 nm.

The DxH 800 housed a flow cell in a Multi Transducer Module which produced three measurement signals: volume, conductivity, and light scatter. Differential, NRBC, and Reticulocyte analysis is based on the VCSn technology using Aperture Impedance, Conductivity, Laser Light Scatter (multiple angles). In the flow cell, a direct current measures cell volume, high-frequency current senses cellular content, light scatter characterizes the size and refractivity of the cells.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

    a. *Precision/Reproducibility:* Precision study was performed in accordance with CLSI

EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods. Acceptance criteria were met as defined in the performance specifications described in the Instructions for Use.

b. Linearity/assay reportable range: Linearity study was performed in accordance with Class II Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells; Final Guidance for Industry and FDA. Linearity was performed on whole blood (WBC, RBC, HGB, and PLT), Body Fluid (WBC and RBC). All results met specifications as described in the Instructions for Use.

c. Traceability, Stability, Expected values (controls, calibrators, or methods): N/A

d. Detection limit: N/A

e. Analytical specificity: N/A

f. Assay cut-off: N/A

2. Comparison studies:

a. Method comparison with predicate device: Normal and clinical samples were analyzed on the test instruments and compared against predicate devices or reference methodologies. Specimens giving non-numeric results and system alarms were excluded from the data analysis. Accuracy testing was performed in accordance with:

- CLSI H20-A2, Reference Leukocytes Differential Count (Proportional) and Evaluation of Instrumental Methods. Approved Standard.
- CLSI EP9-A, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline

Accuracy was performed on whole blood for CBC, Differential, Reticulocyte, NRBC parameters, and on Body Fluids for TNC and RBC count. Acceptance criteria were met as defined in the performance specifications described in the Instructions for Use.

b. Matrix comparison:

- A comparison of the performance of venous whole blood and capillary whole blood specimens was performed. Test results are as follows:

				95% Confidence Limits			95% Confidence Limits		Mean		
Parameter	n	Correlation	Intercept	Lower	Upper	Slope	Lower	Upper	Venous	Capillary	Units
WBC	29	0.963	0.573	-0.12	1.27	0.978	0.87	1.09	6.28	6.71	x10^3/uL
RBC	29	0.940	0.324	-0.34	0.99	0.972	0.83	1.11	4.75	4.94	x10^6/uL
HGB	29	0.917	1.849	-0.39	4.08	0.908	0.75	1.06	14.26	14.80	g/dL
MCV	29	0.994	-0.852	-4.74	3.03	0.995	0.95	1.04	87.43	86.15	fL
PLT	29	0.936	-34.627	-73.77	4.51	1.047	0.89	1.20	247.37	224.48	x10^3/uL
MPV	29	0.944	1.478	0.41	2.54	0.891	0.77	1.01	8.59	9.13	fL
RDW	29	0.960	-0.169	-1.71	1.37	1.010	0.89	1.13	13.27	13.23	CV%
RDW-SD	29	0.964	-5.252	-10.17	-0.33	1.113	0.99	1.23	40.72	40.05	fL
NE%	25	0.978	0.995	-3.91	5.90	0.966	0.88	1.05	56.65	55.58	%
LY%	25	0.974	1.488	-1.52	4.50	0.968	0.87	1.06	30.75	31.46	%
MO%	25	0.949	1.573	0.52	2.63	0.864	0.75	0.98	9.11	9.38	%
EO%	25	0.979	0.039	-0.21	0.29	0.992	0.91	1.07	2.78	2.81	%
BA%	25	0.333	0.551	0.28	0.82	0.309	-0.05	0.66	0.70	0.77	%
NRBC%	27	0.567	0.045	-0.04	0.13	0.30	-0.23	1.24	0.10	0.09	%
RET%	25	0.954	0.078	-0.09	0.25	0.856	0.74	0.97	1.44	1.34	%
MRV	28	0.898	24.299	7.69	40.90	0.784	0.63	0.94	107.13	108.29	fL
IRF	28	0.790	0.078	-0.01	0.17	0.831	0.57	1.09	0.34	0.36	N/A

