UnityPoint Health Pekin
Department of Pathology
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Cerebrospinal Fluid

MANUAL BODY FLUID COUNT AND DIFFERENTIAL
SFCNTX

I. CLINICAL SIGNIFICANCE

This procedure is used to detect any abnormalities in the spinal fluid by means of observation, cell count, and cell differential. Any detected abnormalities may be indicative of, or aid in diagnosing central nervous system disease, intracranial hemorrhage, meningitis, encephalitis, or brain abscess.

II. SPECIMEN

A. A volume of 1-3 ml is requested, but 1 ml is the minimum specimen.
B. If three tubes are received and a CSF Film Array is ordered
    1. Tube #1 – Hematology cell count if a second count is requested - Save specimen at room temp.
    2. Tube #2 - CSF Film Array- remains unopened at room temp and sent to Methodist
    3. Tube #3 – Send Out tech- Aliquot some specimen to Hemo for cell counts. Do a Gram stain. Supernatant to Chemistry. Refrigerate after completion of testing.
C. If four tubes are received
    1. Tube #1 - Hematology to be used if 2 cell counts are requested- Save specimen at room temp.
    2. Tube #2 – Send Out tech- gram stain and give supernatant to Chemistry. Refrigerate after completion of testing
    3. Tube #3 CSF Film Array- unopened at room temp and Culture to Methodist
    4. Tube #4 to Hematology cell counts- Refrigerate after completion of testing
D. Traumatic Tap: The first and last tubes are sent too Hematology to check for a traumatic tap versus a brain hemorrhage by comparing the Red Cell Count in both tubes.
E. 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.
F. Spinal counts must be done STAT, within one hour of collection. This is necessary because of cell degeneration and lysis.

III. REAGENTS/EQUIPMENT

A. Neubauer hemacytometer
B. Hemacytometer cover slip
C. Disposable pipettes

D. Petri dish humidity chamber
E. Cytospin centrifuge
F. Cytospin slide
G. Cytospin funnel and holder
H. Slide stainer
I. Sysmex Analyzer

IV. CALIBRATION

The hemocytometer is checked for accuracy by manufacturer.

V. QUALITY CONTROL

A. Hemocytometer techniques are monitored by commercial materials.
B. Techs performing manual counts must analyze quality controls, obtain results within the acceptable ranges, and document results
C. Two levels of commercial Spinalscopics control materials must be run every 8 hours of patient testing, per tech when a specimen is received
D. Procedure for manual analysis of Spinalscopics control material:
    1. Remove the controls from the refrigerator and allow them to come to room temperature (18-25°C) for 10-15 minutes but no more than 20 minutes. Mix the controls thoroughly by inverting the bottles several times, squeezing the bulb in the cap, 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.
    2. Using glass dropper, charge both sides of the hemocytometer chamber with level 1. Repeat with level 2. Each control level must be tested in duplicate.
    3. Immediately recap the controls. The Spinalscopics Controls should be stored tightly capped refrigerated (2-8°C) when not in use. Do not freeze. Once opened, the controls are stable for six months when stored at 2-8°C between uses.
    4. Allow the cells to settle by placing the hemocytometer in a humidified chamber for 10-15 minutes and no longer than 30 minutes before counting.
        a. In a clean 15mm petri dish, place a square of gauze and slightly wet is with DI water.
        b. Break the plastic handle of an applicator into two pieces and place one on each side of the wetted gauze.
        c. Set the hemocytometer on top of the two plastic pieces, which will keep the hemocytometer off the wet gauze.
        d. Place the lid on the petri dish and let the cells set. The moisture in the chamber will prevent evaporation.
    5. Count the RBC's and WBC's in the 9 squares on each side. The two sides must agree within 20% or 4 cells on a low count. Average the two sides.

Divide average by 0.9 (or multiply by 1.1) and round to the nearest whole number. Result must be within that level's acceptable range or a two-sided re-plate is required for that level.

