HEM.BF.1.0 CEREBROSPINAL FLUID CELL COUNT AND DIFFERENTIAL

PRINCIPLE

To perform the hematologic examination of spinal fluid for identifying macroscopic and microscopic elements and a WBC differential count in percentages of cell types. Specialized instructions are included for specimens from oncology patients.

OWNERS

Manager, St Vincent Indianapolis Hospital Laboratory

SPECIMEN COLLECTION

A. Patient Preparation:
Collection is by an invasive procedure. Be careful to conserve sample as recollection may not be possible.

B. Specimen Type:
1. Cerebral spinal fluid specimens usually consist of 3 to 4 sterile prenumbered vials.
2. Vial #3 is the hematology specimen of choice.

NOTE: Occasionally a request for a cell count on another vial # is received. Verify this order with appropriate personnel and proceed as instructed.

3. Cerebral spinal fluid specimens may on a rare occasion be collected in an anticoagulant-free tube.
4. Fluid should be transported, stored, and tested at room temperature.
5. Process spinal fluids immediately. Cells begin to lyse within one-half hour of collection.
6. Check all CSF specimen tubes for proper identification and labeling.
7. Keep all spinal fluid specimens for one week unless otherwise instructed by the physician.

SAFETY PRECAUTIONS:

Care should be taken when handling all spinal fluids, as with any other specimen.

When spinal fluids are hazy or cloudy (contain an increased number of WBC's) or if the diagnosis is meningitis or suspected meningitis, extra precautions should be taken as follows:

a. Place the used hemocytometer and coverslip in a container filled with 10% bleach for one minute. Remove the hemocytometer and coverslip and rinse thoroughly with distilled water. Dry with a Kimwipe.
b. Be sure to wash hands thoroughly after working with the spinal fluid.

REAGENTS

A. Normal saline

B. BMP LeukoChek (BMP-LUKCHK) 1:100 dilution with buffered Ammonium Oxalate 20 ul capillary pipet and 1.98 ml reservoir

C. Wrights Stain

D. Bovine Albumin 22%. Store at 2-8□C.

NOTE: All fluids must be checked microscopically for contamination. Place a drop of fluid on a glass slide and coverslip and record on logsheet.

E. Brilliant Cresyl Blue Stain

EQUIPMENT

A. Hemocytometer with coverslip
B. Non heparinized capillary tubes
C. Pipettes with tips
D. Falcon tubes
E. Petri dish with moist filter paper
F. Manual counter
G. Single frosted end glass slide
H. Cytocentrifuge
I. Cytocentrifuge filter paper
J. Slide stainer
K. Microscope
L. Kimwipes
M. InCyto – C-Chip
N. Urisystem Slip
O. Coverslip and slide

CALIBRATION

None

CONTROLS

A. Use standardized Neubauer hemocytometer or C-Chip

B. Check all fluids for background count prior to use.

C. Spinalscopics (Quantimetrix Corporation) Spinal Fluid Cell Count Controls Level 1 and Level 2. One cell control is tested once every 8 hours of patient testing.

D. Unopened controls are stored tightly capped at 2-8°C. Do not freeze. When stored unopened at 2-8°C, the controls are stable until the expiration date stated on the label. Once opened, the controls are stable for 6 months when stored at 2-8°C between uses.

E. Note: Discard the controls if there is any evidence of microbial contamination. The level 2 control may appear slightly turbid after mixing.

F. Remove the controls from the refrigerator and replace the cap on the control bottle with the dropper cap included in the control box. Allow the control to come to room temperature (18-25°C) for at least 15 minutes, depending on remaining volume. Mix the controls thoroughly by inverting the bottles several times and by squeezing the bulb in the cap and aspirating and expelling the control through the glass dropper attached to the cap at least 10 times immediately prior to use to assure homogeneity of the contents. (Avoid foaming.)

G. Controls should be treated like a patient. Using the glass dropper, charge both sides of the hemocytometer chamber. Immediately recap the controls, and return to the refrigerator. NOTE: During microscopic examination of the Spinalscopics cell count control, clumps of 5 or more white blood cells may be observed. These clumps should not be included in the final cell count. When the clumps are ignored, cell counts should fall within the package insert range.

