Rigshospitalet

Body Fluids at Copenhagen University Hospital

MARGIT GROME, Dept. of Clinical Biochemistry, Rigshospitalet

Ruth and Annette.



Agenda

- Introduction
- BF's analysed on SYSMEX XN
- Cytospin: Preparation and DM96
- Case stories

Rigshospitalet, Copenhagen





Accreditation:



RH: Den Danske Kvalitetsmodel (DDKM).

 Dept. of Clinical Biochemistry is accredited according to ISO 15189

Number of analyses/year: >5 million



Amount and type of BF's in 2013:

| •CSF | 3220 |
|------|------|
|------|------|

- Peritoneal Fluid (ASC)6
- Synovial Fluid3
- Pleural Fluid 64

Total: 3293

Average per week: 63

RBC - manual count:153 (in 4 months)



BF's can be sent to:

- Dept. of Clinical Biochemistry (24 hours/day)
- Dept. of Pathology
- Dept. of Microbiology
- Dept. of Clinical Immunology (Flowcytometry)
- Other special laboratories



A useful guide:

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

Body Fluid Analysis for Cellular Composition; Approved Guideline

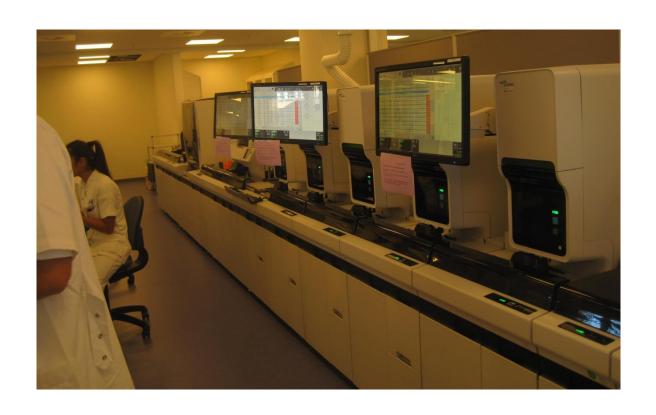
- "...Use properly prepared cyto-centrifuge slides optimally stained with Romanowsky stains (May-Grünwald-Giemsa)."
- "...In the nucleated differential, all cells derived from the Hematopoietic system should be included. The term mononuclear cell should be avoided, since the term does not adequately distinguish monocytes from lymphocytes, a distinction that has diagnostic significance".



From July 1 2013 we have used SYSMEX XN APP: Body Fluid (BF) mode



From June 2014 we have used the complete SYSMEX system: 3 Sysmex XN, 1 SP-10, 1 TS 2000 and (1 Tosoh G8).



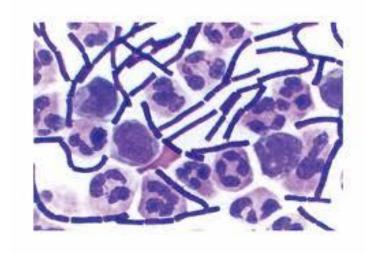


UNITS

In BF cell-count results, we use the unit

- cells X 10⁶/L
- ~ million cells/L

for both nucleated cells (WBC) and RBC.





Reference-intervals:

| NPU | Name | Reference- interval KB3011 10 ⁶ /L |
|-----------|--|---|
| NPU 28838 | Cerebrospinal fluid—Nucleated cells and RBC. | < 5 < 5 |
| NPU 28840 | Pleural fluid—Nucleated cells | < 1000 |
| NPU 28837 | Peritoneal fluid—Nucleated cells | < 100 |
| NPU 28839 | Synovial fluid—Nucleated cells | < 200 |

From: Body Fluid Analysis for Cellular Composition: Approved Guideline. Vol 26, and McPherson RA, Pincus MR, ed. Henry's Clinical diagnosis and management by laboratory methods. 21st ed. Philadelphia: Saunders, 2007:442-3.



The Body Fluid application was validated... and a report written according to SOP.

Method comparison:
 Counting chamber method vs. Sysmex XN.

For CSV: Nucleated cells (WBC) and RBC

For other BF's: Nucleated cells (WBC)

Lower detection limit - nucleated cells (WBC)

Lower limit (Sysmex): 0 x 10⁶/L

- Own analysis
- 4 CSF-samples with low WBC-konc. (<5 x 10⁶/L)
- Every sample analysed 10 times
- Average value and SD (intra-assay) calculated
- Detection limit is set to 0 + 5 SD
- Conclusion: SD: 0,49 x 10⁶/L
- Our lower WBC- Detection limit: 3 x 10⁶/L

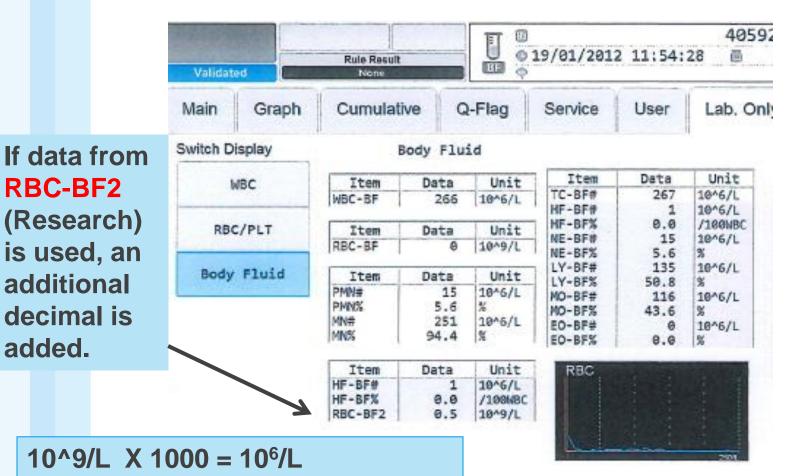
Example: $0.5 \times 1000 = 500 \times 10^6/L$

RBC-BF2

additional

added.

