LETTURA AUTOMATIZZATA DEI LIQUIDI CAVITARI

Alessandra Fanelli
Laboratorio Generale
Azienda Ospedaliera Universitaria Careggi

Origine del versamento

Fisiologicamente le cavità sierose contengono minime quantità di fluido simile al plasma per caratteristiche chimico-fisiche. Esso si forma continuamente per ultrafiltrazione del plasma e viene riassorbito attraverso i capillari delle sierose così che la quantità e la composizione restano costanti

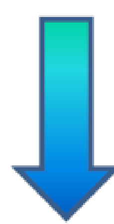
In condizioni patologiche……

La quantità di fluido intracavitario aumenta e le caratteristiche chimico-fisiche e citologiche risultano modificate in funzione della *noxa patogena*

Tipologie di versamento

- **Trasudato:** causato dalla combinazione di un aumento della pressione idrostatica ed una riduzione della pressione oncotica.
- **Essudato:** causato da un'aumentata permeabilità dei capillari sanguigni e linfatici dovuta ad un processo infiammatorio, infettivo o neoplastico.
- **Chiloso:** provocato da un trauma o da una neoplasia (solitamente linfoma) che coinvolge il dotto toracico.

L’analisi dei liquidi biologici rappresenta uno strumento essenziale per la diagnosi e l’inquadramento di numerose patologie



L’esame citometrico dei liquidi, che consiste nel conteggio e nella differenziazione degli elementi nucleati, è cruciale nel determinare l’eziologia del versamento, permettendo di distinguere tra affezioni di origine reattiva, infettiva o maligna.

Body fluids analysis for clinical chemistry and cellular composition: approved guideline

CLSI C49-A 2008 H56-A 2008

C49-A
Vol. 27 No. 14
Replaces C49-P
Vol. 26 No. 21

Analysis of Body Fluids in Clinical Chemistry; Approved Guideline

This document provides guidance for the application of widely available measurement procedures for testing body fluids and for reporting and interpreting those results. It emphasizes defining the common clinical situations for this use; acceptable practice for measuring analytes without extended method verification for abnormal body fluid; influence of biologic and analytic variation on interpretation of results; and variability in comparing results between different instrument manufacturers. This document does not consider serum, plasma, whole blood, or fluids for which assays typically have performance claims in the measurement procedure documentation.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

Clinical and Laboratory Standards Institute (Formerly NCCLS)

IFCC

H56-A
Vol. 26 No. 26
Replaces H56-P
Vol. 25 No. 20

Body Fluid Analysis for Cellular Composition; Approved Guideline

This guideline provides users with recommendations for collection and transport of body fluids, numeration and identification of cellular components, and guidance for qualitative and quantitative assessment of body fluid

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

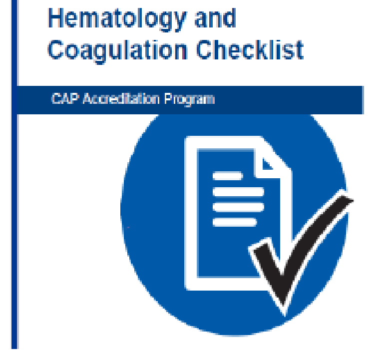
Clinical and Laboratory Standards Institute (Formerly NCCLS)

IFCC

H56-A
Vol. 26 No. 26
Replaces H56-P
Vol. 25 No. 20

Body Fluid Analysis for Cellular Composition; Approved Guideline

Anno 2006



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Hematology and Coagulation Checklist
07/29/2013 Edition

Anno 2013

International Journal of Laboratory Hematology
The Official journal of the International Society for Laboratory Hematology

REVIEW INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

Anno 2014

ICSH guidelines for the verification and performance of automated cell counters for body fluids

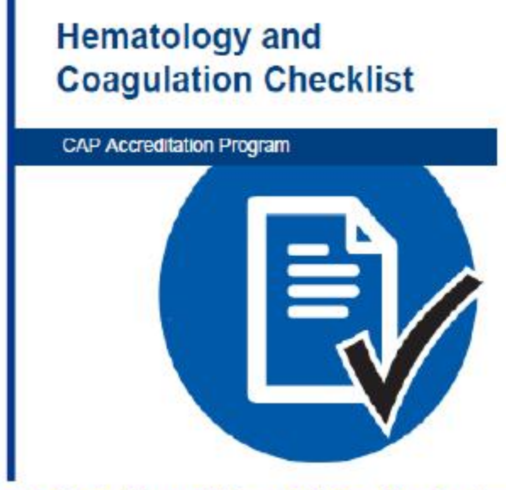
G. BOURNER*, B. DE LA SALLE†, T. GEORGE‡, Y. TABE§,¶, H. BAUM**, N. CULP††, T. B. KENG‡‡,
ON BEHALF OF THE INTERNATIONAL COMMITTEE FOR STANDARDIZATION IN HEMATOLOGY (ICSH)

H56-A
Vol. 26 No. 26
Replaces H56-P
Vol. 25 No. 20

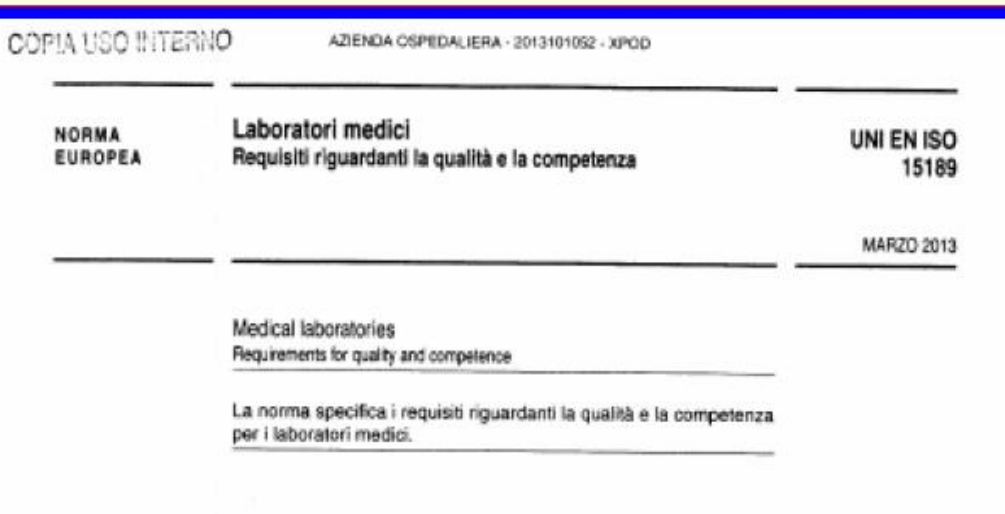
Body Fluid Analysis for Cellular Composition; Approved Guideline

E' compito dello Specialista di Medicina di Laboratorio stabilire opportune/e procedura/e operative per la corretta gestione dell'esame citometrico dei Liquidi in documenti che comprendano le seguenti fasi:

- **Pre-Analitica**
- **Analitica**
- **Post-Analitica**



Check-list di verifica CAP chiede le evidenze oggettive di ciascun punto descritto nel CSLI *document* H56-A



COPIA USO INTERNO AZIENDA OSPEDALIERA - 2013101052 - XPOD

NORMA EUROPEA

Laboratori medici
Requisiti riguardanti la qualità e la competenza

UNI EN ISO 15189

MARZO 2013

Medical laboratories
Requirements for quality and competence

La norma specifica i requisiti riguardanti la qualità e la competenza per i laboratori medici.

FASE PREANALITICA

Tipologia di provetta per la corretta raccolta del campione

Table 2. Specimen Requirements for Serous Fluids**

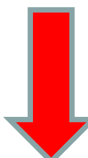
Tests	Anticoagulant	Volume (mL)
Cell count and differential cell counts	EDTA	5-8
Total protein, LD, glucose amylase	Heparin, none	8-10
Gram stain, bacterial culture	SPS*, none, or anticoagulant without bactericidal or bacteriostatic effect	8-10
AFB culture	SPS, none, or anticoagulant without bactericidal or bacteriostatic effect	15-50
PAP stain, cell block	None, heparin, EDTA	5-50

*SPS = Sodium polyanetholsulfonate

** Suggested specimen requirements

FASE PREANALITICA

Tempistica entro la quale deve essere eseguita la conta cellulare



Le provette devono essere trasportate in laboratorio a temperatura ambiente ed analizzate il più rapidamente possibile

Non devono passare più di 4 ore dal momento del prelievo al momento della conta cellulare

FASE ANALITICA

ESAME MACROSCOPICO

Aspetto: limpido, torbido, lattescente, ematico

Colore: rosso, marrone, verde, bianco, giallo

Deve essere segnalata la eventuale presenza di coaguli di fibrina

FASE ANALITICA

ANALISI DELLA COMPONENTE CELLULARE

CONTA DELLE CELLULE NUCLEATE E DEI GLOBULI ROSSI

L'analisi microscopica in camera è il gold standard.