H. Allow the cells to settle by placing the hemocytometer in a humidified chamber for 10 minutes before counting. Count the cells in the 9 squares on both sides of the hemocytometer. Average the number of cells counted on both sides. Calculate the total number of red and white cells per uL using the formula.

$$\frac{\text{Average number of cells counted}}{0.9} = \text{Total cells/uL}$$

I. If any control results are outside the acceptable range:
   1. Recount the chamber.
   2. Charge a new hemocytometer chamber with fresh specimen.
   3. If results are still outside the acceptable range, charge a new hemocytometer using a new vial of quality control.
   4. If results remain out, inform a supervisor; do not report patient results until QC is acceptable.
   5. Document appropriate corrective action.

J. Quality Control Results:
   1. QC data must be recorded in the computer:
      a. Access Function: MEM
      b. Enter appropriate worksheet
      c. At Test-1 press ENTER
      d. Enter control accession number C-SS1 for Spinalscopics Level 1 and C-SS2 for Spinalscopics Level 2.
      e. Enter the results for WBC and RBC.

2. Acceptable ranges for the lot number of QC in use will be verified before reporting patient results.

PROCEDURE—CEREBROSPINAL FLUID CELL COUNT

NOTE: Always mix sample well before performing any cell count.

A. Examine CSF for color, appearance and xanthochromia.
   1. Color -- should be determined against a white background.
   2. Appearance -- of well-mixed uncentrifuged specimen.
   3. Xanthochromia -- done on centrifuged specimen if CSF is not clear and colorless; perform after completing all cell count testing.
   4. If Xanthochromia is present report “Positive”. If Xanthochromia is not present report “Negative”.

      NOTE: Xanthochromia is the brown, green, red, pink, orange, or yellow color of the supernatant. It is indicative of subarachnoid hemorrhage or other factor. If color of centrifuged specimen is different than color reported please note in result.See color chart below.

B. Due to the variety of specimens received in the laboratory, it is not possible to outline a procedure for each specimen. The following are intended as dilution guidelines:
   1. Clear and colorless -- Plate undiluted.
   2. Hazy or slightly cloudy (with no microscopic evidence of RBCs) -- Plate undiluted. If there are too many white cells to count, dilute the WBC with normal saline. Count the RBC undiluted.
   3. Hazy or slightly cloudy with evidence of RBCs (pink color) -- Dilute with normal saline. Count both the WBC and RBC from this dilution. If there is some confusion in differentiating RBCs and WBCs, dilute the fluid with WBC Unopette to count the WBC. This will lyse the RBCs and leave only the WBCs or use phase microscope.
   4. Cloudy specimen with gross blood -- Dilute the WBC count 1:20 or 1:100 with LeukoChek reservoir or with normal saline.

      NOTE: A drop of CSF placed on a slide with a coverslip or urisystem slide well may be examined for necessity of diluting.

C. If diluting is required, prepare the smallest possible dilution for an accurate count.

D. Some diluting guidelines:

Dilution	Sample	Diluent
1:5	100 ul	400 ul
1:10	100 ul	900 ul
1:50	100 ul	4.9 ml

Retain the dilution factor for calculations.

E. Mix prepared dilution well before plating.

F. Hemocytometer

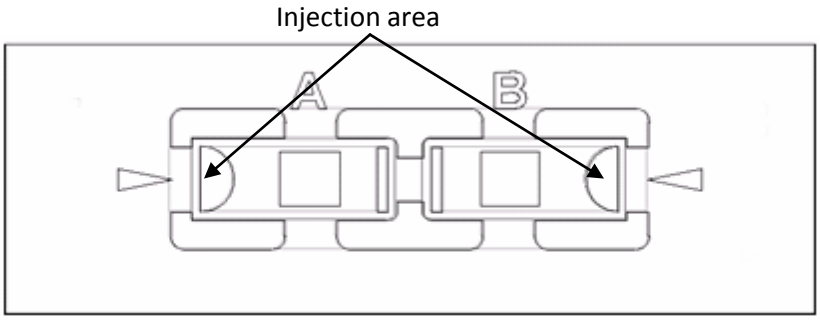
1. Charge both sides of a clean hemacytometer with the specimen using an unheparinized capillary tube or the Unopette assembly.
2. Let specimen settle for 1 to 20 minutes, depending on viscosity of fluid.