- $\frac{\text{Average \# cells counted}}{0.9}$ = Total Cells/µL
- Average # cells counted X 1.1 = Total Cells/µL

6. Document results on the control clipboard
7. Expected Ranges: Check commercial control package inserts for expected ranges.
8. Counts on patient samples should never be done unless QC is within range
9. Expected Ranges:
    a. The expected ranges are based on the average number of cells per µL counted in the nine large gridded squares on both sides of the hemocytometer counting chamber.
    b. Check commercial control package inserts for expected ranges.

E. The WBC and RBC counts are counted in duplicate and must match within 20%.

VI. PROCEDURE:

A. Record all results on the Body Fluid Worksheet prior to entering them into the LIS
B. Tech receiving the tubes will deliver the correct tubes to each Department
C. Record on Worksheet the total volume of all tubes in mL, as well as Color and Clarity
    1. Color:
        a. Colorless, Yellow, Straw, Amber, Red (bloody), Pink,
        b. If the color is Red/Pink, spin an aliquot of the CSF down. Add a comment to the color result ("Supernatant is ___").
    2. Clarity
        a. Clear, Slightly Hazy, Hazy, Cloudy, Turbid, Opaque

D. Before analyzing, determine the quality of the specimen
    1. Place a drop of the body fluid on a slide with a cover slip and examine under a microscope
    2. If clumps of cells are observed but cells are intact, add a note stating the "results may be inaccurate due to partial clotting, cell clumps or debris"
    3. If deteriorated cells are primarily seen do not try to count on hemocytometer as cell counts would be inaccurate
        a. Append a comment – "Specimen unsuitable for quantitative cell count due to low quality of specimen"
        b. Create a cytospin and determine if a differential can be done.

4. A cell differential should be done on all specimens whether a cell count can be performed or not.

E. Manual counting using a hemocytometer:
1. Mix the specimen well.
2. Label hemacytometer with a small LIS label or hand write accession number with a pencil
3. Using a disposable pipette, charge both sides of the hemocytometer chamber with specimen, taking care not to overfill the chamber.
   - If specimen is cloudy, perform a dilution using Nerl saline
   - Perform a background check with just the saline to confirm that no cells or debris is seen prior to making dilution.
4. Allow the cells to settle by placing the hemocytometer in a humidified chamber for 10 minutes, but no longer than 30 minutes, before counting.
5. Count the RBC's and WBC's on each side. The two sides must agree within 20%, or a two-sided re-plate is required for that level.
6. Average the counts from both sides, divide by 0.9 (or multiply 1.1) and round to the nearest whole number. The volume counted will be 0.9 cu mm, so the total number of cells counted can be calculated as:

$$\frac{\text{Average \# cells counted}}{0.9} = \text{Total Cells/}\mu\text{L}$$

7. Write out calculations on the Body Fluid worksheet to ensure accuracy. See the Hemocytometer protocol for further information and calculations
   a. For undiluted counts, all nine large squares (0.9 mm^3) are counted on each side and the two totals are averaged and rounded to the nearest whole number.
   b. For diluted counts, multiply the result by the dilution factor.
8. If no cells are seen on either side:
   a. Re-plate the undiluted specimen and count again.
   b. If only one cell is found on both sides, report as 1 cell/µL
   c. If no cells are seen, still create a cytospin. If cells are observed on the cytospin, report WBC count as 1 cell/µL
9. Cerebrospinal fluids can be run through the hematology analyzer as a procedural control as long as the results are within linearity. RESULTS MAY NOT BE TURNED OUT.

F. Perform a differential if WBC
1. Prepare two cytospins slide (Refer to Cytospin procedure).
2. Stain well dried slides with Wright's Giesma stain.
3. Read and record a differential.

a. Cells: Neutrophil, Lymphocyte, Monocyte, Eosinophil, and Other
b. Other cells include: Basophils/Mast Cell, Blasts, Lymphoma Cells, Plasma Cells, CSF lining cell (newborns-rare, adults- very rare) or any Unidentified cell.