- A comparison of the performance of whole blood and pre-dilute specimens was performed. Pre-dilute mode provides only CBC results. Test results are as follows:

				95% Confidence Limits			95% Confidence Limits		Mean		
Parameter	n	Correlation	Intercept	Lower	Upper	Slope	Lower	Upper	Whole Blood	Pre-Dilute	Units
WBC	57	0.999	-0.341	-0.60	-0.08	1.079	1.07	1.09	14.856	15.690	x10^3/uL
RBC	57	0.999	-0.010	-0.06	0.05	1.043	1.03	1.06	3.67	3.82	x10^6/uL
HGB	57	0.999	0.165	0.03	0.30	1.041	0.01	1.03	10.95	11.57	g/dL
MCV	57	0.996	-2.686	-4.87	-0.50	1.004	0.98	1.03	90.42	88.11	fL
PLT	57	0.998	3.127	-3.39	9.64	1.003	0.99	1.02	281.69	285.70	x10^3/uL
MPV	57	0.940	0.349	-0.43	1.12	0.924	0.83	1.01	8.47	8.18	fL
RDW	57	0.995	-0.625	-1.08	-0.17	1.005	0.98	1.03	16.86	16.31	CV%
RDW-SD	57	0.991	-1.054	-2.90	0.79	0.964	0.93	1.00	52.38	49.44	fL

3. Clinical studies:

   a. *Clinical Sensitivity:* Analysis of normal and clinical samples was performed for internal validation.

   b. *Clinical specificity:* Analysis of normal and clinical samples was performed for internal validation.

   c. *Other clinical supportive data (when a. and b. are not applicable):* N/A

4. Clinical cut-off: N/A

5. Expected values/Reference range:

   Reference range was established in accordance with CLSI C28-A2, How to Define and Determine Reference Intervals in the Clinical Laboratory, Approved Guideline. Whole blood samples were collected from 273 donors (133 males and 140 females) from the Beckman Coulter blood donor program. Reference intervals for each parameter were calculated using 95% confidence limits. Reference intervals for Overall, Male, and Female were listed in the Instructions for Use.

N. Instrument Name: UniCel® DxH 800 Coulter® Cellular Analysis System

O. System Descriptions:

1. Modes of Operation:

   - Single tube station: open and closed vial sampling for whole blood and body fluid, open vial sampling for pre-dilute whole blood:
   - Automated cassette: closed vial sampling for whole blood

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes __X__ or No ____

3. Specimen Identification: Barcode and manual key board entry

4. Specimen Sampling and Handling: Open tube, pierced cap

5. Calibration: Coulter commercial calibrator, whole blood

6. Quality Control: Coulter commercial control materials

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

1. Whole blood sample age and storage: Two sets of specimens from donors were collected. For each specimen tested, a set of sample tubes was stored at controlled room temperature (18-26º C) and another set was stored refrigerated (2-8º C).

   Room temperature samples were analyzed at t = 1.5, 8, 16, 24, and 32 hours. Refrigerated samples were analyzed at t = 1.5, 8, 16, 24, 32, 48, 56, 64, and 72 hours. Reticulocyte parameter was analyzed up to 72 hours, NRBC parameter to 24 hours, using t=1.5 room temperature as reference. WBC differential flagging ability was evaluated on the specimens up to 32 hours at room temperature and 72 hours refrigerated.

Donor samples are stable up to:

- 24 hours at room temperature and 48 hours at refrigerated temperature for WBC, RBC, HGB, MCV, RDW%, RDW-SD, PLT, MPV, NE%, LY%, MO%, EO%, BA%
- 24 hours at room temperature and refrigerated temperature for NRBC%
- 24 hours at room temperature and 72 hours at refrigerated temperature for RET%, MRVfL, and IRF

2. Sample stability on pre-dilute whole blood: Specimens collected from donors were stored at controlled room temperature (18-26º C) and tested at t = 0, 15, 30, 45, and 60 minutes after dilution to verify pre-diluted sample stability. The pre-dilute samples are stable up to one hour as specified in the Instructions for Use.