NOTE: Hemocytometer can be placed in a petri dish with moistened filter paper if drying is a concern.

G. C-Chip (DHC-N01)

NOTE: The C-Chip is for single use only. Do not reuse. It should be used immediately after unsealing.

1. Mix samples well
2. Load 10ul of sample into the sample injection area, Fig 1., so that it fills the chamber by capillary action.
3. Let settle for 1 to 20 minutes, depending on viscosity of the fluid.



4. Continue with counting as outlined for the hemocytomer.

H. Determine the area of the hemocytometer to be used for counting. Some counting guidelines:

1. Low count -- Count all 9 squares on each side.
2. Moderate count -- Count entire center square.
3. High count -- Count RBC counting area (5 small squares in the center square).
4. If cells are too numerous to count or are overlapping, a higher dilution is needed.
5. If there are not very many cells present, a lower dilution may be needed.

NOTE: For picture of hemocytometer counting areas, see calculations.

I. Using 10x or 40x magnification, count the cells on each side of the hemocytometer.

J. Average the counts obtained on the each side of the hemocytometer.

NOTE: Retain this number for calculations.

K. For counts less than 100 cells the sides must agree within 20%. For counts greater than 100 cells, the sides must agree within 10%. If counts do not agree recharge hemocytometer and recount.

L. If WBCs and RBCs are hard to distinguish from each other, use phase microscope. If phase microscope is unavailable, Brilliant Cresyl Blue Stain can be used or the specimen can be sent to Regional Hematology where a phase scope is available.

1. Place a small amount of undiluted or diluted well-mixed body fluid into a test tube.
2. Rinse a disposable pipette with the filtered Brilliant Cresyl Blue stain and discard any excess. Wipe the outside of the pipette to remove as much stain as possible.
3. Rinse the same pipette with the well-mixed body fluid. The negligible amount of stain left in the pipette will not significantly dilute the specimen and aids in differentiating white and red blood cells.
4. Let the stain/fluid mixture stand for 10 minutes for proper staining.
5. Stain SpinalScopics Spinal Fluid Cell Count Controls in the same manner as described above in steps 1 -4.
6. Refer to procedure steps F – K for details of the cell count process.

M. Report WBC and RBC counts as number of cells per cubic millimeter (mm^3).

N. Calculations:

1. All calculations require:
   a. Total number of cells counted
   b. Total number of square mm counted
   c. Any dilution factor
2. Cells in 1 mm^3 = $\frac{\text{number cells counted}}{\text{\# squares x volume 1 square (area counted in } mm^3 \text{ x depth)}}$ x dilution factor

Volume in one large WBC square = 0.1 mm^3
Volume in one small RBC square = 0.004 mm^3

Example: If undiluted, use standard formula for hemacytometer with dilution factor of 1