4. Optimal differential should be done on 100 cells. No fewer than 10 cells should be used for a differential. However, if cell count is low in the body fluid and 100 cells are not available, click on the Count Terminate key (Term =) to get a percent of 100.
5. Alert the pathologist if any "Other" cells are seen. The pathologist will determine if cytology, flow cytometry, path review or other additional studies are needed. The pathologist will guide you on how to report out the specimen.
6. Refrigerate any remaining CSF for one week.
7. Save slides in the CBC slide box for that day.

G. Disinfect and clean the hemacytometer.
1. Cover hemacytometer and coverslip with disinfectant or alcohol.
2. Soak two to five minutes.
3. Rinse with tap water and alcohol, and dry.
4. Air dry or polish with lint-free material.
5. Before reusing, use alcohol and lens paper to polish surfaces.

VII. REPORTING RESULTS

A. Report in Result Entry:
1. Tube Number used for counts
2. Total Collected Volume, including specimen volume used in any other testing
3. Clarity (Clear, Slightly Hazy, Hazy, Cloudy, Turbid, Opaque)
   a. Note any other observations, e.g. present of clots
4. Color (Colorless, Yellow, Straw, Amber, Pink, Red)
5. Record White Blood Cell count
   a. <u>If only one cell is found on both sides, report as 1 cell/µL</u>
   b. <u>If no cells are seen, but WBCs are observed on the cytospin, report WBC count as 1 cell/µL</u>
   c. If clots or debris are present in the specimen, comment that the WBC may be inaccurate due to presence of clots or debris.
6. Record Red Blood Cell count

B. Result the differential under Differential Result Entry:
   1. Use Differential Result Entry. Keyboard YCSF
      a. Neutrophils, Lymphocytes, Monocytes, Eosinophils, and Other Cells
   2. Comment under "Other Cells" the type(s) seen and in what numbers. Ask the Pathologist for guidance with resulting "other cells" and potential path review.

C. Print a cumulative report and leave the slide(s), with the Body fluid Worksheet and calculations for Supervisor Review.

VIII. PROCEDURAL NOTES/PROBLEM-SOLVING TIPS

A. If WBC count is abnormal, greater than 5 cells/cmm, call results to infection control.

B. Normal white cell counts are:
   < 1 year = 0 - 30/cu mm
   1 - 4 years = 0 - 20/cu mm
   4 years to 12 years = 0 - 10/cu mm
   Adult = 0 - 5/cu mm

C. Reference ranges:

WBC Cell Type	Adults	Neonates
Lymph	40 - 80%	5 - 35%
Mono	15 - 45%	50 - 70%
Seg	0 - 6%	0 - 8%

D. Most common types of cellular reactions are:
   1. Neutrophilic indicates meningitis usually from neisseria, haemophilus, pneumococcus, and streptococcus.
   2. Mixed indicates subacute bacterial meningitis, tuberculosis, mycotic meningitis. Usually segs, lymphs and monos are seen.
   3. Monocytic indicates viral encephalitis, multiple sclerosis, tuberculosis, fungal or syphilitic meningitis. May see monocytes and lymphocytes. The lymphocytes will have a "monocytic" appearance.

E. 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.

F. Add a note to the results for any body fluid that has a clot, clumped cells, or other debris present; indicate that the counts may be inaccurate

G. REFERENCES

A. Galagan, KA, Blomberg, D, Cornbleet, PJ, Glassy, EF. Color Atlas of Body Fluids. CAP 2006

B. Kjeldsberg, C.R. and Knight, J.A., Body Fluids, 3rd Edition, p 265-301, 309-324. ASCP, Chicago

C. College of American Pathologists (CAP) Hematology-Coagulation Checklist, 8/21/2017
D. Quantimetrix, Spinalscopics Product Insert, MO46031A 10/16

POLICY CREATION :	***Date***
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REVISION HISTORY			
Rev	Description of Change	Author	Effective Date