Lower RBC-detection limit (Acc. Sysmex): 1000 x 10⁶/L, which was unacceptably high for us.



Lower detection limit RBC:

- Analysis of 5 samples with low count of erythrocytes (<500 x 10⁶/l)
- Detection limit is set to 0 + 5SD
- Average SD: 52,7 x 10⁶/L
- Lower detection limit: $264 \times 10^6/L = 300 \times 10^6/L$
- A detection limit of 300 x 10⁶/L is higher than the current limit, but it is clinically acceptable ... or so we thought ...



Alarm system Sysmex XN:

| Alarms: | | |
|-----------------------------|---------------------------------------|---|
| @ | Next to result | Dilute – or analyze as blood. |
| WBC Abn Scattergram | IP message | Check number of nucleated cells by manual count |
| " * " (STAR) or (3 dots) | Next to result – or instead of result | Very, very rare – but check number of nucleated cells by manual count |

Conclusion:

- The method fulfils all of the established quality demands.
- And we started using the Sysmex XN for BF's.
- BUT...

We received an e-mail:

- It turns out that normal atraumatic spinal fluids are now given with erythrocyte numbers <300 x 10^6/L. Previously, they were given as <3 x10⁶/L with normal spinal fluids.
- This is of considerable importance to our department, as a certain number of RBC >10 x 10⁶/L equals a traumatic puncture in our protocols. For leukaemia, a traumatic lumbar puncture at the time of diagnosis means an increased risk of spread to CNS, and this will often trigger extra intrathecal chemotherapy.
- At present, we thus find ourselves unable to distinguish between these situations (traumatic versus atraumatic lumbar puncture).



Change of handling – CSF RBC < 300 x 10⁶/L

- With samples from certain wards (children, oncology and haematology), we count RBC manually if the concentration of RBC in CSF is < 300 x 10⁶/L (counted on SYSMEX XN).
- SYSMEX is working on solving the problem.

New NPU-codes and parameter names for BF cell count and diff count?

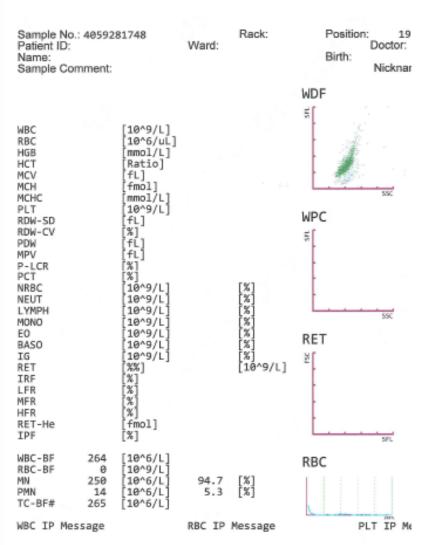
- We do not believe that WBC and other nucleated cells can be separated from each other by automated or manual methods.
- (Tumour cells, erythroblasts, fungi, microorganisms, and more).
 This can only happen with manual differential count in the microscope/Cellavision DM96.
- Therefore, we decided to change the name in all BF's so Instead of WBC-concentration, from July 1 2013 we have used:

E.g.: CSF-Nucleated cells; conc. (Unit: x 10⁶/L) IFCC-IUPAC-code: NPU028838

... and we will use the total count (TC-BF#) as result.



Print – SYSMEX XN: WBC-BF or TC-BF#?



WBC-BF: 264 x 10⁶/L

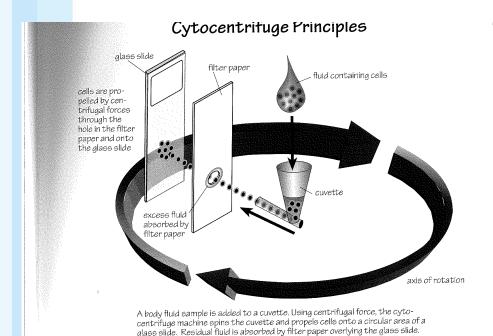
TC-BF: 265 x 10⁶/L

We always use TC-BF#.



Cytocentrifuge-preparation (we have used this for many years)





Introduction

Shandon Cytospin:

Optimal amount WBC's per pellet:

Approximately 5000.

Optimal amount of liquid:

300-500 μl (Max. 500 μl).

We always add:

1 drop of 20 % Albumin.

Speed:

13

1000 (700) RPM.

(Optimized in 2008)



Manual diff count of BF's

If the concentration of nucleated cells is

 $> 10 \times 10^6/L$

we will make a differential count.



The Cytospin slides are stained in Sysmex SP-10

Staining method: May-Grünwald – Giemsa

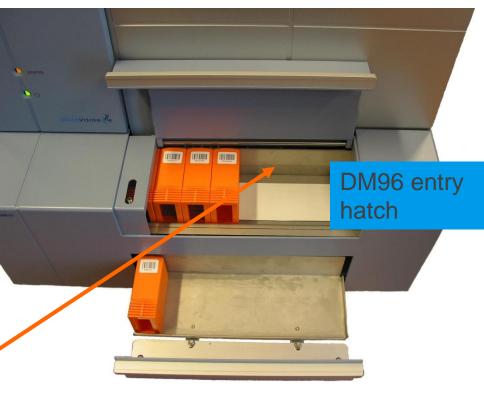


CellaVision DM96 for BF-diff 's since 2008

CSV-Cytospin-prep. stained in SP1000i:

Loading of the preparation:



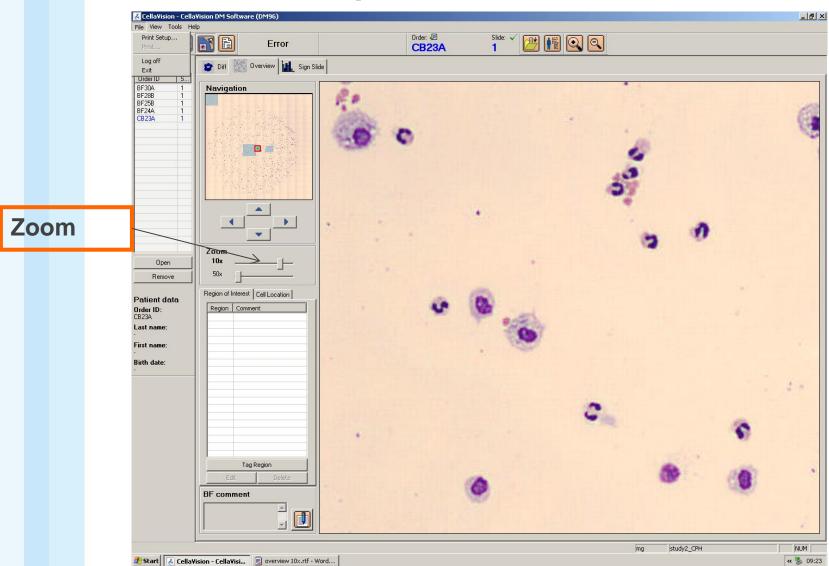


NOTE!:

BLUE cassette for body fluids.

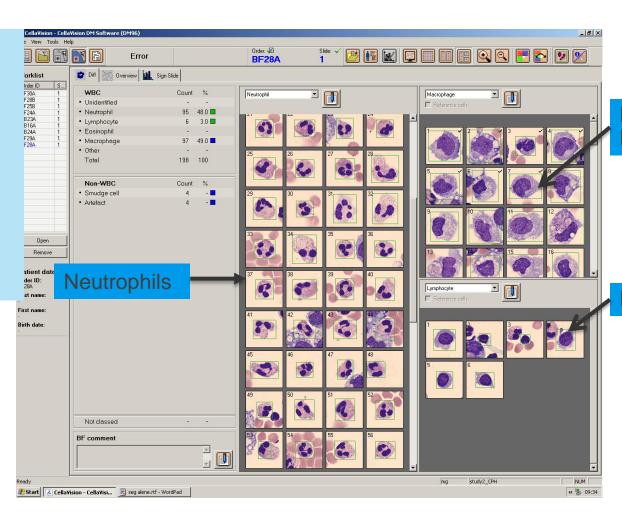


Overview of pellet



BF differential count in CellaVision DM 96 - screenprint where 3 cell classes are shown.

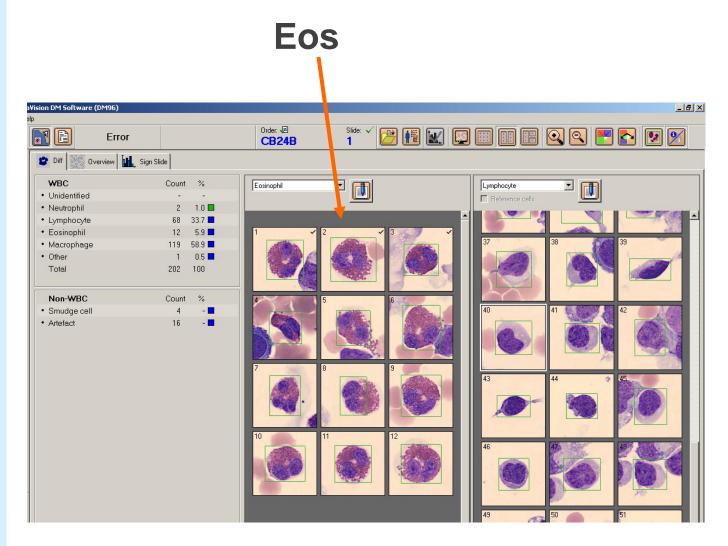
The neural network prevalidates cells, and Lab Technician checks and moves cells if necessary and signs.



Monocytes/ Macrophages

Lymphocytes

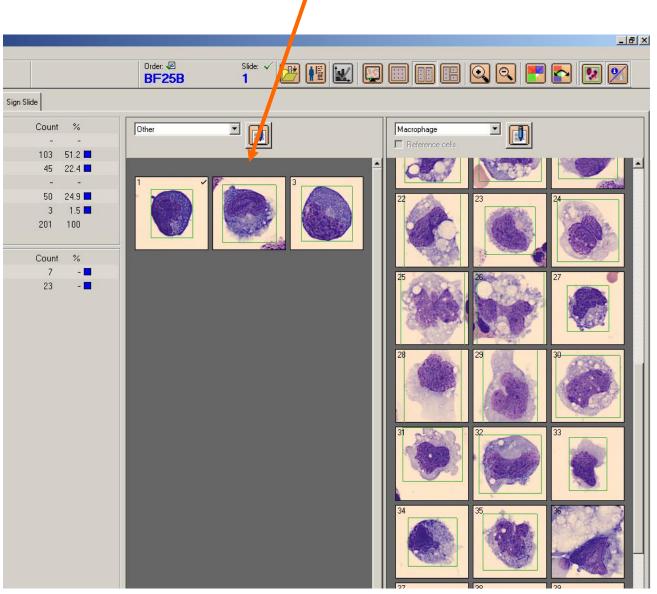




Bodyfluids and DM96 KB3011



et Plasmacells



OBS.

