CSF CELL COUNT

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☒ St. Clare Hospital Lakewood, WA	☐ Highline Medical Center Burien, WA	☐ PSC

PURPOSE

To provide instruction for performing manual analysis of cerebrospinal fluid specimens.

BACKGROUND

Analysis of cerebrospinal fluid is indicated in the differentiation of meningeal infection, subarachnoid hemorrhage, CNS malignancy, and demyelinating diseases. Automated cell counts for CSF have not been validated.

RELATED DOCUMENTS

R-W-HEM-1437	Hematology CJD Protocol
R-W-SPC-0215	Spinal Fluid Processing
R-W-HEM-1405	Hemocytometer Counts
R-W-HEM-1428	Cytocentrifuge Use
R-PO-HEM-0108	Pathologist Review of Blood and Body Fluids-Criteria
R-W-HEM-1401	Body Fluid Cell Count

SPECIMEN REQUIREMENTS

HANDLING: Deliver ASAP to lab. Store at 2-8°C. Specimens held 1 week.
NOTE: If you receive a CSF specimen labeled CJD PROTOCOL, refer to protocol HEMATOLOGY CJD PROTOCOL for specimen handling precautions.

VOLUME: Minimum volume: 1.0 ml.

ALIQUOT: Distribute tubes as follows, unless specified differently by physician:
Tube #1: Specimen for Hold, or 2nd Cell Count/Diff (Cell Count CSF, Tube 1)
Tube #2: Chemistry
Tube #3: Microbiology
Tube #4: Hematology, 1st Cell Count/Diff

STABILITY: CSF fluids are performed stat within 1 hour to prevent cell lysis.

EQUIPMENT/SUPPLIES

- Hemocytometer (Improved-Neubauer): glass or disposable
- Plastic petri dish with moist cloth, calibrated pipette, as needed
- Wright-Giemsa stain, slide stainer
- Microscope and Tally counter
- Cytocentrifuge, cytospin chambers, filters and slides, 6% Albumin

- Hematology diluent, CSF or Turk's Diluting Fluid, as needed
- 10% bleach, if CJD PROTOCOL in effect

QUALITY CONTROL

1. One Level of manual body fluid QC is performed every 8 hours of patient testing. Results are recorded in the LIS or the location according to your site.

2. Hemocytometer is inspected for integrity and cleanliness. Results are documented in the LIS or on the worksheet.

3. Certified pipettes are used to dilute specimens. This is documented in the LIS or on the worksheet.

4. CSF or Turk's Diluting Fluid may be used to hemolyze RBCs for cell counting and to enhance the nucleus of nucleated cells, when the distinction between RBCs and nucleated cells is difficult.

5. Quality check of diluting fluids. Diluting fluids, stains and lysing agents are visually inspected under the microscope each day of use for clarity, cellular elements, and debris. The background count must be less than 3 cellular elements and free of debris. Results are recorded in the LIS or on the worksheet.

6. Cell counts are performed in duplicate by counting both sides of the chamber. Counts must agree within 20%. This is documented in the LIS or on the worksheet.

7. Cells are evenly distributed in the hemocytometer chamber. This is documented in the LIS or on the worksheet.

8. Cytospin or manual slides are submitted for pathologist review if 5 or more WBC's are reported. Refer to Pathologist Review of Blood and Body Fluids-Criteria.

PROCEDURE STEPS

Preparation of Specimen for Testing

1. Check that the Body Fluid sample has been correctly ordered before proceeding.

2. Record all results and observations on a Body Fluid worksheet or in the LIS.

3. Visually inspect the fluid in the aliquot tube and record in the LIS and on the worksheet any clots or visible cell clumping. When a result of "Yes" is entered in the result field "Check specimen integrity- Clots Present?", the LIS will automatically append the comment BFCLOT, which reads "Body Fluid specimen has clots present."

4. Determine the color of the fluid and appearance.

5. Record the tube # and volume of the total specimen collection for all tubes.

6. Centrifuge an aliquot if indicated to check for xanthochromia. The supernatant may appear pale orange-yellow color. Report as Present or Absent in the LIS.

Cell Count (Hemocytometer)

1. Fill a small capillary tube about ¾ full of well mixed fluid.

2. Charge both sides of a hemocytometer and allow to settle at least 2 minutes.

3. Scan the counting area on 10X for even cell distribution and presence of cell clumps. Change to 40X lens and count sufficient squares to obtain a count of at least 50 nucleated cells on each side. Use CSF or Turk's Diluting fluid, if necessary, to distinguish between RBCs and nucleated cell types by making a 1:2 dilution or higher (refer to table below of frequently used dilutions). Counts on the two sides must agree within 20%. If not, mix the specimen thoroughly, replate, and recount.
   - If the nucleated count is elevated, estimate the number of cells present and determine an appropriate dilution. See Hemocytometer Counts procedure.
   - Determine if RBC crenation is present. Report as a percentage of the total count. Rule out crenation due to contaminants on the chamber surface

4. Perform a manual cell count for both RBCs and Total Nucleated Cells (TNC). TNC includes WBC's, and all other nucleated cells seen on the hemocytometer and will be reported in the LIS as WBC's. See work Instruction, Performing a Hemocytometer Count.