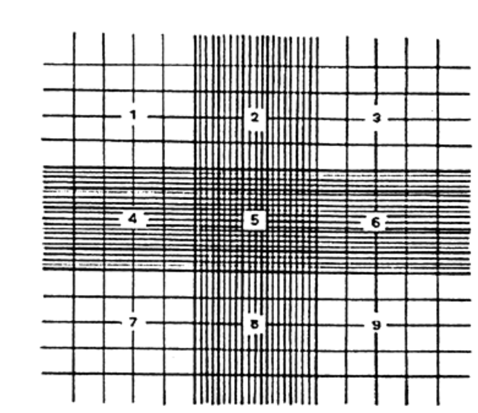


Figure 1. Hemocytometer Counting Area
Reprinted with permission from Medical Center Laboratory (www.MedicalCenterLab.org).

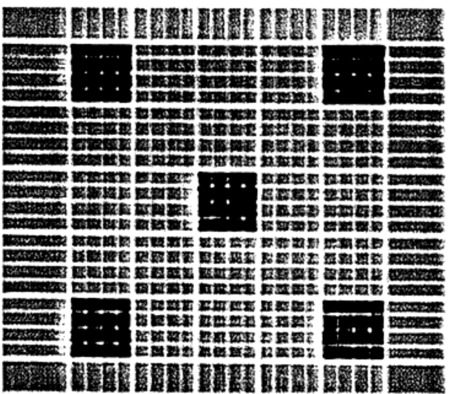


Figure 1A. Red Cell Counting Area
Reprinted with permission from Professor Richard A. Bowen, Dept. of Biomedical Sciences, Colorado State University.

CONTA MICROSCOPICA IN CAMERA

Conta in camera Nageotte
Cellule a fresco

La conta microscopica in camera presenta alcuni svantaggi:

Alta imprecisione, scarsa riproducibilità e standardizzazione

Risultato operatore-dipendente

Time-consuming (media tra più letture)

Personale qualificato h24

CONTA AUTOMATIZZATA CON I CONTAGLOBULI

Table 1. List of hematology analyzers most commonly used to perform automated body fluid counts

Analyzers	Fluids	Parameters reported
Beckman LH 750/780	Serous, synovial, cerebrospinal fluid (CSF)	WBC, RBC {WBC = TNC}
Beckman DxH 800	Serous, synovial, CSF	TNC, RBC
Sysmex XE 2100, XT 1800i/2000i,	Serous, synovial, CSF	WBC, RBC
Sysmex XT-4000 and XE-5000	Serous, synovial, CSF	BF Mode:WBC-BF, TC-BF, RBC-BF, 2 part diff (mononuclear/ polymorphonuclear)
Advia 2120, 2120i	Peritoneal, pleural, peritoneal dialysate	TNC, RBC
	CSF	TNC, RBC, 5 part diff and PMN/MN%
Iris iQ200 and iRICELL systems	CSF, pleural, peritoneal, peritoneal lavage, peritoneal dialysate, pericardial, synovial, general serous	Nucleated count, RBC

VANTAGGI METODO AUTOMATIZZATO

-Migliore riproducibilità ed accuratezza

- Rapidità d'esecuzione
- Non richiede una particolare formazione del personale tecnico

Int J Lab Hematol. 2018 Feb;40(1):26-33.

Two-site evaluation of the diagnostic performance of the Sysmex XN Body Fluid (BF) module for cell count and differential in Cerebrospinal Fluid.

Buoro S[1], Peruzzi B[2], Fanelli A[2], Seghezzi M[1], Manenti B[1], Lorubbio M[2], Biagioli T[2], Nannini S[2], Ottomano C[3], Lippi G[4]

XN-BF provides rapid and accurate counts in clinically relevant ranges of CSF values, thus providing a valuable alternative to conventional LM analysis. However, microscopic review remains advisable in samples with abnormal cell counts or high fluorescent (HF-BF) cell parameter exceeding 5×10^6 cells/L

Clin Chim Acta. 2016 Jan 15;452:92-8.

Cell Population Data and reflex testing rules of cell analysis in pleural and ascitic fluids using body fluid mode on Sysmex XN-9000.

Buoro S[1], Mecca T[2], Azzarà G[2], Seghezzi M[2], Dominoni P[2], Crippa A[2], Ottomano C[3], Lippi G

Our results suggest that the XN-BF module on Sysmex-9000 may be a suitable alternative to OM for screening BF samples, especially when specific validation rules are used

Am J Clin Pathol 2010 Feb;133(2):291-9.

Use of the Cell-Dyn Sapphire hematology analyzer for automated counting of blood cells in body fluids.

De Smet D, Van Moer G, Martens GA, Nanos N, Smet L, Jochmans K, De Waele M.

Clin Chim Acta 2013, feb 12

Performance evaluation and results comparison of the automated hematology analyzers Abbott CD 3700, Sysmex XE 2100 and Coulter LH 750 for cell counts in serous fluids**.**

Yang D, Zhou Y, Chen B.

J Clin Lab Anal. 2016 Sep;30(5):381-91.

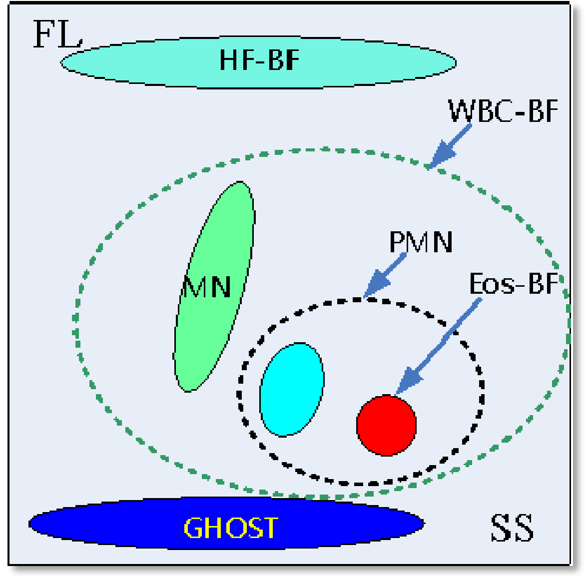
Automated Cerebrospinal Fluid Cell Counts Using the New Body Fluid Mode of Sysmex UF-1000i.

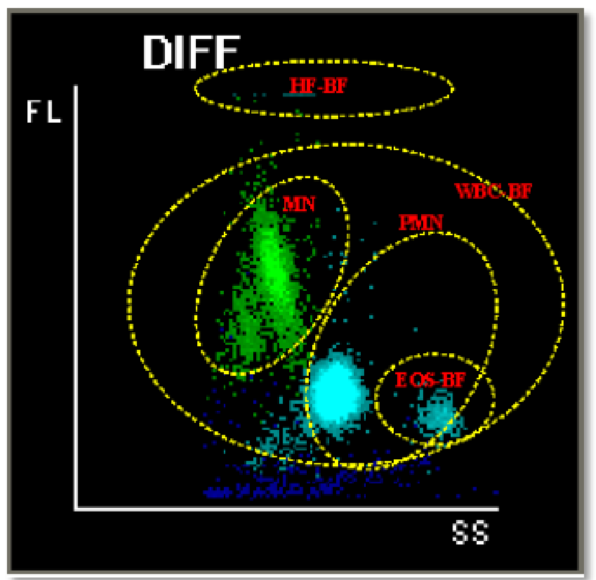
Buoro S[1], Apassiti Esposito S[2], Alessio M[2], Crippa A[2], Ottomano C[3], Lippi G[3]

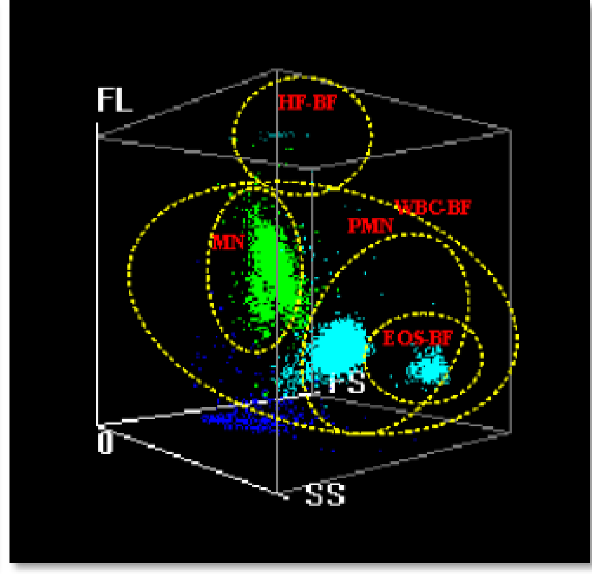
The UF1000i-BF provides rapid and accurate WBC and RBC counts in clinically relevant values of CSF cells. The use of UF1000i-BF may hence allow to replace routine optical counting, except for samples displaying abnormal WBC counts or abnormal scattergram distribution, for which differential cell counts may still be required

Principio di analisi BC-6800

Il canale DIFF fornisce il conteggio del numero totale dei WBC (WBC-BF) ed il numero totale degli elementi nucleati (TC-BF).
Vengono evidenziate le sottopopolazioni leucocitarie, cellule mononucleate (MN) e polimorfonucleate (PMN). Per solo uso di ricerca, il BC- 6800 fornisce risultati differenziali per: eosinofili , neutrofili e cellule ad alta fluorescenza (HF-BF).







