KAISER PERMANENTE®
COLORADO LABORATORY

CEREBROSPINAL FLUID EXAMINATION (CELL COUNT AND DIFFERENTIAL)

PURPOSE

To describe the examination of cerebrospinal fluid (CSF) in the Medical Office Laboratories

CEREBROSPINAL FLUID (CSF)
Cerebrospinal fluid serves to protect the underlying tissues of the central nervous system. Analysis of the CSF can provide helpful diagnostic information and differentiation for conditions such as meningitis (infection of the meninges), subarachnoid hemorrhage, and disorders affecting the blood brain barrier.

SCOPE

All Medical Technologists and Medical Laboratory Technicians working at the Medical Office Laboratories

POLICY

1. CSFs are prioritized STAT and must be performed within 1 hour after it has been received in the technical laboratory due to cellular and glucose deterioration.
2. CSFs may be infectious – gloves and protective clothing must be worn.
3. If cytology is ordered, make a cytospin slide **without** albumin and stain on the Hematek stainer. Send the slide and the remaining fluid to Cytology. Add an equal amount of CytoLyt to the remainder of the specimen; secure the specimen lid and wrap the cap in parafilm and send the CSF to the Cytology department with a Health Connect Non-GYN requisition for further analysis. If CytoLyt is not available, keep the specimen refrigerated. Cytology CSF specimen must be taken from the last tube collected. Use a #2 pencil to label the slides. Labels are destroyed in the staining process at RRL.

SPECIMEN

Routine tests on CSF may include cell count, differential, glucose and protein level, and gram stain with culture.

1. CSF is routinely collected by lumbar puncture and collected in 3 or 4 sterile tubes. The tubes are usually collected sequentially.
   Tube #1 – Cell count and differential
   Tube #2 – Glucose and protein
   Tube #3 – Used for microbiological exam and gram stain – requires centrifugation
   Tube #4 – Used for cell count, differential and cytology
2. If only one cell count is ordered, perform the counts from the last tube – tube #4 (or tube 3 if only 3 tubes are received). If two counts are ordered, perform the counts on the first and last tubes.
3. Cellular constituents and glucose deteriorate quickly so specimens should be transported or assayed as soon as possible after collection.

4. Prepare smears as soon as possible after sample collection to help prevent cellular distortion and degeneration of any cells present. Cytocentrifugation yields the best preparation.
5. A *traumatic lumbar puncture* (traumatic tap) gives a pink or red color to the fluid due to the presence of RBCs from trauma during the collection process. The color will clear as subsequent tubes are collected. A clot may be seen in fluids that are extremely bloody from a traumatic tap.
6. Specimens collected sequentially from patients with a *subarachnoid hemorrhage* will appear evenly pink/red in all tubes collected, and supernatant after centrifugation appears xanthochromic (faint pink, orange or yellow) caused by released hemoglobin from hemolyzed RBC's.
7. When the volume of specimen collected is insufficient for the usual processing protocol, a decision as to the priority of the testing should be made by the provider.
8. If the fluid is partially clotted, perform the cell count and differential. Append a qualifying comment "BFCLOT" (Sample clotted, interpret with caution) to the WBC count.
9. If the fluid is completely clotted and cannot be reamed with a wooden applicator stick, the test is credited in the "ORV" application with the cancel code of "Clotted".

REAGENTS AND SUPPLIES

1. Plastic test tube
2. Plastic transfer pipette
3. Cytocentrifuge with appropriate specimen chamber and filter
4. Frosted glass slide
5. Hemacytometer cover slip
6. Neubauer counting chamber
7. Non-heparinized plastic Microhematocrit tubes
8. Microscope with 10 x and 40 x dry objectives.
9. Automated slide stainer.
10. Adjustable pipetor
11. 1:100 WBC unopette system (1.98 ml. of diluent mixture and 20 ul)
12. 1% Ammonium oxalate – Available from Rocky Mountain reagents
13. Sterile Saline
14. 5% bovine serum albumin. Store at 2-8 ^{0}C. Expiration date on the bottle.
15. Petri dish. The bottom should be lined with moist gauze.

