MERIDIAN HEALTH SYSTEM
Jersey Shore University Medical Center
Laboratory
Neptune, New Jersey 07753

SECTION: Core Lab - Hematology	TITLE: CEREBROSPINAL FLUID EXAMINATION
EFFECTIVE Oct 2003 DATE:	APPROVED BY: Laboratory Director or Designee
SUPERSEDES: CSF Oct 2015 DISCONTINUED DATE:	PREPARED BY: Richard Hull

PRINCIPLE:

The most important role of a lumbar puncture is in the diagnosis of bacterial, fungal, mycobacterial, and amebic meningitis. Due to its high specificity, the lumbar puncture is useful in diagnosing malignancy involving the meninges, although sensitivity is only about 70% (Marton, 1986). The examination of the CSF includes a nucleated blood cell count and differential if the WBC's are >5/µL. Lumbar puncture also has value in diagnosis of subarachnoid hemorrhage, multiple sclerosis, and demyelinating disorders.

SPECIMEN COLLECTION:

CSF should be collected in sterile plastic tubes without preservatives. The specimen should be walked to the laboratory. Ideally, the specimen should be divided into three samples and placed in sterile tubes, which are labeled sequentially. Tube #1 should be used for chemical and immunologic studies, tube #2 for cell count, and tube #3 for microbiologic examination unless otherwise specified by the doctor.

Since cells deteriorate quickly in this fluid **all CSF cell counts must be performed within one hour.** If any delay occurs in the testing it should be noted with the results. **Specimens must be refrigerated (maximum of 8 hours) if testing is to be delayed.**

A clot may be seen in the CSF with a very bloody traumatic tap. When partially clotted, a cell count may be done as long as a statement is added to the report indicating that the cell count may be inaccurate due to partial clotting. Please append the following code: PCS-CAUT (partially clotted specimen, interpret with caution)

REAGENTS & EQUIPMENT:

- Neubauer hemocytometer and coverglass
- Phase microscope
- MLA pipettes for dilutions
- Spinal Diluting Fluid
    - A commercially prepared diluting fluid that hemolyzes all red cells present and stains all white cells so that they are easily seen. Store at room temperature. Use until the manufacturer's expiration date. Discard expired reagent
- Cell Pack for RBC counting only
    - This is the diluent used on the Sysmex instrument. It is stored at room temp and good until the expiration date indicated on the box.

QUALITY CONTROL OF REAGENTS:

Each day of use perform a background count on the Spinal Fluid Diluting Fluid and the Cell Pack (if used) to check for contamination:

- Place a drop of diluting fluid (or Cell Pack) on a clean glass slide and coverslip. Examine it microscopically for particulates.
- If particulates are present, filter the diluting fluid. Recheck for particulates and if still present, **the diluting fluid must be discarded**.
- This background check must be logged in the fluid worksheet.

QUALITY CONTROL:

Quality Control Using the Spinalscopics spinal fluid cell count control:
Intended use:
Spinalscopics spinal fluid cell count control is intended for monitoring total cell counts performed manually using a hemocytometer to validate quantitation of red and white blood cells in patient CSF samples. Control materials having known component concentrations are an integral part of diagnostic procedures.

Product Description:
Spinalscopics spinal fluid cell count controls are supplied in two levels. They are ready to use liquid, requiring no reconstitution or dilution. They are prepared in a human protein matrix fortified to target levels with purified chemicals and stabilized human red and white blood cells. Preservatives have been added to inhibit microbial growth.

Storage and Stability:
The Spinalscopics Controls should be stored tightly capped refrigerated (2-8ºC) when not in use. When stored unopened at 2-8ºC, the controls are stable until the expiration date stated on the label. Once opened, the controls are stable for 6 months when stored at 2-8ºC between uses.

Procedure:

1. QC is performed on each shift of patient testing.
2. Remove the controls from the refrigerator and allow them to come to room temperature (18-25ºC) for at least 15 minutes, depending on remaining volume.
3. 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. Thorough mixing with each use is important in order to obtain reproducible results. Avoid foaming.
4. Treat the controls as you would a patient sample.
5. Charge both sides of the hemocytometer chamber.
6. Recap the controls immediately
7. Allow the cells to settle by placing the hemocytometer in a humidified chamber for10 minutes before counting.
8. Count the cells in the 9 squares on both sides of the hemocytometer.
9. Average the number of cells counted on both sides.
10. Calculate the total number of red and white blood cells per µL as follows:

$$\frac{\text{Average No. of cells counted} \times \text{volume (10)} \times \text{dilution}}{\text{\# of squares counted}} = \text{Total cells/µL}$$

Expected Ranges:

The obtained results of the cell count should fall within the expected ranges published for each lot #. If the QC result is out of range, repeat the assay, making sure the control material is mixed well. If the results are still out of range, use a new bottle of control material. If the control is still out of range, call Tech support at 310-536-0006 ext. 112 for an update of expected values. Notify a supervisor.

QC results must be entered into the LIS system and documented on the fluid worksheet.