Are placed in "Other"

Bodyfluids and DM96 KB3011

Differential count of body fluids:

| | Previously (Manually) | Since Dec. 2008 |
|------------------|---------------------------|--------------------------|
| Number of cells: | 100 (3) | 200 (5) |
| Cell classes: | Neutrocytes | Neutrocytes |
| | Lymphocytes and monocytes | Lymphocytes |
| | "Other" | Monocytes and macrofages |
| | | Eosinophiles |
| | | Other |
| | | (Unidentified) |

Smudge cells?

- Are a problem.
- We "include" them in the result if they are > 20%-
- We place them in "Other" and give a comment.
- "Other" we use to so many other cell-classes:
 Mesothelia-cells, blasts, basofiles, plasmacells

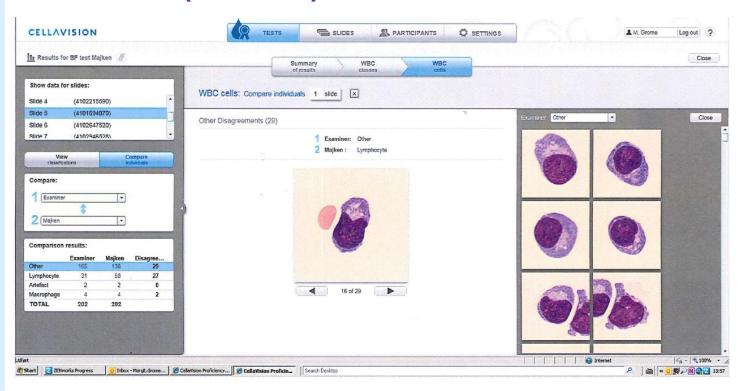
So: New cell type:

- DNK35265 Plv(spec.)—Smudge celler; antalk. = ? x 10⁶/L
- DNK35264 Csv—Smudge celler; antalk. = ? x 10⁶/L
- DNK35266 Asc—Smudge celler; antalk. = ? x 10⁶/L
- DNK35267 Ledv(spec.)—Smudge celler; antalk. = ? x 10⁶/L
- DNK35269 B—Smudge celler; antalk. = ? x 10⁹/L
- "Celler" = Danish word for cells.



Training? Blood- Diff.: 22 technologists CSV- Diff: 32 technologists

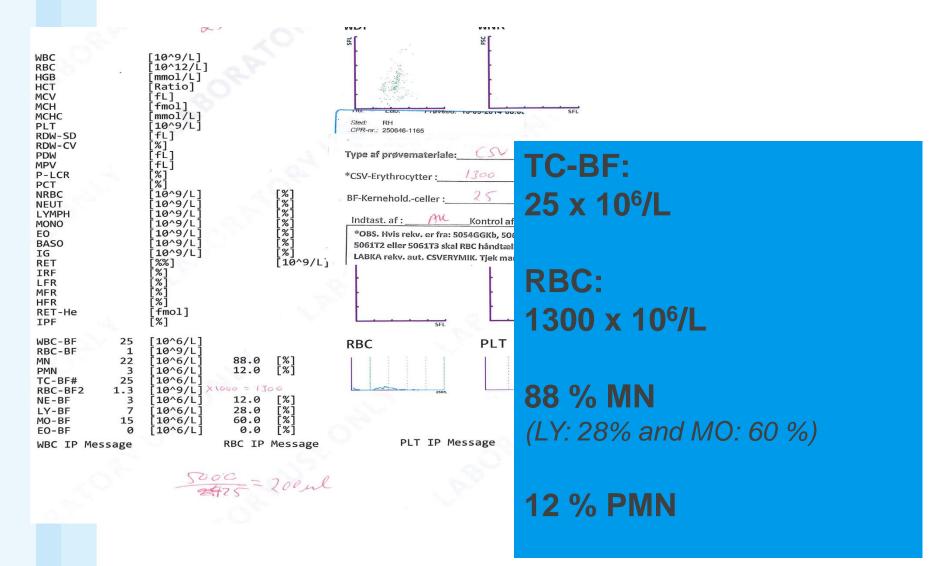
CellaVision Competency software/ Proficiency Software (BF-test)



Cases:

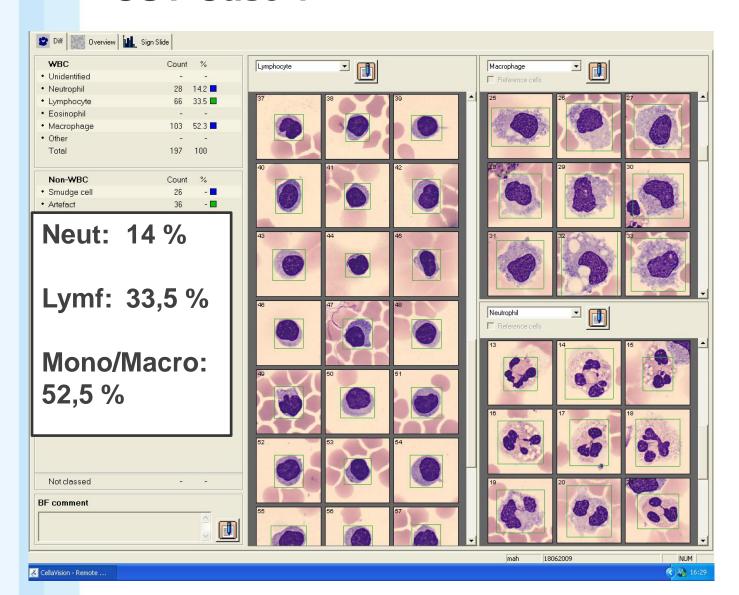


CSF Case 1: Dept. of Neurosurgery





CSV Case 1



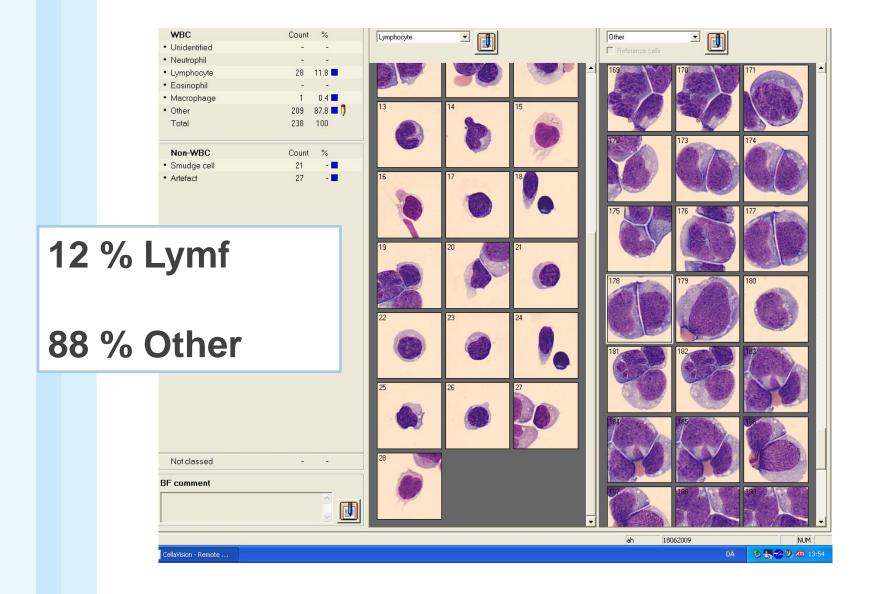
Diagnosis:

Glioblastoma in occipital region.

Infection in shunt?

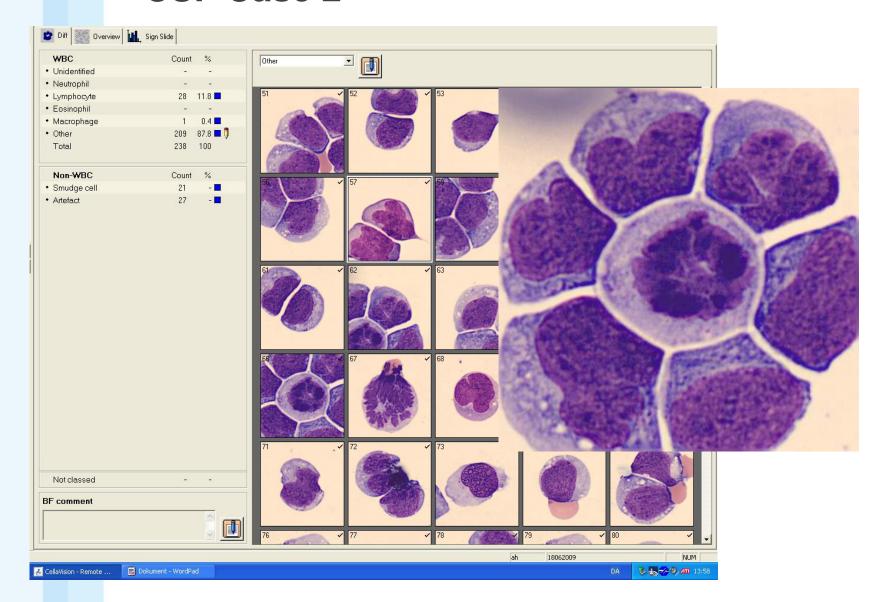


CSF Case 2





CSF Case 2





CSF Case 2 LIS (LABKA) Print

LABORATORIEUDSKRIFT

Fra Søg svar
Bestillingsformål= Behandling

Dannet 16-09-2014 kl. 15:02:59

| - 1 | Rigshospitalet | 1883 |
|------------------------|---|--|
| | WW - 2 2 2 2 3 1 1 1 3 1 | |
| 40556 65687 2012 | | |
| mar | 3 | Rigshospitalet |
| 26 | Kinisk Bio | kembik afdeling |
| 14:45 | | |
| 2094NI | | ф |
| 2094NI | D. | NAK |
| , www. | | , |
| New 2012/06/2012 184 | 12.24 | |
| 35 | ×10 ⁶ /1 | <=1 |
| 4,0 1 | mmoV | 2,2-3,9 |
| Udført | | |
| | | |
| ~ ° | ×10 ⁶ /1 | |
| 165 | ×10 ⁶ /1 | |
| 22 1 | ×10 ⁶ /1 | <5 |
| 1 , | ×10 ⁶ /1 | |
| 0 | ×10 ⁶ /1 | <1. |
| 188 f | ×10 ⁶ /1 | <5 |
| _ 0,86 1 | g/l | 0,15-0,50 |
| | 35 4.0 1 Udfart 35 4.0 1 Udfart 35 4.0 1 Udfart | CRH CRH |

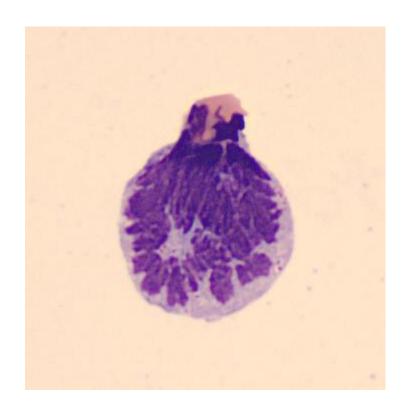
CSV- WBC (nucleated cells): 188

CSV-RBC: 35

A note to "Leukocytter (uspec.) – "OTHER": "Dominated by immature, mononuclear cells. Mitosis is detected. "