QUALITY CONTROL

1. Manual QC control is performed every eight hours – Refer to the Hemacytometer QC procedure

TEST CODES:

Cerner Test Code	Cerner Description	Health Connect Code	Health Connect Description
CSFCC	Cell Count w/ Diff	89051A	Cell Count w/ Diff

PROCEDURE

1. Record all results on the body fluid worksheet.

2. SOURCE
   - Record source as CSF on the worksheet and indicate CSF tube # as well.

3. APPEARANCE
   - Record the appearance of the CSF and report as follows:
     - Clear (newsprint can be read through the tube)
     - Hazy (newsprint can be read through the tube, but not clearly)
     - Cloudy (newsprint cannot be read through the tube)
     - Bloody (visual presence of RBC's)

4. COLOR
   - Record the color of the spun CSF supernatant as:
     - Colorless
     - Red
     - Pink
5. WBC CLUMPING
   - Before performing the cell count on the hemacytometer, check for presence of WBC clumping while on the 10X dry objective. Record on the worksheet. This is not reported in the LIS.

6. CELL COUNT
   - DO NOT run CSF through the Hematology instrument. Current Hematology analyzers have NOT been validated for CSF automated counts.
   - Counts must be performed in duplicate and must agree within 10% - record results on the worksheet.
   - Use the following chart to determine the type of dilution

Appearance	**Dilution**	**Amount of Sample**	**Amount of Diluent**	**Type of Diluent**
Clear	No Dilution needed			
Hazy	1:2	100 *ul*	100 *ul*	Normal Saline
Hazy	1:10	50 ul	450 ul	• Normal Saline • 1% Ammonium Oxalate or Unopette diluent – This will lyse the RBCs.
Hazy to Cloudy	1:20	25 ul	475 ul	• Normal Saline • 1% Ammonium Oxalate or Unopette diluent – This will lyse the RBCs.
Cloudy to Bloody	1:100	20 ul	1980 ul (Unopette reservoir)	• Normal Saline • 1% Ammonium Oxalate or Unopette diluent – This will lyse the RBCs.

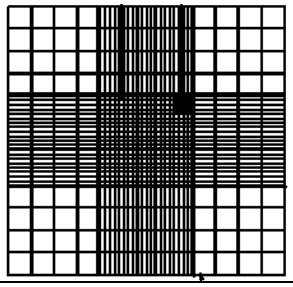
a. Undiluted WBC and RBC counts
   - Fill a non-heparinized plastic coated hematocrit tube with well mixed CSF
   - Charge both sides of the counting chamber with fluid. Be sure to discard the 1st drop before charging the counting chambers.
   - Place the counting chamber in a Petri dish with moist gauze for 5 minutes to allow the cells to settle.
   - See step g. below for number of minimum squares to count.
   - Record these values on the body fluid worksheet and enter into the LIS.
   - Results will be calculated in the LIS based on the following formula:

$$\frac{\text{\# cells X dilution X depth (10)}}{\text{\# squares counted}}$$

b. 1:100 dilution using the unopette system
   - Using the protective shield on the capillary pipette, puncture the diaphragm of the reservoir.
   - Fill the capillary with the CSF until it reaches the top of the capillary bore. Wipe excess fluid from the outside of the capillary.
   - Transfer the fluid to the reservoir. Squeeze the reservoir slightly to expel air. Maintain the pressure on the reservoir. Cover the top of the capillary pipette with your index finer, and gently insert the pipette into the reservoir neck. Release pressure on the reservoir. Gently squeeze the reservoir 2-3 times to rinse the capillary bore. Be careful not to force fluid out the top of the pipette.
   - Mix the fluid with the diluent and let it stand for 5-10 minutes to allow the red cells to hemolyze. Leukocytes remain stable for up to three hours.
   - Remove the pipette from the reservoir, invert and reseat in the neck of the reservoir.
   - Invert the reservoir and gently squeeze out 3-4 drops to clean out the capillary bore.
   - Proceed to step c.