CSF CELL COUNT
PROCEDURE

Character and Xanthochromia must be noted on all specimens.

Examine a well- mixed sample and result the character based on the following codes:

Appearance	**LIS CODES**
Clear and colorless	CC
Cloudy	CLDY
Milky	MILKY
Turbid	TRBD
Slightly bloody	SLY BLDY
Bloody	BLDY
Grossly bloody	GB

XANTHOCHROMIA

Xanthochromic	XANTH
No xanthochromia	NOX

Bloody spinal fluids require an additional step: Spin down a small aliquot of the specimen and note the xanthochromia of the supernatant. The supernatant will have either Xanthochromia or no Xanthochromia. This information is useful in determining if the blood is from a hemorrhage or a "traumatic tap".

Any clots or partial clots or debris present should also be noted on the report.

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MICROSCOPIC EXAMINATION—RBC's

Cell Count Procedure:

Fluid RBCs are reported as a semi-quantitative statement:

1) Add a well-mixed drop of specimen into both chambers of a hemocytometer and allow to settle for 10 minutes.
2) Approximate the number of RBCs by examining all 9 squares of both chambers of the hemocytometer. (examine with a 40x lens)
3) From this **undiluted** specimen make a semi-quantitative statement about RBCs using a nomenclature ranging from:

RBC's per 40 x field:	Sunquest Code to be Used:	Sunquest Code Translation
0 RBC' per 40 x field	FLNONE	(0/uL)
1 – 3 RBC's per 40 x field	FLOCC	Approx. 45 – 150/uL
4 – 10 RBC's per 40 x field	FLMOD	Approx. 151 – 500/uL
11 – 50 RBC's per 40 x field	FLMANY	Approx. 501 – 2,300/uL
> 50 RBC's per 40 x field	FLTNTC	Approx. >2,300/uL

Note in the report if the RBC appear fresh or crenated. **If a numeric RBC count is requested, follow the protocol stated later in this procedure.**

MICROSCOPIC EXAMINATION --WBC's:

If no RBC's are present in the previous counting chambers, count the WBCs (nucleated cells) in all 9 squares of one chamber of the hemocytometer. Repeat the WBC count in all 9 squares for the other chamber of the hemocytometer. Average your results. **Differences of more than +/- 10% (or 2 cells, whichever is greater) between the two counts indicate poor cell distribution, which requires the entire procedure to be repeated!**

Example:
Total WBCs on side # 1 = 15
Total WBCs on side # 2 = 17
Average WBCs counted = 16

Calculation:

$$\frac{\text{Average No. of cells counted} \times \text{volume (10)} \times \text{dilution}}{\text{\# of squares counted}} = \text{Total cells/µL}$$

Example:

$$\frac{16 \times 10 \times 1 \text{ (no dilution used)}}{9 \text{ squares counted}} = 17.7 \text{ WBC/µL}$$

The Total WBC count is 18 WBC/ µL

****Cell counts are reported in WHOLE numbers****

If RBC's are present:

Dilute the CSF with Spinal Fluid Diluting Fluid to lyse the RBC's and stain the WBC's as follows:

1. Using a MLA pipette, mix 100µl of the specimen and 100µl of " Spinal Fluid Diluting Fluid " into a clean 13x75 tube.
   a. Allow this 1:2 dilution to sit for approx. 3-5 minutes to allow all RBCs to lyse.
2. Add a well-mixed drop of the 1:2 dilution into the hemocytometer. Charge both of the chambers of the hemocytometer.
3. Allow the cells to settle in the chambers for at least five minutes before counting.
4. Count the WBCs (nucleated cells) in all 9 squares of the one chamber of the hemocytometer. Repeat the WBC count on the other chamber of the hemocytometer. Average your results. **Differences of more than 10% (or 2 cells, whichever is greater) between the two counts indicate poor cell distribution, which requires the procedure to be repeated!**

Example: Total cells on side # 1= 12
Total cells on side # 2= 14
Average total cells counted= 11

Calculation:

$$\frac{\text{Average No. of cells counted} \times \text{volume (10)} \times \text{dilution}}{\text{\# of squares counted}} = \text{Total cells/µL}$$

Example:

$$\frac{11 \times 10 \times 2 \text{ (1:2 dilution used)}}{9 \text{ squares counted}} = 24.4 \text{ WBC/µL}$$

The Total WBC count is 24 WBC/ µL

****Cell counts are reported in WHOLE numbers****

If the WBC count appears to be greater than 500/uL dilute using the Leuko-tic method and perform a WBC count as per MANUAL WBC COUNT USING LEUKO-TIC in this manual.

A differential is required on all CSF specimens with 5 or more WBC.