CONTA AUTOMATIZZATA CON I CONTAGLOBULI

Adattare un apparecchio nato per contare e differenziare le cellule presenti nel sangue pone problemi per i seguenti motivi:

- **La presenza oltre le normali cellule ematiche di elementi diversi (cellule mesoteliali, macrofagi, elementi neoplastici isolati o in gruppi).**

- **Le cellule si trovano in ambienti solitamente diversi per costituzione da quelli plasmatici.**

- **In alcuni liquidi le conte sono talmente basse per cui è difficile avere risultati accurati con analizzatori ematologici.**

International Journal of Laboratory Hematology
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ORIGINAL ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

ICSH guidelines for the verification and performance of automated cell counters for body fluids

G. BOURNER*, B. DE LA SALLE†, T. GEORGE‡, Y. TABE§,¶, H. BAUM**, N. CULP††, T. B. KENG‡‡, ON BEHALF OF THE INTERNATIONAL COMMITTEE FOR STANDARDIZATION IN HEMATOLOGY (ICSH)

© 2014 John Wiley & Sons Ltd, *Int. Jnl. Lab. Hem.* 2014, **36**, 598–612

CONTA AUTOMATIZZATA CON I CONTAGLOBULI

Prima di inserire nella diagnostica routinaria la conta automatizzata degli elementi cellulari nei liquidi cavitari ogni laboratorio deve:

- **Verificare il limite funzionale dello strumento in uso, cioè il numero più basso di cellule nucleate e di eritrociti che lo strumento conta in modo accurato CV<20%**
- **Verificare come sono contate le cellule non leucocitarie**
- **Verificare come sono identificate le particelle non cellulari**
- **Definire e condividere all'interno del laboratorio regole di accettazione e validazione degli esiti del conteggio ed eventuali reflex test da eseguire in camera**
- **Validare l'intero procedimento analitico**

ANALISI DELLA COMPONENTE CELLULARE
VALUTAZIONE DELLA MORFOLOGIA

- La conta differenziale dei globuli bianchi nei liquidi cavitari è uno strumento essenziale per la diagnosi e il trattamento dei pazienti.

Citocentrifugazione con colorazione di May Grunwald Giemsa o Wright Giemsa modificato

Serie eritroide, mieloide e linfoide
Serie monociti/macrofagi
Lining cells
Cellule neoplastiche
Cellule varie
Microorganismi

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Vol. 25 No. 20

Body Fluid Analysis for Cellular Composition; Approved Guideline

8 Morphology Assessment

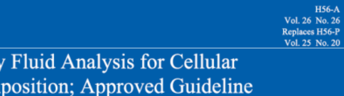
8.1 Slide Preparation

8.1.1 Cytocentrifugation

Wedge smears (push smears) should not be used with fluids because of their inferior ability in preserving intact cells. The cytocentrifuge preparation is recommended for air-dried body fluid slides, because this technique concentrates the cells, minimizes cell distortion, and produces a monolayer of cells. Romanowsky-type stained slides of cytocentrifuged CSF and other body fluids show excellent morphologic detail, and cells appear similar to their counterparts in blood or bone marrow. Cells typically are randomly dispersed in a small circular area, and a microscopic differential can be performed to subclassify the nucleated cells. When malignancy is suspected, the whole cellular area should be evaluated microscopically on each prepared slide, since malignant cells may be present in low frequency.

The cytocentrifuge instrument generally contains a centrifugation bowl with multiple slide assembly units. The assembly consists of a filter card placed upon a slide and a chamber to hold the sample, secured together by a clip. The outlet arm of the chamber is apposed to a hole in the filter card, exposing a round area on the glass slide. In the resting position, the fluid specimen in the chamber does not contact the glass slide. During centrifugation, the fluid and cells are forced out of chamber outlet onto the slide. The filter absorbs the fluid, while the cells are deposited on the slide.

Cells are concentrated approximately 20-fold by cytocentrifugation.[23] Even hypocellular samples with a chamber cell count of zero can have a yield of approximately 35 cells per slide.[23] The quantitative yield, however, varies from 30 to 75%,[24] and smaller cells, such as lymphocytes, may be underrepresented.[25] The speed and time of centrifugation, the amount of sample in the chamber, and the filter paper absorbance are factors that can influence both the cell yield and morphology.



Although the cytocentrifuge is not a complex instrument, some sample processing and instrument techniques can enhance slide quality[23]:

- Fresh, unfixed specimens should be used for slide preparation. Cells may begin deteriorating in a few hours, particularly in body fluid samples with low protein content, such as cerebrospinal fluid.[26] If there is a prolonged delay in preparing cytocentrifuge slides (i.e., more than four hours for CSF), the report should include a statement that the differential count may be inaccurate, due to cellular degeneration.

- Pleural, pericardial, peritoneal, and synovial fluid samples may contain fibrin and other proteins that can clog the filter card, reducing cell yield and affecting cell distribution on the slide. Washing the cells before cytocentrifugation, by centrifuging an aliquot of the sample and resuspending in saline, can improve both the cell yield and morphology.

- If clots are present, both the cell count and differential may be inaccurate. However, slides can be prepared and examined for malignant cells. The clots should be agitated gently to free trapped cells before aliquoting a portion of the sample for washing and cytocentrifugation.

- Viscous synovial fluids can be liquefied by adding 400 units of the enzyme hyaluronidase (solution or powder form) to approximately 1 mL of fluid, and incubating at 37 °C for ten minutes. Washing the cells after liquefaction also is helpful.

- Cellular samples or bloody samples need to be diluted with saline before cytocentrifugation to avoid overcrowded slides. Overcrowded slides are difficult to interpret due to clumping of cells and distortion of morphology. By using a standardized scheme for sample dilution based on cell counts, a slide with a uniform monolayer of cells can be obtained on every sample. The appropriate dilution will depend upon the amount of sample in the chamber and the cytocentrifuge speed and time. Alternately, for bloody samples, some laboratories prefer to gently lyse the erythrocytes before cytocentrifugation.

- Adding a drop of sterile, 22% albumin to the sample chamber before adding the sample enhances adherence of cells to the glass slide and reduces cell smudging or disintegration, particularly for low protein specimens, such as cerebrospinal fluid.

- Proper alignment of the sample chamber outlet port to the hole in the filter card is essential to optimize cell yield.

- Residual fluid remaining in the cell chamber after cytocentrifugation must not be allowed to flow back onto the slide. Air-dried cytocentrifuge slides for Romanowsky-type stain must be kept free of moisture until fixing and staining. If unfixed slides become wet, artifactual change occurs, resulting in a "shrunken" or "rounded-up" appearance to the cells.

8.1.2 Other

Alternative methods of cell concentration for morphologic evaluation include sedimentation methods,[14,27-30] and centrifugation with smears made from the resuspended sediment. These methods are difficult to standardize and produce smears of variable quality.[14] These alternatives are inferior to cytocentrifugation and are not recommended. Filtration methods[27,30-33] that are widely used in cytopathology laboratories are less practical for the hematology laboratory because they involve prefixation in ethanol, which precludes Romanowsky-type staining of air-dried smears.

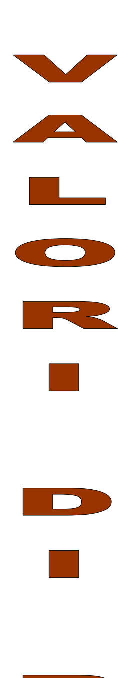
8.2 Identification of Morphologic Constituents

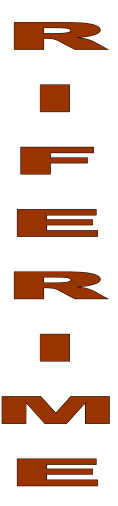
The following descriptions apply to properly prepared cytocentrifuge slides optimally stained with Romanowsky stains.[14,34,35] Differences from typical blood or bone marrow aspirate morphology are emphasized. Because cytocentrifugation produces a thin cell monolayer, cells may be slightly larger than their counterparts in blood or bone marrow aspirate smears. More intense staining of basophilic cytoplasm and azurophilic cytoplasmic granules also may occur.