Dilutions

1. Manual Dilutions: Make an appropriate dilution as determined by the estimation from the direct plating. Pipette the appropriate volumes of fluid and diluent into a clean, labeled tube. Cap and mix well.

2. Frequently used dilutions:

Ratio	Volume of fluid	Volume of diluent
1:2	100 µL	100 µL
1:5	100 µL	400 µL
1:10	100 µL	900 µL
1:20	50 µL	950 µL
1:50	20 µL	980 µL
1: 101	20 µL	2 mL

Differential

1. If the WBC Count is greater than 5 cells/mm^3, prepare one or more cytospin or push slides. See work instruction, Cytocentrifuge Use. If a cytocentrifuge is not available, make a manual slide using sediment from a spun sample if sample volume is sufficient.

2. Stain slides with Wright-Giemsa Stain on the manual slide stainer.

3. Assess slide quality. Scan the slide on 10x for general cell distribution. Cells should be adequate in number, intact, evenly distributed and with good stain color. If not, remake the slides.

4. Scan the slide on 10x for the presence of clumps of abnormal or possibly malignant cells. Correlate the number and proportion of cells on the cytospin slide with the manual cell count results.

5. Count 100 nucleated cells and document on the worksheet or in the LIS. Note: The worksheet template includes the following result fields:
   - Fluid Polys
   - Fluid Lymphs

- Fluid Monos
- Fluid Mesos
- Fluid Eosinophils
- Fluid Basophils
- Fluid Other: Use for cells not in one of the categories, unidentified cells, or suspected malignant cells. This would also include macrophage, plasmacytes or nucleated RBC's. Add a comment identifying other cell types.
- Comment: Use for comments on cell inclusions, bacteria, or fungal elements.
- Path Review

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If the WBC Count is 5 cells/mm³ or less, a differential is not necessary. The LIS will automatically add the comment BF DIF (WBC=5 or less per microliter. Differential not performed). Slides for pathology review are not indicated if 5 WBC's or less reported. The Pathology Review will need to be cancelled in the LIS.

If bacteria or fungus is noted (confirm with gram stain or Microbiology), determine if it is intracellular or extracellular. Rule out stain or slide contamination as the cause. Result findings as a comment in the LIS. **NOTE:** The presence of bacteria/fungus (non-contaminant) in CSF is a Critical Result and must be called to the ordering physician or unit STAT.

CALCULATIONS

See work Instruction Hemocytometer Count.

INTERPRETATION (Reference Ranges)

Cell Counts (CSF)	WBC	0-10 /mm³
	WBC (neonates)	0-30 /mm³
	RBC	None
Differential	Neutrophils	0-5%
	Lymphocytes	50-100%
	Monocytes	0-30%
	Other	not usually present and may indicate an abnormality
Appearance	Clear	
Color	Colorless	
Xanthochromia	Absent	

ORDERING AND RESULTING

- Orderable tests for cerebrospinal fluids include: CSF Panel, Cell Count CSF, Cell Count CSF Tube 1, if requested)
- Results are reported in the LIS.

TECHNICAL NOTES

1. Dilutions: Use the smallest possible dilution, so that the total number of nucleated cells counted on the hemocytometer is between 50/mm² and 250/mm².

2. If the specimen contains clots but can still be pipetted, perform the analysis including the differential. Use LIS smart phrase: BF CLOT (Body fluid specimen has clots present) or BF CLUMP (Body fluid specimen has cell clumps present).

4. Cell Identification confirmation techniques:
   - Turks Diluting Fluid or WBC Diluting Fluid may be used to better distinguish between RBCs and nucleated cell types. Both enhance the cell nucleus for better identification. If used, you must initially count all cells, then repeat the count using the lysed fluid sample.

   - Diluted Methylene blue stain may be used. If used, the count should be compared in number and proportion to the cells on the cytospin preparation.

   - For staining or lysing techniques, refer to reference manuals.

MANUAL CALCULATION FOR CELL COUNTS

See work instruction Hemocytometer Counts

REFERENCES

1. Susan King Straasinger, “Urinalysis and Body Fluids”, Third Edition, 1994, F.A. Davis Company.
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4. Ross and Neely. Textbook of Urinalysis and Body Fluids. Appleton-Century-Crofts, Connecticut: 1983.
5. Henry, John Bernard. Clinical Diagnosis and Management by Laboratory Methods, 19th edition. W.B. Saunders, Co., 1996, pp556-557, pp.469-47