$$\frac{48 \text{ WBC's}}{9 \times 0.1} \times 1 = 53 \text{ WBCs per } mm^3$$

Example: 25 RBCs counted in 4 large "WBC" squares with a 1:10 dilution

$$\frac{25 \text{ cells} \times 10}{4 \times 0.1} = 625 \text{ cells/mm}^3$$

3. The diagram below shows another formula used to calculate cells/mm^3.

Volume Factor for whole chamber 1.1 = $\frac{1}{9 \times 0.1}$

4 WBC (W) squares = 2.5 = $\frac{1}{4 \times 0.1}$

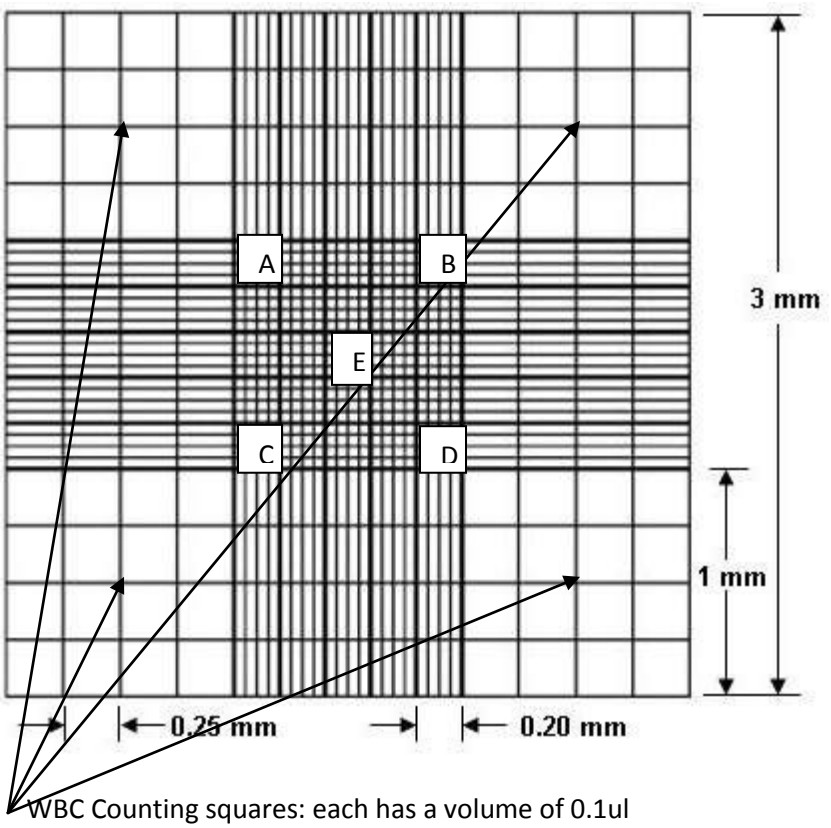
Center square - 10 = $\frac{1}{25 \times .004}$

5 RBC (R) squares = 50 = $\frac{1}{5 \times .004}$

Depth from hemocytometer coverslip is 0.1 mm.

Number of cells counted x dilution factor x volume factor = cells / mm^3.

Hemacytometer or C-Chip
(Neubauer ruling of chamber)



WBC Counting squares: each has a volume of 0.1ul

A,B,C,D,E = RBC counting squares: each has a volume of 0.004ul

Hemacytometer Calculations:

$$\frac{\text{No. of cells counted X dilution}}{\text{No. of squares counted X volume of each square}} = \text{Total Cells/ul}$$

PROCEDURE—CEREBRAL SPINAL FLUID WBC DIFFERENTIAL

A. Perform a WBC differential on all CSF specimens with a WBC of 5 or more cells.

- For patients <18 years of age, a WBC differential must be performed on all samples, even those where the WBC count is between 0-5.
- All CSF oncology cell count specimens must have a cytospin differential performed and be reviewed by a pathologist.

B. Make two slides labeled with accession number, patient name, source and date.

NOTE: Make two smears whenever sample volume permits.

C. The primary method for making smears for CSF differentials is by cytocentrifugation.

NOTE: See Procedure Preparation of Body Fluid Differential and Gram Stain Smears Using the Cyto-Tek Centrifuge.

D. An alternate method for making smears if cytocentrifuge is not usable.
1. Place an aliquot of CSF in falcon tube and spin at the recommended speed by the manufacturer for 5 minutes.
2. With a disposable pipette draw off supernatant. The sediment may not be visible if the count is low.
3. Add one drop of bovine albumin to CSF sediment, mix well and make two smears. Let slides air dry completely.

E. Stain smears using normal hematology Wright stain method.

F. Perform a 100 cell differential if possible, scanning entire area of both cytospin differential slides.
1. Differentiate each cell into appropriate cell type. Segmented and band neutrophils should be reported together under the "SEGS" category.
2. Report all cell types as a percentage.
3. If less than 100 cells are seen, divide the total count for each cell type by the total cells counted and then multiply by 100. Round each to a whole number so that the sum of the cell types equals 100.