Limiti

Tutti quelli di una metodica manuale

apparentemente semplice, operatore dipendente, Specialista di Laboratorio esperto in morfologia cellulare dei liquidi cavitari e liquor, imprecisione elevata ecc.





Appendix C. (Continued)

Table C2. Reference Intervals for Pleural Fluid. (From Noppen M, De Waele M, Li R, et al. Volume and cellular content of normal pleural fluid in humans examined by the pleural lavage. *Am J Respir Crit Care Med*. 2000;162:1023-1026. Official Journal of the American Thoracic Society. ©American Thoracic Society. Reprinted with permission.)

Volume (mL/cavity)	4.1-12.7 mL
Nucleated cell count	1395-3734/µL
Macrophages	64-80%*
Lymphocytes	18-36%*
Neutrophils	0-1%*
Mesothelial cells	0-2%*

*Results expressed as interquartile range.[7]

Table C3. Peritoneal Dialysate Cell Count and Differential in Noninfected Drainage Fluids (n=29). (Modified from Rubin J, et al. Peritonitis during continuous ambulatory peritoneal dialysis. *Ann Intern Med*. 1980;92:7-13. Reprinted with permission from the American College of Physicians.)

Red blood cells/µL	24 ± 48*
Total nucleated cells/µL	36 ± 48
Leukocytes/µL	21 ± 27
Neutrophils (%)	18 ± 15.8
Lymphocytes (%)	24 ± 26
Monocytes (%)	35 ± 26
Eosinophils (%)	7 ± 7
Basophils (%)	3 ± 2

*Results expressed as mean ± SD.

Liquido ascitico

- Un cut-off per i neutrofili <250 cellule/µL è diagnostico di ascite sterile
- Un cut-off per i neutrofili di ≥250 cellule/µL è diagnostico di peritonite batterica
- Un conteggio di elementi nucleati totali ≥ 1000 elementi/µL con predominanza di linfociti è diagnostico di peritonite tubercolare

Liquido pleurico

- Conteggio degli elementi nucleati totali > 10000/µL sono associati con versamenti parapneumonici
- neutrofili >50% indicativo di infiammazione acuta o versamento parapneumonico
- eosinofili > 10% indicativo di pneumotorace o embolia polmonare o malattie parassitarie o funginee o allergie o sindrome di Churg-Strauss.
- linfociti >50% indicativo di tubercolosi , carcinoma, malattia linfoproliferativa o versamento chiloso

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AM68

ANALYTIC PERFORMANCES OF SYSMEX XE5000, SIEMENS ADVIA 2120I AND CELLAVISION DM96 FOR COUNTING AND CLASSIFYING CELLS OF PERITONEAL DIALYSIS EFFLUENT

Mayumi Idei[1], Yoko Tabe[1], Kazunori Miyake[1], Chieko Hamada[2], Hiroyuki Takemura[3], Hiroaki Io[2], Kiyoshi Ishii[3], Takashi Horii[3], Yasuhiko Tomino[2], Akimichi Ohsaka[3], Takashi Miida[1]
[1]Department of Clinical Laboratory Medicine, Juntendo University School of Medicine Tokyo, Japan, [2]Division of Nephrology, Department of Internal Medicine, Juntendo University School of Medicine Tokyo, Japan, [3]Division of Clinical Laboratory, Juntendo University Hospital Tokyo, Japan

Objectives: Peritonitis and encapsulating peritoneal sclerosis (EPS) are the serious complications of peritoneal dialysis (PD). The total nucleated cell (TNC) count and the differential count of polymorphonuclear cells (PMNs) / mononuclear cells (MNs) of the peritoneal dialysis effluent (PDE), usually performed by manual microscopic method, is critical for diagnosis of peritonitis. Whereas a large mesothelial cell is a useful predictive marker for EPS, the conventional microscopic method is labor-intensive and imprecise. The objective of this work was to evaluate the ability of the automated analyzer systems Sysmex XE-5000 and Siemens ADVIA2020i and the digital microscopy system CellaVision DM96 to count and classify cells in PDE.

presence of large mesothelial cells. **Conclusions:** These results confirmed that Sysmex XE-5000 and Siemens ADVIA2020i-BASOmode give accurate and precise TNC count and PMNs / MNs differentiation in PDE test. The automated digital image processing utilizing the overview option by CellaVision DM96 may be useful for further cell classification and non-hematological mesothelial cell detection. Observation of mesothelial cells for prediction of EPS should be performed in non-peritonitis phase.

Morfologia con analizzatore di immagini: vantaggi analitici

- **Standardizzazione della zona di analisi**
- **Conteggio rapido e meno faticoso**
- **Pre classificazione dei globuli bianchi con possibilità di confronto con immagini di riferimento**
- **Maggiore oggettività morfologica**
- **Maggiore sensibilità nel riconoscimento di cellule rare**
- **Possibilità di analizzare diversi tipi di materiali (liquor, liquidi cavitari, midollo osseo)**

Morfologia con analizzatore di immagini: vantaggi fella connettività

- Visualizzazione a distanza delle immagini da operatori diversi
- Progetti di telemedicina
- Archiviazione di immagini semplice e non deteriorabile
- Progetti di insegnamento
- Competency software per armonizzare la professionalità degli specialisti
- Sussidio all'addestramento e alla formazione continua del personale di laboratorio

Morfologia con analizzatore di immagini: vantaggi operativi

- Standardizzazione dei processi con miglioramento del workflow
- Totale tracciabilità dei dati

Morfologia con analizzatore di immagini: svantaggi

- Visualizzazione ottimale richiede attrezzature di alta qualità e competenze
- Hardware e software richiedono standardizzazione
- Gli utilizzatori devono avere esperienza con la lettura dei vetrini al microscopio e confidenza con le prestazioni strumentali
- Non viene visualizzato tutto il vetrino

EasyCell

Aperio

Bloodhound™ System

FDA-approved Hematology Digital Cell Image Analyzers

Capability	CellaVision DM96*	CellaVision DM1200	Medica EasyCell
Slide Handling	12 slide per magazine. Up to 8 magazines on board	12 slide per magazine Batch analysis	Carousel for up to 30 slides
Target Market Slide Volume	50+ Slides per day	25 - 150 slides per day	15-50 slides per day
On Board Capacity	96 Slides	12 Slides	30 slides
Slide Scan Time (WBC, RBC, PLT)	~ 2 minutes	~ 2 minutes	~ 4 minutes
Throughput (WBC,RBC,PLT)	Up to 30 slides/hour	up to 20 slides/hour	up to 15 slides/hour
STAT Capable	NO	NO	YES
Power used for imaging	10x, 50x 100x	10x, 50x 100x	10x & 100x
Body Fluid Analysis (Optional)	YES	YES	NO
Approximate price	$135K	$100K	$65K