3. Sample storage on body fluid: Per established literature, the Instruction for Use recommends that the body fluid samples should be stored at room temperature and analyzed within one hour of collection.

4. Carryover study:

    a. Whole blood CBC (WBC, RBC, HGB, and PLT), Diff, and Retic: carryover was determined by analyzing three normal whole blood samples, followed by 3 diluent samples.

    b. Body Fluid: carryover was evaluated by analysis of a normal blood followed by a diluent sample analyzed as a body fluid. Carryover is assessed by achieving background count on the diluent.

    The results for whole blood and body fluid met the acceptance criteria as stated in the specification in the Instructions for Use.

5. Mode to mode comparison study: Twenty five specimens collected from normal donors were analyzed as closed vial and open vial specimens. The results are follows:

				95% Confidence Limits			95% Confidence Limits		Mean		
Parameter	**n**	**Correlation**	**Intercept**	**Lower**	**Upper**	**Slope**	**Lower**	**Upper**	**Closed Vial**	**Open Vial**	**Units**
WBC	25	0.9982	0.135	-0.04	0.30	0.974	0.95	1.00	6.435	6.404	x10^3/uL
RBC	25	0.9936	0.136	-0.10	0.37	0.973	0.93	1.02	4.83	4.84	x10^6/uL
HGB	25	0.9912	1.213	-0.22	0.65	0.985	0.95	1.02	13.72	13.73	g/dL
Hct	25	0.9882	0.810	-1.89	3.51	0.980	0.91	1.05	41.14	41.15	%
MCV	25	0.9927	2.152	-2.22	6.52	0.974	0.92	1.03	85.29	85.22	fL
MCH	25	0.9863	1.166	-0.80	3.13	0.959	0.89	1.03	28.46	28.45	Pg
MCHC	25	0.8949	1.929	4.83	8.69	0.943	0.74	1.15	33.35	33.38	g/dL
PLT	25	0.9906	-0.288	-16.56	16.11	1.011	0.95	1.07	265.03	267.81	x10^3/uL
MPV	25	0.9913	0.671	0.22	1.12	0.919	0.87	0.97	8.53	8.51	fL
RDW	25	0.9344	1.568	-0.43	3.57	0.879	0.73	1.02	13.82	13.72	CV%
RDW-SD	25	0.8766	10.782	3.58	17.99	0.731	0.56	0.90	41.58	41.16	fL
Neut %	25	0.9877	-0.837	-4.97	3.30	1.019	0.95	1.09	59.27	59.53	%
Lymph %	25	0.9809	-1.013	-3.67	1.65	1.031	0.94	1.12	29.54	29.44	%
Mono %	25	0.8794	0.530	-1.24	2.30	0.923	0.71	1.14	8.02	7.93	%
Eos %	25	0.9756	-0.073	-0.37	0.22	1.022	0.92	1.12	2.49	2.48	%

Baso %	25	0.7909	0.080	-0.11	0.27	0.796	0.53	1.06	0.68	0.62	%
NRBC %	25	0.8899	0.017	-0.02	0.05	0.752	0.59	0.92	0.15	0.13	%
Retic %	25	0.9112	0.187	-0.04	0.42	0.849	0.68	1.01	1.35	1.33	%
MRV	25	0.9584	-0.467	-14.0	13.07	1.009	0.06	0.88	104.32	104.75	fL
IRF	25	0.8569	0.069	-0.01	0.59	0.801	0.59	1.01	0.36	0.36	N/A

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

1. The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
INSTRUMENT ONLY TEMPLATE

A. 510(k) Number:

K080595

B. Purpose for Submission:

Expanded intended use

C. Manufacturer and Instrument Name:

CellaVision AB, CellaVision DM 96 with the body fluid application

D. Type of Test or Tests Performed:

White Blood Cells (differential), RBC characterization and Platelet estimation

E. System Descriptions:

1. Device Description:

CellaVision DM96 consist as of a slide feeder unit, an optical unit consisting of a microscope and camera (referred to as a slide scanning unit), a computer system contains the acquisition and classification software CellaVision DM software.