G. For any slide with questionable and/or atypical cells, and all pediatric (<18 years) CSF differentials.
1. Print out an IRA report of the results.
2. Submit both cytospin slides and IRA report to a Pathologist for review.

REPORTING RESULTS

FUNCTION	**WORKSHEET**	**METHOD**	**TEST**
MEM	_____	_____	_____
Answer:			

Colors		Appearance		Xanthochromia	
Color	**Canned Comment**	**Turbidity**	**Canned Comment**	**Supernatant Color**	**Canned Comment**
Brown	BROWN	Clear	CLEAR	Positive	POS
Colorless	CLRLS	Slightly Cloudy	SCLDY	Negative	NEG
Green	GRN	Cloudy	CLDY		
Orange	ORNG	Very Cloudy	VCLDY		
Pale Pink	PALE-PINK	Turbid	TURB		
Red	RED	Bloody	BLDY		
Yellow	YEL	Clotted	CLTD		
White	WHIT	Milky	MILK		

Colorless	Slightly Cloudy	Cloudy	Very Cloudy	Turbid

Xanthrochromia and Turbidity Chart

REPORTING RESULTS (continued)

RBC -- numerical value
WBC -- numerical value
SEGS -- numerical value in %
LYMPHS -- numerical value in %
MONOS -- numerical value in %
EOS -- numerical value in %
OTHRS (Example: Baso 1) -- Footnote each type with the numerical value in %.
If prompt does not have a result or is not applicable to final report, type "HIDE" for the answer.

If physician requests a differential on specimen with less than 5 WBC's do differential and tag results with CSFMD -- CSF WBC differential performed at MD's request. If specimen is partially clotted do cell count and tag results with PCLOT -- Cell count inaccurate due to partially clotted specimen.

Critical results must be called. Computer result must be tagged with time of call and who critical result was reported to.

EXPECTED VALUES

Normal Leukocyte Counts:

Adults:	0-5 mononuclears (lymphs and monos)	
Children:	1 yr.	0-30/mm^3
	1-4 yrs.	0-20/mm^3
	5 yr.	0-10/mm^3

Differentials:

	Adults	Neonates
Lymphs	60+/- 20%	20+/- 15%
Monocytes	30+/-15%	70+/-20%
Neutrophils	2+/-4%	4+/-4%
Others	rare %	rare %

RBCs -- Not normally present in CSF. A few may be found due to contamination by blood from vessels injured during the lumbar puncture.

Nucleated RBCs – Not normally present in CSF. When nRBCs are seen they are indicative of blood or bone marrow contamination. Do not perform a corrected WBC count. Include the count in the report and make a comment: "WBC count should be interpreted with caution".

PROCEDURE NOTES

A. Any questionable slides reviewed by a pathologist and confirmed for atypical cells should be resulted with pathologist's comment and the pathologist's name.

B. Specimens containing atypical cells should be correlated with cytology results of Ameripath by the pathologist.

INTERPRETATION

An increase of cells in the spinal fluid is called pleocytosis. An increase in red blood cells is usually due to recent hemorrhage. Xanthochromia can be seen in newborns and in patients with an old hemorrhage. A cloudy or turbid spinal fluid indicates an increase WBC count. In tuberculosis, neurosyphilis, polio, multiple sclerosis and encephalitis, a predominance of lymphs can be seen. An increase of polys is seen in pyogenic meningitis, influenzal meningitis and brain abscess.

LIMITATIONS

A. Clotted specimens result in decreased cell counts, as many cells will be trapped in the clot.

B. Cells in CSF degenerate quickly, always process as a STAT.

C. Use universal precautions.

REFERENCES

A. Kjeldsberg / Knight, Body Fluids, 3rd Edition.
B. Clinical Diagnosis by Laboratory Method, Davidsohn and Henry.
C. Urinalysis and Body Fluids, Susan King Strasinger, 3rd Edition
D. Shandon Operating Manual.
E. Cyto-tek Operating Manual 8/97
F. Spinalscopics Spinal Fluid Cell Count Control Package insert, Quantimetrix Corporation. 8/2009.
G. InCyto C-Chip DHC-N01, Instruction Manual, 2004.