- See procedure CYTOSPIN PROCEDURE FOR BODY FLUID SEDIMENTS AND DIFFERENTIAL COUNT.
- Review slide for correlation of cell count results.
  - If discrepancies are noted between cell count and cell concentration on the cytocentrifuge slide, the cell count must be repeated to verify results.
- Do not perform a differential from the chamber count as it is considered unsatisfactory as one cannot be certain of the cell types in a wet prep.
- Make sure the cell count and differential are performed from the same tube
- All CSF specimens should be treated with extreme care because they can be highly contagious. These specimens could potentially harbor viruses or other infectious organisms.
- Stain the smear in the same manner as a peripheral smear, using the slide stainer.
- Scan the slides using both the low power and high power objectives. Note the distribution of various cell types and look for clumping of cells. Be alert for organisms and crystals (which are often seen on stained body fluid smears).
- Use the oil immersion objective to perform a differential cell count. If possible count 100 cells; if not, count all cells present in the concentrate. (note: if the cell preparation is too concentrated for accurate identification or if the cytospin preparation does not correlate with the cell count, repeat procedure.)
- Abnormal cells may be found in CSF. (e.g. Plasmacytoid lymphs, plasma cells, blasts, macrophages. Pure monocytosis is rarely seen. Consult the Body Fluid reference books located in Hematology

Note: The Pathologist will review any slide with malignant or unusual cells. Please submit reports from all departments to pathologist for review.

RBC COUNT:

A RBC count is only performed when specifically ordered by the physician.

1. Place a well-mixed specimen in a hemocytometer and allow it to sit for 5 minutes.

2. Count all the RBC in all 9 squares on both sides of the hemocytometer and average your results. **Differences of more than 10% (or 2 cells, whichever is greater) between the two counts indicate poor distribution, which requires the procedure to be repeated!**

Example: Side #1 RBC = 24
Side #2 RBC = 26
Average RBC = 25

Calculation:

$$\frac{\text{Average No. of cells counted} \times \text{volume (10)} \times \text{dilution}}{\text{\# of squares counted}} = \text{Total cells/µL}$$

Example:

$$\frac{25 \times 10 \times 1 \text{ (no dilution used)}}{9 \text{ squares counted}} = 27.7 \text{ RBC/µL}$$

The Total RBC count is 28 RBC/ µL

****Cell counts are reported in WHOLE numbers****

4. If the RBC count appears to be greater than 100 you can make a dilution.

What is the appropriate dilution?

If the specimen appears to be grossly bloody make a 1:50 dilution. If the specimen is only slightly bloody, but the RBC's are too many to count accurately without dilution, make a 1:10 dilution.

5. **Procedure for a 1:10 dilution:**

a. In a clean 13mm x 75 mm tube pipette 900µl of Cellpack.
b. Add 100µl of CSF
c. Mix gently
d. Place a drop of this dilution on a hemocytometer and allow it to sit for 10 minutes.
e. Count the RBC's in the **large center square** (divided into 25 smaller squares, each divided into 16 tiny squares) of both sides of the hemocytometer and average your results.

Calculations:

Total average number counted x 100 = RBC /cumm

Example:
Total number counted on side # 1 = 10
Total number counted on side # 2 = 8

Total **average** number counted = 9
9 x 100 = 900 RBC/cumm

6. **Procedure for 1:50 dilution:**

a. In a clean 16mm x 100m test tube pipette 4,900µl of Cellpack
b. Pipette 100µl of CSF into the **4,900** µl **of** Cellpack
c. Mix gently.
d. Place a drop of this dilution on a hemocytometer and allow it to sit for 10 minutes.
e. Count the RBC in the **large center square** (divided into 25 smaller squares, each divided into 16 tiny squares) of both sides of the hemocytometer and average your results.

Calculation:
Total average number RBC counted x 500 = RBC / cumm

Example:
Total number counted on side # 2 = 42
Total number counted on side # 1 = 38
Total **average** number counted = 40

40 x 500 = 20,000 RBC / uL

NORMAL VALUES:

MICROSCOPIC EXAMINATION:

Total WBC Normal Values:	Mononuclear cells
Adults:	0 – 5 cells/cmm (uL)
Newborns:	0 – 30 cells/cmm (uL)

Differential:

	ADULTS	NEONATES	<5yrs:	5 – 15yrs:
Lymphs	40%-80%	5%-35%		
Monocytes	15%-45%	50%-90%		
Neutrophils	0-6%	0-8%		
Mononuclear cells:	Up to 5	Up to 30.0	Up to 20	Up to 10

Interpretation:
An increase in mononuclear cells is suggestive of viral infection. Increased neutrophils suggest bacterial infection.

Function: MEM
Test Code: CSFCT
Worksheet: CSFW
PATHC: CSF Pathology Review Codes

REFERENCES

Body Fluids, third Edition
Carl R. Kjeldsbert & Joseph A, Knight
1993 American Society of Clinical Pathologist

Clinical Diagnosis & Management by Lab Methods
18th Edition
John Bernard Henry, MD
1991 W>B> Saunder Co.

Hematologic Analysis of Body Fluids
Patricia F. Garrity MS SH MT (ASCP)

Jerelyn Walters BS SH MT (ASCP)
Clinical Laboratory Education
1995

Stasinger, Susan King, Urinalysis and Body Fluids.
2nd edition; F A Davis Company, Philadelphia,
1989, p 176-178

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