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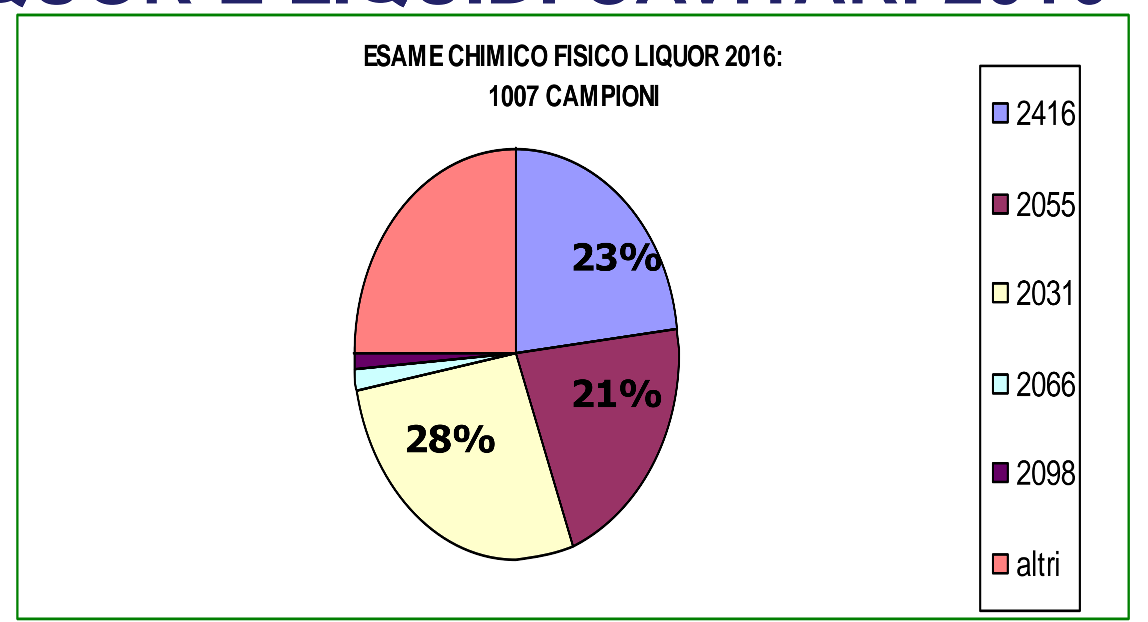
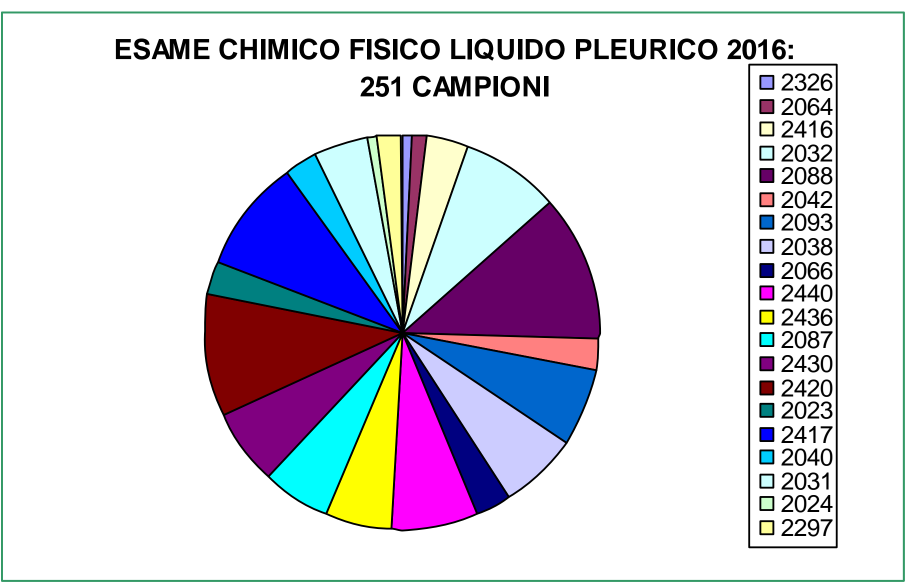
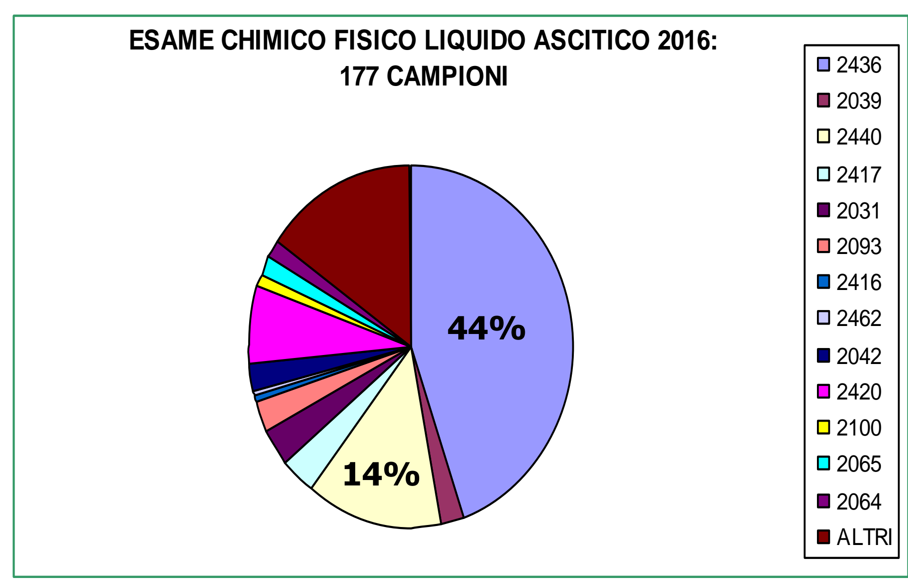


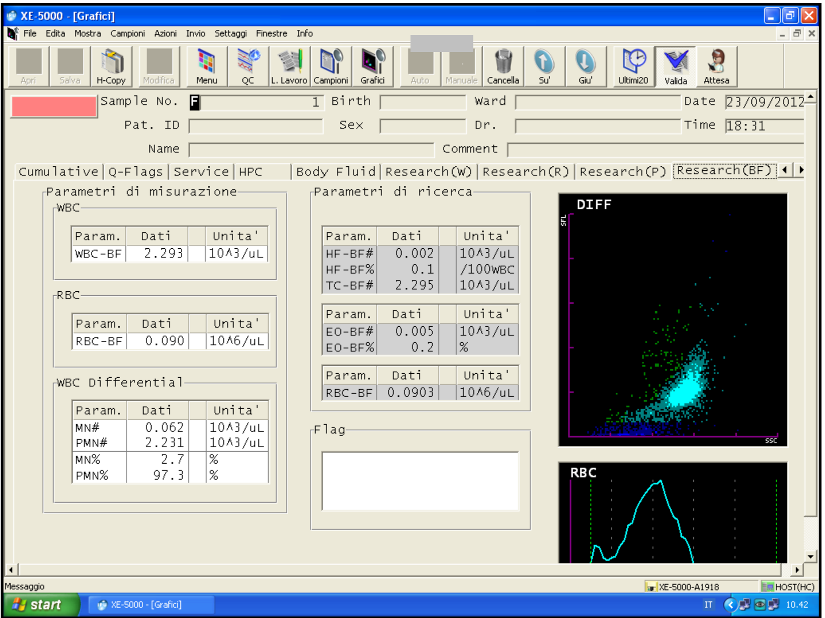




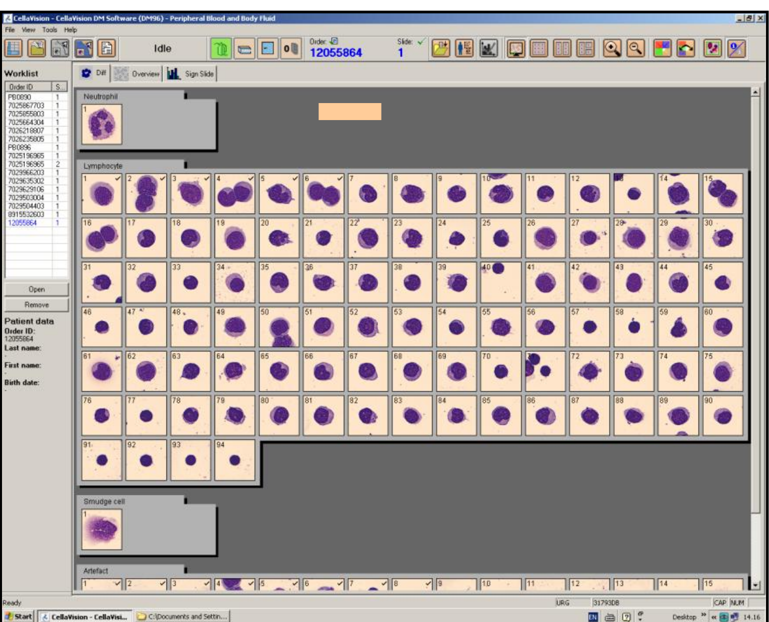
Performance evaluation of DM Body fluid software module using the automated digital cell morphology analyzer CellaVision DM96

[1]Peruzzi Benedetta, [1]Caporale Roberto [1]Gelli Anna Maria Grazia and [1]Fanelli Alessandra,
[1]General Laboratory – Flow Cytometry Unit, AOU-Careggi.

