UnityPoint Health
Methodist | Proctor

Effective Date: August 3, 1999
Date Reviewed/ Date Revised: 03/07/18

CSF
MANUAL BODY FLUID COUNT AND DIFFERENTIAL
SFCNTX

I. CLINICAL SIGNIFICANCE

To quantitate the cell types in spinal fluid and differentiate the white blood cells.

II. REAGENTS

Disinfectant
Saline/cellpack
2% acetic acid
Albumin

III. Supplies

MLA Pipette and tips
Disposable pipettes
Hemocytometer (glass or plastic)
Petri Dish
Cytospin slide
Cytospin funnel and holder

IV. Equipment

Cytocentrifuge
Slide Stainer
Micropscope/Phase Microscope

V. Sample

Labeled collection tube of CSF, preferably the second tube of three tubes. If more than one tube is to have a count, make sure that there are two cell counts ordered. Append a comment to the result that tells which tube was used, if multiple counts are ordered.

VI. Quality Control

We use procedural Quality Control for body fluids.

1. Check the diluting fluid (if making a dilution) for contaminants.
   A. Saline and Cellpack should be plated on a Hemocytometer and do a count. Acceptable performance is 0.0
   B. 2% acetic acid should be checked by placing a drop on a glass slide and coverslip. Look for contamination. No contamination is acceptable performance.
   C. If the diluting fluid is contaminated, it should be discarded and replaced.

D. Record the results on the body fluid worksheet provided in the department.

2. The WBC and RBC counts are counted in duplicate and must match within 20%.
3. Review the ratio of RBC to WBC on the cytospin. This is a gauge to see if you identified your cells correctly.

VII. PROCEDURE:

1. Use the body fluid worksheet for recording results before entry in Sunquest.
2. Record the volume of fluid in all tubes.
3. Receive the sample in General Lab and observe all testing that is needed throughout the Lab.
4. Retrieve the labels and deliver the samples to other areas for testing.
   Tube #1 to Chemistry
   Tube #3 for Microbiology
   Be sure to make cytospin slides for CSF Cytology.
5. Describe the appearance of the fluid.
   A. Color: Colorless, xanthochromic (yellow),bloody. If the specimen is bloody, spin down an aliquot of CSF and comment on the color of the supernatant. Append -; supernatant is ____ Tube 2.
   B. Clarity: Clear, cloudy, clotted or turbid. A specimen that is Bloody will need a comment on the clarity of the supernatant.
5. Put a drop of CSF on a glass slide with a coverslip to check the specimen for clumps or debris that would give a false low or high result. If clumps are seen, append a comment to the results. "Count may be inaccurate due to clumps of _____ ."
6. Take an aliquot of the fluid and use our cytospin to make a slide for a differential. Stain the CSF as we do peripheral blood. Refer to the Cytospin procedure in this manual.

Smears for Cytology

7. If CSF cytology is ordered, make a second cytospin slide, labeled "cytology". Stain with our CSF differential slide on the Aerospray. Put the slide in a slide carrier with the order and deliver to Histology.
8. Perform a red cell count and a white cell count on all spinal fluids. The count should be performed twice as a procedural control and match within 20%. You may use the phase microscope to help differentiate the RBC's from WBC's.
9. Mix the sample well.
10. Charge a clean glass hemocytometer directly with CSF using a disposable pipette.
11. Count the RBC and WBC in 10 sq mm (10 large squares) of the chamber.(9 on one side and 1 on the other) use the High dry (40x) objective to distinguish the cells. RBC's appear as concave discs or may be crenated. WBC's are a little larger and have nuclear material. To help in distinguishing RBC's from WBC's new methylene blue or phase microscopy may be used.
12. Clean the glass hemocytometer and coverslip with 20% Clorox or other approved Lab disinfectant for 10 min. Rinse with water and clean with lense cleaner. Throw away any scratched slides or hemocytometers. (Alert supervisor if you need to dispose of a hemocytometer)

13. Average the two counts before performing the calculation.

    RBC and WBC Count Calculation:

    $$\frac{\text{Average \# (RBC or WBC) X Dilution}}{\text{\# Large Squares x 0.1 (depth)}}$$