Case 2: NK-T-cell lymphoma with CNS-infiltration. (Confirmed by dept. of pathology)





CSF Case 3:

Male, 19:

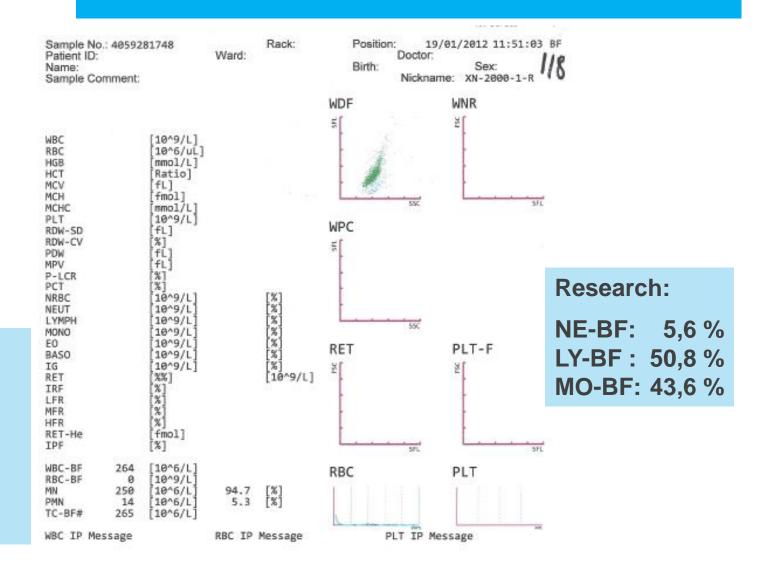
• 2007: Pre B-ALL

April 2010: Finished treatment

August 2010: Isolated CNS



Case 3: CSF-sample with blasts. Sysmex XN-print (2012):

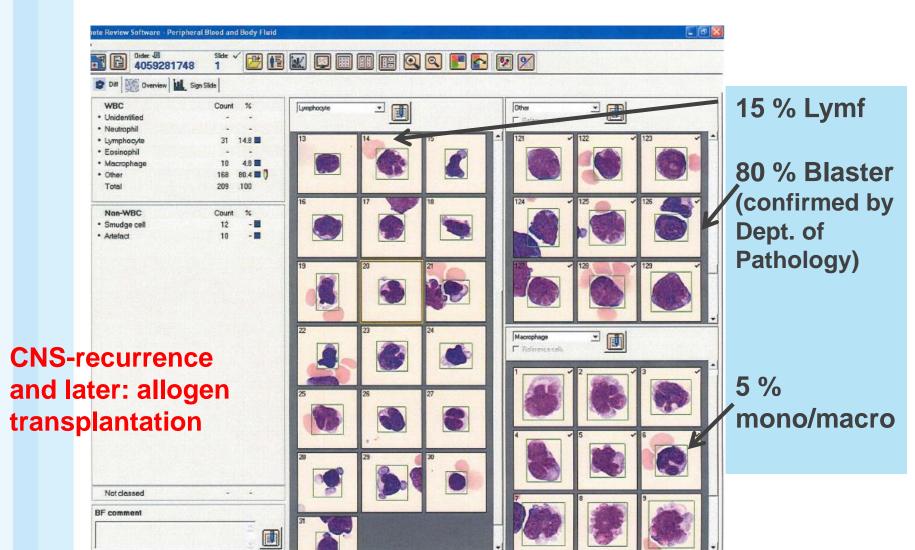


No alarms TC-BF#: 265

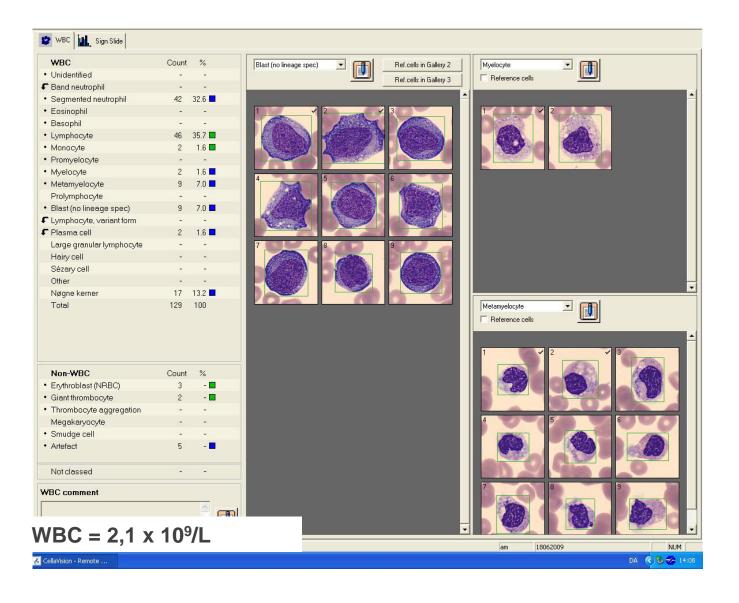
MN: 95 %

PMN: 5%

Case 3: Same sample with blasts (CellaVision DM96-screenshot)