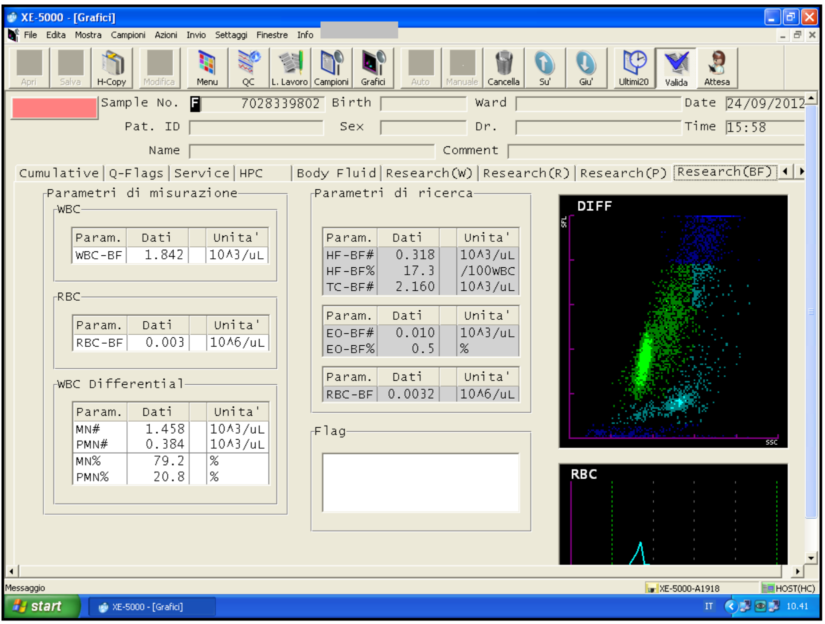
Materials and Methods: A total of **40 body fluids samples (10 CSF, 12 AF, 16 PIF, 1 SF, 1 PeF)** were analysed with the **XE-5000** to obtain cell count and WBC differentiation into Mononuclear and Polymorphonuclear cells. A **cytospin preparation** (Cytofuge2, Iris Int.) were performed using 250-300 ul of a cells suspension (20 cells /ul) to obtain a cell pellet of approx. 5000 cells; slides were left to air-dry and stained with the Wright-Giemsa method. **Slides were analyzed using the DM96**. PMN% and MN% reported by the XE-5000 were compared with PMN% (Neutr.+Eos.) and MN% (Lymph.+Mono/Macroph.) obtained at the DM96 after cells re-classification. Statistical analysis was performed by Pearson correlation and by Bland-Altman plot. % of Other cells classified with the DM96 (eg. Mesothelial, Tumor cells) were compared by Spearman correlation with % HF (High Fluorescence cells) reported by the XE-5000.



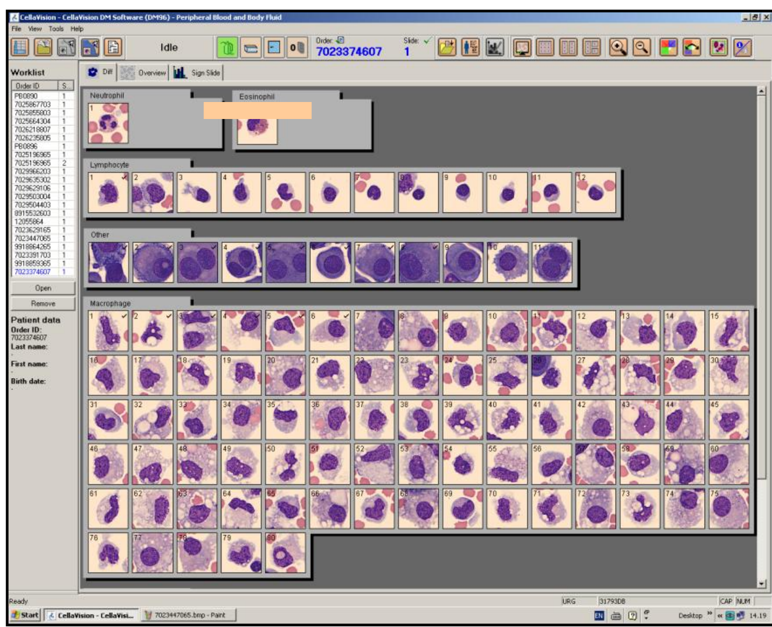
Examples of scattergrams from Sysmex XE-5000 analysis.



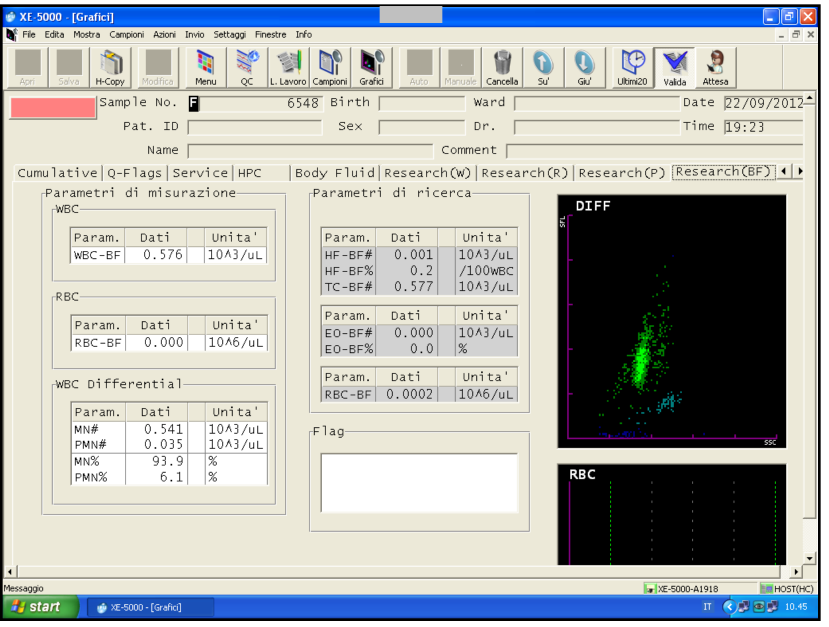
Figures of white blood cells presented on the CellaVision DM 96 computer screen



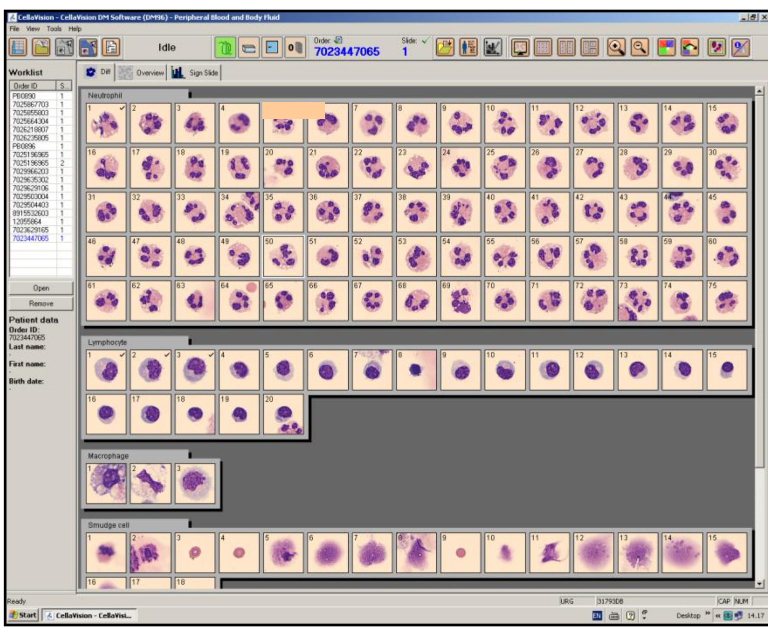
Examples of scattergrams from Sysmex XE-5000 analysis.



Figures of white blood cells presented on the CellaVision DM 96 computer screen



Examples of scattergrams from Sysmex XE-5000 analysis.



Figures of white blood cells presented on the CellaVision DM 96 computer screen

Results: The comparison between XE-5000 and DM96 show very good correlation for PMN% (r=0,97; p<0,0001) and MN% (r=0,96; p<0,0001).
A slight positive bias of the XE-5000 (3,71; 0,87 to 6,54; p=0,032) was observed for low PMN counts.
HF-BF% shows a significant correlation with Other cells (r=0,50; p=0,0011).

Conclusion: From our experience the use of the XE-5000 in combination with the DM96 can significantly improve automation and standardization in the diagnostic work-up of body fluids analysis. Furthermore, **DM96 represents a valuable tool to increase diagnostic accuracy, overall quality in morphological analysis, documentation of analysis results (digital archiving) and of the identification of the responsibility of slide interpretation.**

White blood cell analysis (WBC differential count) in body fluids: comparison between the Sysmex XE5000 and manual flow cytometry

[1]Peruzzi Benedetta, [1]Gelli Anna Maria Grazia,[1]Fanelli Alessandra and [1]Caporale Roberto
[1]General Laboratory – Flow Cytometry Unit, AOU-Careggi.

Materials and Methods: WBC differential and count cells of a total of **43 BFs (5 cerebrospinal, 23 ascitic, 15 pleural fluids)** were performed on the **XE5000** using the dedicated BF mode.
Flow cytometry analysis of all samples was assess in a **FACS Canto II using moAb CD45 FITC**; the analysis was performed using a gate strategy of a combination of **side scatter vs CD45** expression to differentiated granulocytes (PMN) from monocytes/macrophages and lymphocytes (MN). Debris were excluded by eliminating all the events that were smaller than lymphocytes.