14. If the plastic hemocytometer is used, refer to the procedure in this manual.

    RBC and WBC Count Calculation:

    $$\frac{\text{Average \# (RBC or WBC) X 90 X Dilution}}{\text{\# Small squares counted}}$$

15. If the WBC or RBC is too high to count straight, dilute the fluid. Refer to the procedure for manual WBC or RBC in this procedure manual. Spinal fluids can be run through the hematology analyzer as a procedural control as long as the results are within proven linearity and the background count was 0. Results may not be turned out.
16. Do a 100 cell differential
17. Function: Differential Result Entry Keyboard: YCSF
18. Enter Accession No.
19. Identify the cells as Neutrophil, Lymphocyte, Macrophage/Monocyte, Eosinophil,) or Other. Other cells include: Basophils/Mast Cell, Blasts, Lymphoma Cells, Plasma Cells, CSF lining cell (newborns-rare adults- very rare) or any Unidentified cell.
20. Begin at the top left of the first circle and scan all fields of both circles to find enough cells to do a 100 cell diff. If there are not 100 cells, do a % of 100 by pressing the = key on the keyboard when done. This is the count termination key and it will automatically give you a percent.

    Calculate % Manually

    $\frac{\text{\# lymphs x 100}}{\text{Total \# cells}}$ $\frac{\text{\#segs X 100}}{\text{Total \# cells}}$ $\frac{\text{\# mononuclear X100}}{\text{Total \# cells}}$

VIII. Interpretation of Results:

A clear supernatant (after the CSF is spun) indicates fresh blood, probable traumatic. A xanthochromic supernatant indicates older blood (>2 hours), suggestive of a significant CNS bleed. It is essential to document the tube # and the supernatant color.

.

IX. Reference Intervals:

Spinal fluid should be clear and colorless. Normal ranges are as follows:

Age Range	Reference Interval
Erythrocytes	
Newborn preterm (<37 weeks gestation)	0 - 1000/ul
Newborn term (day 0)	0 - 800/ul
Neonate (0 - 1 month)	0 - 50/ul
>1 month	0 - 5/ul
Leukocytes	
0 - 1 month	0 - 27/ul
1 month – 16 years	0 - 7/ul
>16 years	0- 5/ul
Leukocyte Differential	
Neonates (0 – 1 month)	
Lymphocytes	28 - 96%
Monocytes/Histiocytes	55 - 99%
Neutrophil	0 – 8%

X. Limitations of the Procedure:

If the spinal fluid is partially clotted or has cell clumps, make sure to report that the results may be inaccurate due to the clumps or clot.

XI. Result Reporting:

1. Function: Result Entry
2. Enter the result for: Tube number being used, Color, Clarity , Volume , Red blood cell count and White blood cell count. If no WBC's are seen on count, but cells are seen on the cytospin, report out as "1" WBC.
3. Do not report out the RBC or WBC count without verifying the count with the Cytospin.
4. When there is a request to count more than 1 tube, there must be an accession number for each tube of CSF fluid.
5. Use Differential Result Entry, Keyboard YCSF.
6. If there are less than 100 cells, use the count termination key = and click on QA review. The results will automatically be converted into percent and the number of cells counted will appear with the results.
7. If there are "other" cells, ask the Pathologist if they want an interpretation ordered. If yes, order PATHSM to the accession number.
8. Print a cumulative report and leave the slides, with calculations for Supervisor Review.

XII. References:

Clinical Laboratory Methods, 9th Edition, pp 754-760
Body Fluids, 2nd Edition, pp 31-48
Urinalysis and Body Fluids, pp 134-142
CAP Color Atlas of Body Fluids, pp40-61.

Procedure Created by: _____ Date: _____

Medical Director Approval: _____ Date: _____

Change of Medical Director: _____ Date:_____

REVISION HISTORY			
Rev	**Description of Change**	**Author**	**Effective Date**
0	Initial Release	Cindy Schroeder	1/22/2016
1	Change in keyboard to YCSF	Cindy Schroeder	10/14/2016
2	Slides will only be reviewed by pathologist if abnormal cells are seen	Sheanea LaCock	10/17/2017
3	Added reference ranges	Sheanea LaCock	03/07/2018

Reviewed by

Lead	Date	Coordinator/ Manager	Date	Medical Director	Date
Sheanea LaCock	10/17/2017				
Sheanea LaCock	03/07/2018				