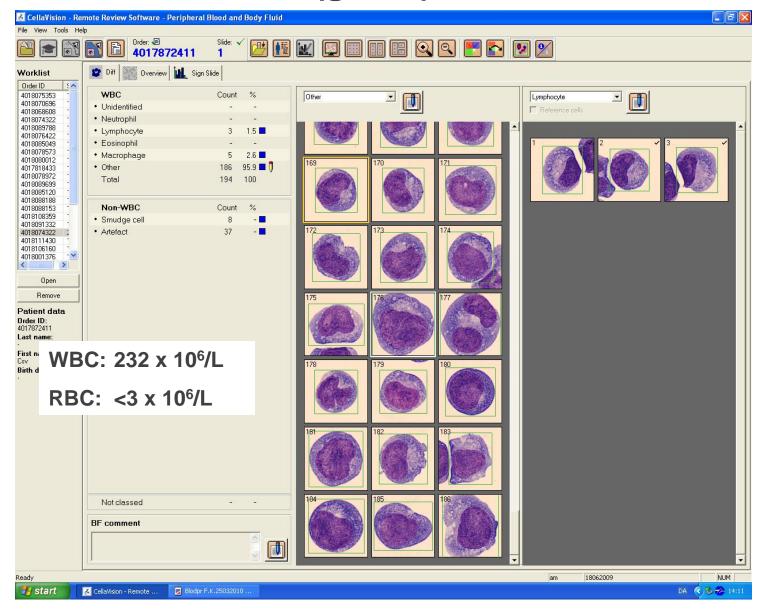


Case 4: Blood diff (girl, 7) - AML



Rigshospitalet

Case 4: CSF diff (girl, 7)





Case 4: CSF diff (girl, 7) - AML

- Our comment:
 - Other: Large, immature cells
- Saturday evening: We made 9 Cytospin-slides and sent them to Dept. of Pathology
- Their result was:
 - Cell-rich cytospin with many large cells, primarily blast cells and early myeloid precursors. Few RBC's. CNSleukaemia



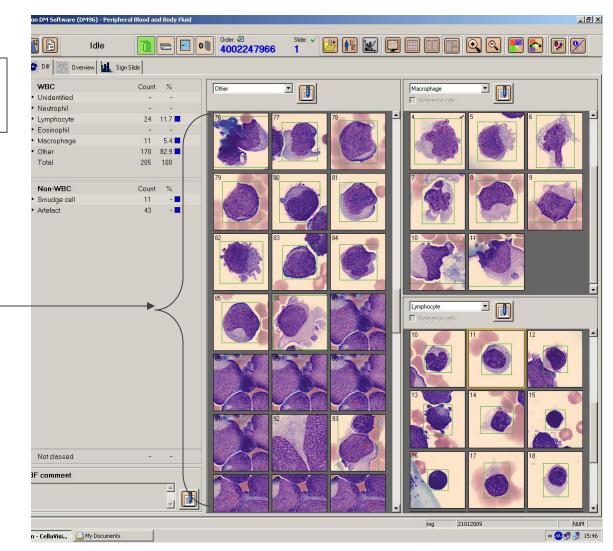
Case 5:

Diagnosis: Brain tumour (Neuroepiteliom)

CSF RBC: 931 x 10⁶/l CSF WBC: 50 x 10⁶/l

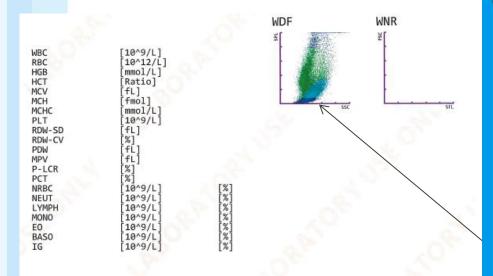
Comment to "other":

Immature, "blast-like" cells.





Case 6: CSF

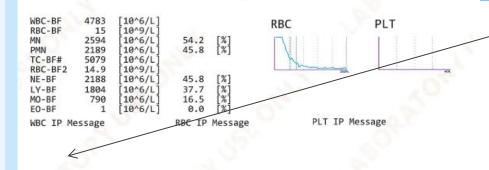


WBC-BF: 4783- 4916

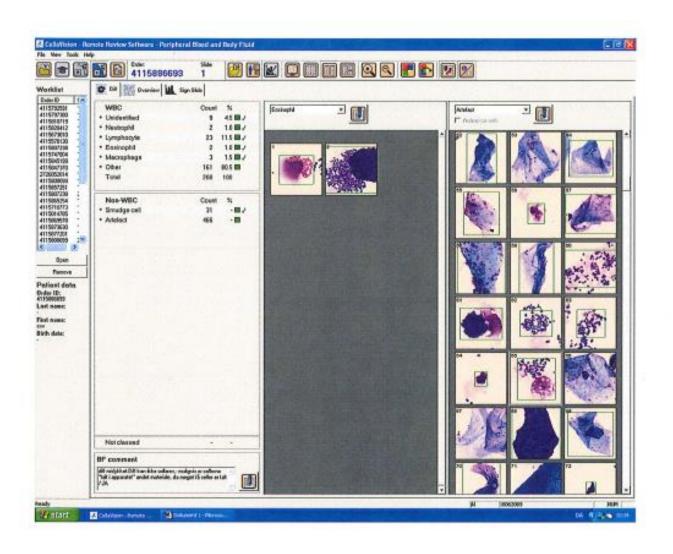
TC-BF: 5079-5264 -

RBC: 14900

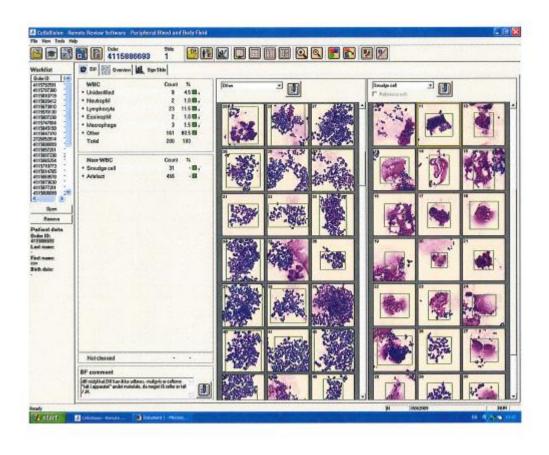
Lots of debris – no alarm (Abn. Scattergram)



Case 6:



Case 6:



Lots of bacteria and some smudge cells.