Results:

The comparison between XE5000 and CFM show very good correlation for:

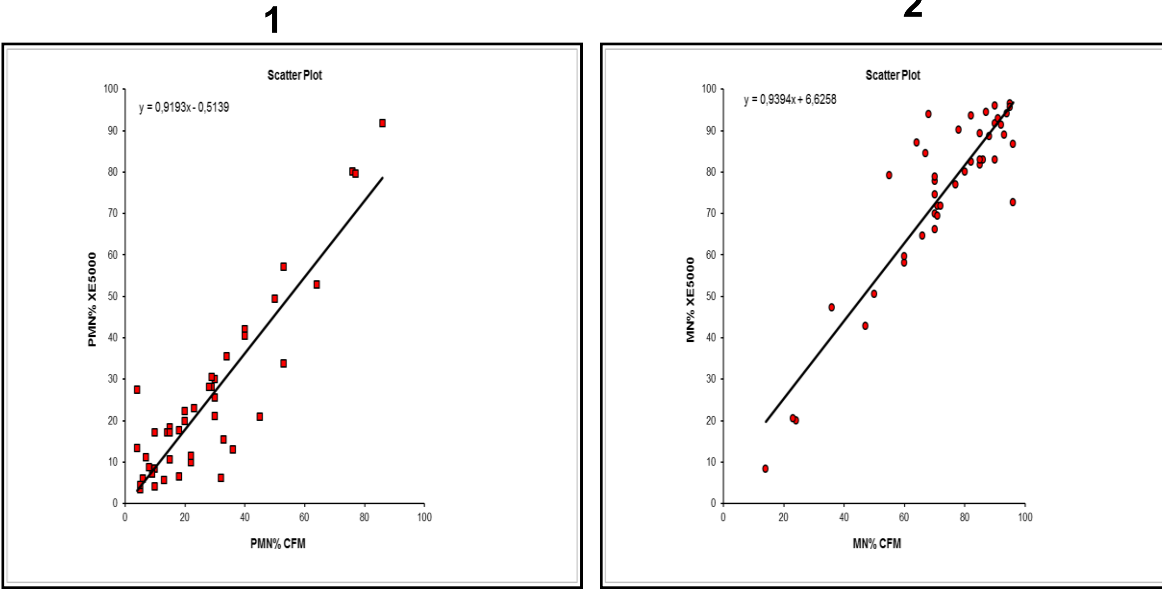
1. PMN% (r=0,90; p<0,0001); mean bias -2,30 (95% IC from -5,02 to 0,41; p-value=0,0944)

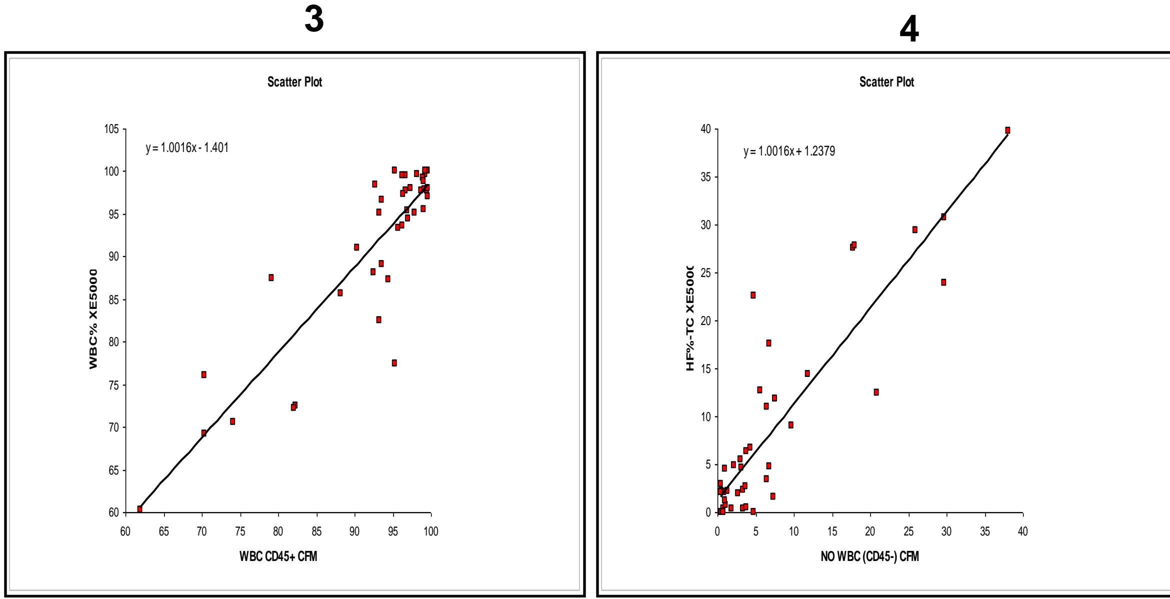
2. MN% (r=0,91; p<0,0001); mean bias 1,77 (95% IC from -0,81 to 4,35; p-value=0,1741)

3. WBC% XE vs %WBC CFM (CD45+cells): r=0,89; p value <0,0001; mean bias -1,25 (95% IC from -2,73 to 0,23; p-value=0,0961)

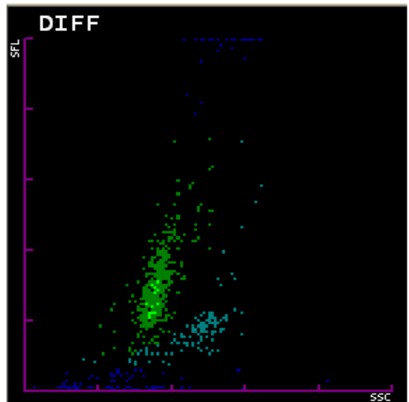
4. %HF_TC XE vs % NO WBC CFM (CD45- cells): r=0,89; p-value<0,0001; mean bias 1,25 (95% IC from -0,23 to 2,73; p-value=0,0961)

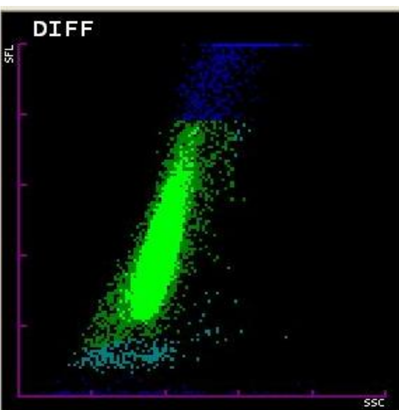
All those analysis shown no significant differences.



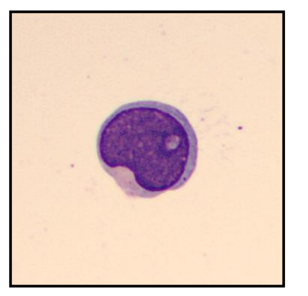


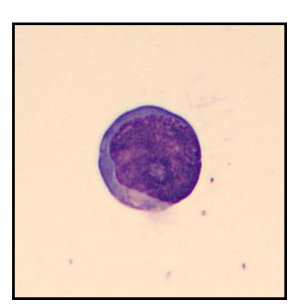
Conclusion: Our works demonstrate that the BF mode of the Sysmex XE5000 is a suitable tool for a rapid and reliable screening of WBC differential counts in Body Fluids analysis, providing also a good discrimination between WBC and non WBC cells.