CellaVision DM96 with the body fluid application is substantially equivalent to the DM96 for peripheral blood regarding technology and functionality. The intended use of the body fluid application is substantially equivalent to the Romanowsky stain manual light microscopic method of cell classification.

The body fluid application functionality:

- Presents an image on a screen of every located cell or object
- Organizes and suggests cell classification (reclassification) for the located blood cells
- Makes it possible to identify, confirm or modify (reclassification) the suggested classification of the located cells

2. Principles of Operation:

Twelve slides can be loaded into each magazine. The magazine used for the body fluid application is identified through the color and the barcode on it.

The magazines are put onto a conveyer belt and are automatically transported to the slide scanning unit. The analysis process consists of an overview image processing and a cell-location step. The body fluid overview image displays the entire sample area. The overview image can be used to find cells of interest and for getting an overall impression of the sample. The overview image can either have one 10x zoom level or both 10x and 50x zoom levels. The cell-location step uses the optical unit and a camera taking images of the identified images and stores the images of the located cells and the results in a database, and displays the images in an organized manner.

3. Modes of Operation:

   The DM96 is an automated cell-locating device. The system also has Remote Review Station capability with network access to the main DM96 device.

4. Specimen Identification:

   Glass microscope slides are labeled with barcodes. Twelve slides fit into a barcoded labeled magazine.

5. Specimen Sampling and Handling:

   Body fluid is cytocentrifuged and stained outside the device on standard rectangular glass slides by standardized stained with Romanowsky stain.

6. Calibration:

   Not applicable.

7. Quality Control:

   The system performs self-tests during startup of the software, and at certain points during the operation of the system. Both hardware and software components are tested. The Cell location test is used to verify the slide preparation process and the system hardware. Running the test once or twice a day is a recommended interval at the high-load laboratory.

8. Software:

   FDA has reviewed applicant’s Hazard Analysis and Software Development processes for this line of product types:

   Yes __X____ or No________

F. Regulatory Information:

1. Regulation section:

   21 CFR 864.5260, 21 CFR 864.5220

2. Classification:

   Class II

3 Product code:

   JOY, GKZ

4. Panel:

   Hematology (81)

G. Intended Use:

1. Indication(s) for Use:

   DM96 is an automated cell-locating device.

   The body fluid application is intended for differential count of white blood cells. The system automatically locates and presents images of cells on cytocentrifuged body fluid preparations. The operator identifies and verifies the suggested classification of each cell according to type.

   DM96 is intended to be used by skilled operators, trained in the use of the device and in recognition of blood cells.

2. Special Conditions for Use Statement(s):

   Not applicable.

H. Substantial Equivalence Information:

1. Predicate Device Name(s) and 510(k) numbers:

   CellaVision DM96, K033840
   Manual Light Microscope (Romanowsky stain process), Class I

2. <u>Comparison with Predicate Device</u>:

Similarities			
Item	DM96 with Body Fluid Application	Manual light microscope process	DM96
Intended Use	Automated cell-locating device for identification of cytocentrifuged body fluids, for in-vitro diagnostic use. Verification of results by skilled human operator.	Manual method for cell-location and identification of blood smears and cytocentrifuged body fluids, for in-vitro diagnostic use. Verification of results by skilled human operator.	Automated cell-locating device for identification of cytocentrifuged body fluids, for in-vitro diagnostic use. Verification of results by skilled human operator.
Specimen type	Body fluids such as cerebrospinal and, serous fluid. Peripheral blood	Peripheral blood and body fluids.	Peripheral blood
Sample preparation	Body fluid samples are prepared by using a cytocentrifuged and stained with Romanowsky stain.	Romanowsky stained blood film on glass slides of peripheral blood. Body fluid samples are prepared using a cytocentrifuged and stained with Romanowsky stain.	Romanowsky stained blood film on glass slides of peripheral blood.
Analysis technique	White blood cells: Cells are located/counted by moving according to the battlement track pattern. Cells images are analyzed using standard mathematical methods. The cell images are pre-classified and the operator verifies the classification.	White blood cells: The examiners usually located/counted by moving according to the battlement track pattern on the smear and distinguish between classes of white blood cells.	White blood cells: Cells are located/counted by moving according to the battlement track pattern. Cells images are analyzed using standard mathematical methods. The cell images are pre-classified and the operator verifies the classification.
Overview image	The device presents an overview image. The image gives the operator possibilities to get an overview on parts of or the whole slide in different magnifications.	The operator scans the slide to get an overview on parts of or the whole slide in different magnifications.	The device presents an overview image. The image gives the operator possibilities to get an overview on parts of or the whole slide in different magnifications.

I. Special Control/Guidance Document Referenced (if applicable):

EP9-A2 *Method Comparison and Bias Estimation Using Patient Samples*, Approved Standard-Second Edition, NCCLS
EP5A *Evaluation of Precision Performance of Clinical Chemistry Device Approved Guideline*, NCCLS
H20-A *Reference Leukocyte Differential Count (Proportional) and Evaluation of Instrument Methods, Approved Standard*, NCCLS
H56-A *Body Fluid Analysis for cellular composition; Approved guideline, CLSI*

J. Performance Characteristics*:*

1. Analytical Performance:

   a. *Accuracy*:

A mixture of 156 samples (89 CSF and 69 BF) was identified according to EP9A-2 from two sites. All samples were initially analyzed on a cell counter or counted in a hemocytometer to get the leukocyte concentration. From each sample two cytocentrifuged smears were prepared. 200-cell differential counts were performed (400 cells/sample) with both methods and analyzed. The same examiners analyzed the same slides. The accuracy was tested through scatter-plots for each cell class.

Samples included in the study.

Defined as in study	Type	number
CSF	CSF	89
Serous	Peritoneal fluid	24
Serous	Pleural fluid	46

Results for all samples included are as follows:

Cell Class	Accuracy	95% CI Slope	n
Neutrophils	y= 1.0166x - 0.0030 r^2= 0.9903	1.0006 - 1.0326	156
Lymphocytes	y= 1.9840x - 0.0011 r^2= 0.9788	0.9609 - 1.0070	156
Macrophages	y= 0.9554x - 0.0113 r^2= 0.9648	0.9264 - 0.9845	156
Eosinophils	y= 1.1352x - 0.0018 r^2= 0.9737	1.1055 - 1.1649	156
Other cells	y= 1.0808x - 0.0019 r^2= 0.9566	1.0442 - 1.1174	156

Cells pre-classified as Basophils, Lymphoma cells, Atypical lymphocytes, Blasts and Tumor cells are automatically are forwarded to the cell class *Other*.

b. *Precision/Reproducibility*:

Short-term imprecision is equivalent for the test and reference method.

Short term imprecision results found on clinical evaluation on 156 samples

Cell Class	Test Method	Reference Method
	SD%	SD%
Neutrophils	3.4	3.6
Lymphocytes	0.8	1.0
Macrophages	6.0	6.1
Eosinophils	6.3	6.1
Other cells	2.2	1.6

c. *Linearity*:

Not applicable.

d. *Carryover*:

Not applicable

e. *Interfering Substances*:

Not applicable.

2. Other Supportive Instrument Performance Data Not Covered Above:

Not applicable.

K. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

L. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.