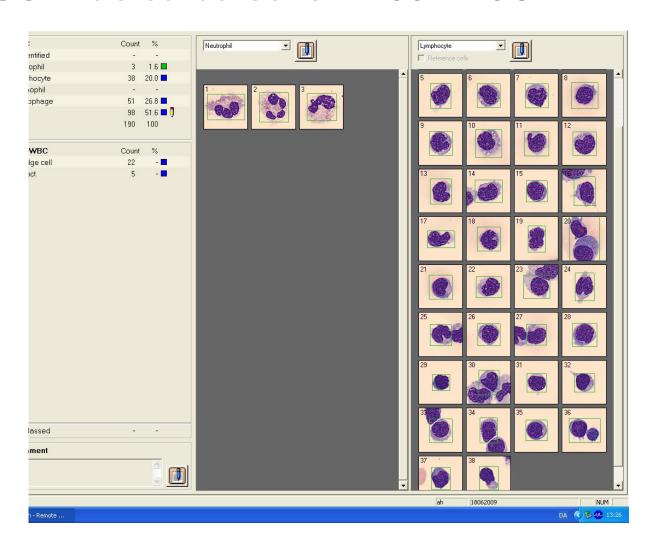
Case 6:

- Patient from Dept. of Neurosurgery.
- Woman, 43. Postoperative complication in ventricular chamber. Previously congenital hydrocephalus. Now VP shunt.
- 1 month previously: Surgical removal of infected bone plate.
- Dept. of Microbiology found: A few neutrophils, numerous Gram-positive rods in clusters.
- Problem: No alarm (abn. Scattergram). Sysmex is involved, and are at the moment looking into the problem in Japan.



Case 7:

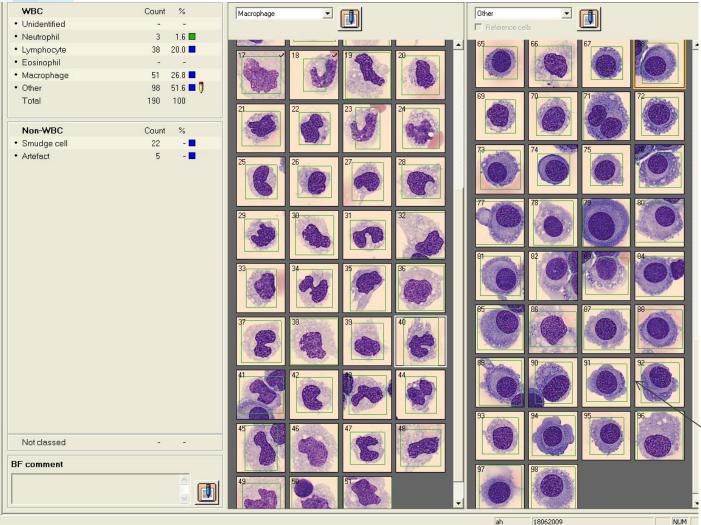
ASC: Nucleated cells: 1130 x 10⁶/L





Rigshospitalet

Case 7: Patient with chronic Hepatitis C (alcoholic)



Dept. of Pathology:

Blood – and mesothelia cells. No malignancy.

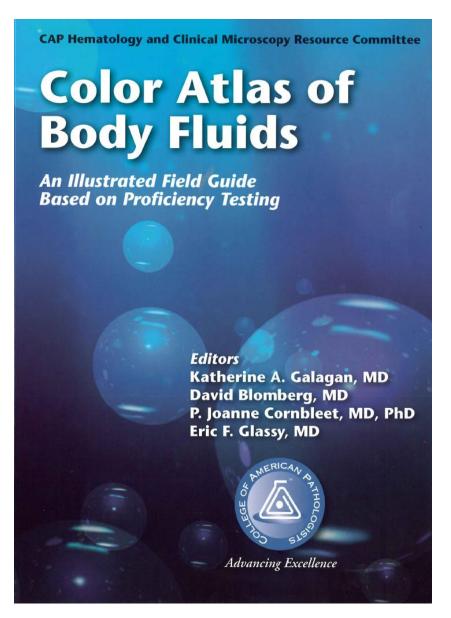
Other =
Dominated
by
Mesothelia
cells











From CAP

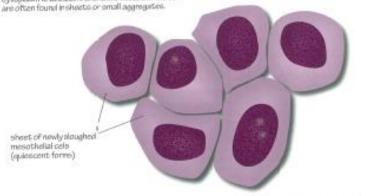
ISBN: 0930304918

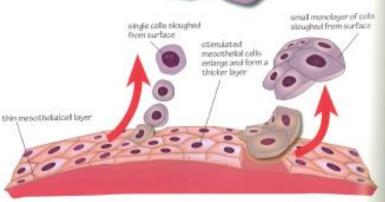
THANK YOU FOR YOUR ATTENTION.

Mesotherial cells:

Formation

The serious membranes are surfaced by a single layer of flat resting mesoth eltal cells. In response to a variety of stimuli, the layer thickens, and single or small sheats of cells may be shed into the serious cavity. At this point, the masothelal cells are called splescent they are small in size, ranging from 20 to 50 µm in dameter. The nucleus is often expentitionally-placed and is round to oval with condensed nuclear chromatis, a thick ruclear membrane, and inconspicuous maleoli. The ight blue or dark thus cytoplaem is aburdant and lacks vacuoles. The cells





"The layer of flat cells of mesodermal origin that lines the embryonic body cavity and gives rise to the squamous cells of the peritoneum, pericardium, and pleura."