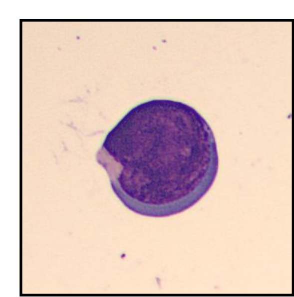


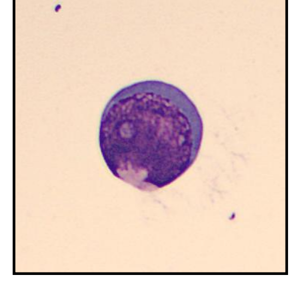


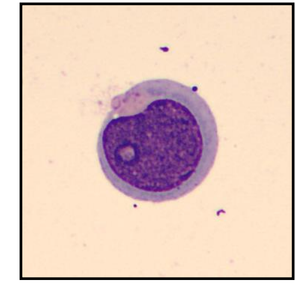
Case report

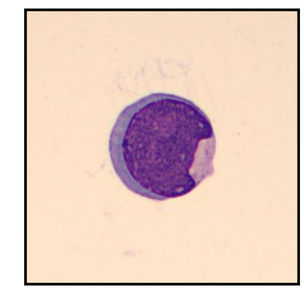




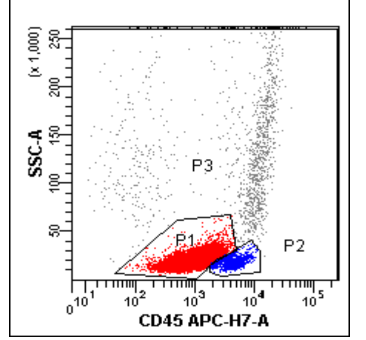


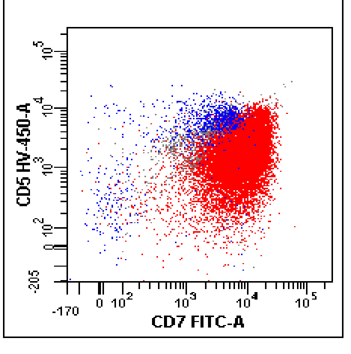


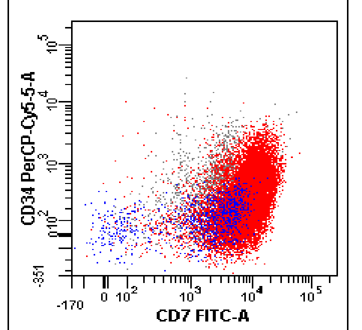


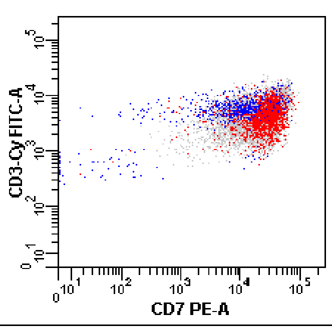


We report on a case of **ALL- T diagnosis made through BF study** on a 31 years-old man admitted to the Emergency Room in August 2012 due to chest pain, dyspnea, cough and fever. The initial blood count was normal (WBC 8.55 X 109/L, RBC 4.71 X 1012/L, Hb 14.3 g/dL Plt 194 X 1012/L). **There was no morphological evidence of atypical cells in the peripheral blood smear.** No lymph node enlargement or petechiae was noted.









SCOPO

La presente procedura ha lo scopo di definire un algoritmo "appropriato" che tenga conto di tutte le possibilità diagnostiche in corso di sospetta meningite batterica ottimizzando le risorse disponibili ed i test disponibili.

Tale algoritmo è basato sul consenso di esperti microbiologi clinici, chimico-clinici e clinici e sulla revisione delle evidenze di letteratura.

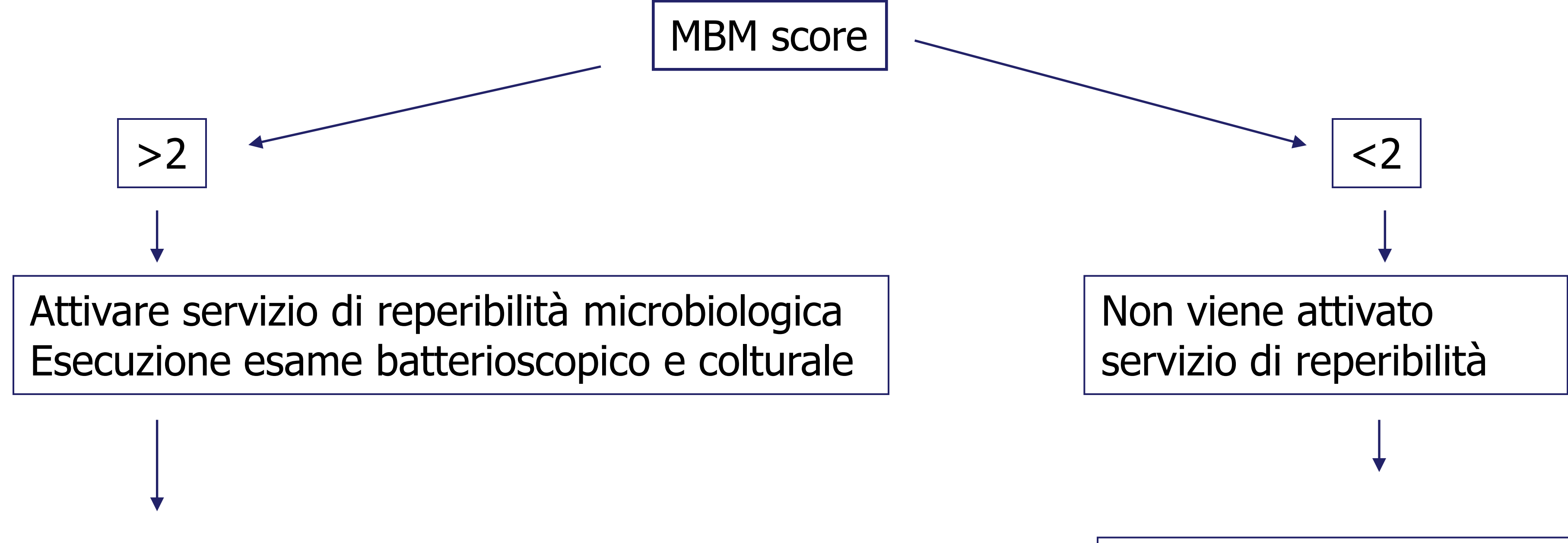
Procedura attiva dal 2014 per tutti i liquor provenienti da DEA Careggi

MBMS (Modified Bacterial Meningitis Score), è stato validato retrospettivamente su 463 campioni di liquor pervenuti dal DEA della AOUC dal 01.01.2010 al 31.12.2012 con sospetta diagnosi di meningite batterica, avvalendoci dei risultati chimico-fisici refertati dal Laboratorio Generale della nostra Azienda e dei dati microbiologici forniti dal nostro laboratorio. Nel corso dei tre anni relativi allo studio la diagnosi di meningite batterica è stata confermata nel 3.5% (15/463) dei campioni esaminati. Tutti i casi di meningite batterica accertata hanno dimostrato di avere uno score ≥4

Modified Bacterial Meningytis Score

analita	valore	punteggio
Conta cellule nucleate del liquor	>50/µl	2
Proteine totali liquor	>0.8 g/L	1
Lattato nel liquor	>3.3 mmol/L	1
Glucosio liquor/ glucosio siero	<45%	1
Conta neutrofili sangue perferico	>10.000/µl	1

Diagramma di flusso



Indagini molecolari (PCR) a discrezione del microbiologo o concordate con il reparto richiedente

Il Dirigente di guardia invia mail al Laboratorio di microbiologia
Viene segnalato l'arrivo del campione e dove vengono conservate le aliquote di liquor e flaconi per emocoltura

Esame chimico-fisico maggio 2014/ maggio 2015

180
160
140
120
100
80
60
40
20
0

70%
9%
8%
4%
3%
2%
3%

0 1 2 3 4 5 6

mbm score

	Cellule liquor n° /microlitro	Lattato mmol/L	Proteine g/L	Rapporto glucosio liquor/glucosio siero %	Neutrofili n° / microlitro	mbm Score	Agente eziologico
1	1231 Prevalenza Polimorfonucleati	11,3	4,6	ndt	12600	6	Streptococcus pneumoniae
2	1243 Prevalenza Polimorfonucleati	18,7	4,41	4	45000	6	Streptococcus Pneumoniae
3	4088 Prevalenza Polimorfonucleati	14,4	2,25	2	17000	6	Enterococcus faecium
4	250 Prevalenza Polimorfonucleati	8,1	2,97	24		6	Streptococcus Gruppo B
5	Tappeto di cellule con netta prevalenza di polimorfonucleati	17,3	12,5	ndt	14000	6	Streptococcus Pneumoniae
6	2680 Prevalenza Polimorfonucleati	7,1	1,18	**46**	25000	5	Neisseria meningitidis
7	870 Prevalenza Polimorfonucleati	13	10,16	18	41000	6	Escherichia Coli
8	792 Prevalenza Polimorfonucleati	16,8	4,06	ndt	38000	6	Streptococcus pneumoniae

La conta dei globuli bianchi e la loro differenziazione morfologica effettuate con l'analisi automatizzata non sembrano superare completamente, nelle attuali strumentazioni automatizzate, i limiti dell'analisi manuale.

Infatti, analogamente alla conta manuale, anche i grafici e le immagini fornite dagli strumenti necessitano in alcuni casi di un occhio esperto in grado di fornire una corretta interpretazione.

LG H56-A: Fase Post-analitica

Nel **referto** deve essere sempre riportato:

- Il tipo li liquido esaminato
- La descrizione macroscopia
- Per l'esame citometrico: il conteggio totale degli elementi nucleati e la differenzione come previsto. Se disponibili i cut-off di cellularità patologica per tipo di liquido e se indicato per età. Se presenti cellule atipiche devono essere segnalate ed indicata la necessità di approfondimento.
- Per gli esami biochimici, gli esiti e dei dosaggi e delle RATIO quando disponibili e relative soglie di patologia

H56-A IFCC 2006 "B*ody fluid Analysis for Cellular Composition; Approved Guideline*"

C49-A IFCC 2007 "*Analysis of body Fluids in Clinical Chemistry, Approved Guideline*"

Nel referto devono essere chiaramente indicati tutti i tipi cellulari identificati e contati in valori percentuali

Ogni altro ritrovamento morfologico deve essere riportato e commentato