c. Other dilutions using diluent from the unopette system
   - Using the protective shield on the capillary pipette, puncture the diaphragm of the reservoir.
   - Use the chart above to make the appropriate dilution. With an adjustable pipette, aliquot the correct amount of diluent and CSF into a plastic test tube then mix by gently tapping the bottom of the test tube.
   - Let stand for 5-10 minutes to allow the red cells to hemolyze. Leukocytes remain stable for up to three hours.
   - Proceed to step d.

d. Clean the hemacytometer and cover slip or use the disposable hemacytometer chamber.
e. With a non-heparinzed plastic microhematocrit tube, charge each side of the chamber with the undiluted or diluted CSF specimen. Discard the first drop.
f. Place the hemacytometer on moistened gauze in covered Petri dish for 5 minutes to allow the cells to settle.
g. Count the nucleated cells on both sides of the chamber based on the criteria below. This is the minimum number of squares to be counted. However, in the interest of accuracy, a tech may choose to count more squares.



# of cells seen	Count
<10	9 large squares on each side of the chamber (Undiluted only). If there are <10 total cells seen on a dilution, a smaller dilution must be made.
10-20	9 large squares on each side of the chamber
>20	4 large squares on each side of the chamber

h. Differences of more than 10% between the 2 sides of the chamber indicate poor distribution, which requires the procedure to be repeated. However, differences of more than 10% may be observed with very low counts. This is acceptable if there is only 1 cell difference between the two sides.
i. If, after dilution, there are less than 10 cells observed in the 18 squares on both sides of the hemacytometer, a smaller dilution must be made. If the number of nucleated cells is too numerous to count, a greater dilution should be considered.
j. Record the results on the body fluid worksheet and enter into the LIS.

7. DIFFERENTIAL
   a. If the total number of cells counted <150 cells, a differential is not performed.
      i. Access the "ARE" application of CERNER
      ii. Click in the comment result field
      iii. Click on the comment icon
      iv. Click on Edit while on the result comment tab
      v. Type the following information in the comment field: "Within reference range – Differential not performed"
      vi. Click on OK
   b. Prepare a cytocentrifuged slide of the CSF – See Wescor Cytocentrifuge – body fluid cell preparation procedure. Cytocentrifuged slides are air dried. With a pencil, label the slide with the patient's name, MRN, Type of fluid and date.
   c. Stain the cytocentrifuged slide on the automated slide stainer.
   d. Scan the slide using the 10 X dry objective and note the distribution of cell types and look for clumping of cells.
   e. Switch to the appropriate oil-immersion objective 50X or 100X to verify the abnormal findings noted on scan and to perform the differential count.
   f. Count 100 cells or all cells present and report the percentage of each cell type.
   g. Differential cell descriptions:

Cell Type	Description
Monocytes / Macrophages	• Usually seen in variable numbers in body fluids. • Vary in size and have a diameter of 15 to 25 um. • Cytoplasm is pale gray, cloudy and usually vacuolated. NOTE: If hemosiderin granules (blue-black pigment seen within macrophages as a result of RBC breakdown) are noted, report as comment.
Lymphocytes	• Have a similar appearance to their counterparts in blood, and become transformed in a similar fashion when confronted by an antigen. • May be small, medium or large in size • May have an immature appearance suggesting lymphoblastic leukemia or lymphoma. *Must be confirmed by a Pathologist*.
Plasma cells	• Presence suggests an inflammatory process. Seen in patients with acute viral diseases and certain chronic inflammatory conditions such as TB, syphilis, sarcoidosis, subacute sclerosing panencephalitis and multiple sclerosis. • *Must be confirmed by a Pathologist*.
Neutrophils	• Predominance suggests bacterial infection. In infected effusions, Neutrophils may show evidence of degeneration in the form of vacuolization, loss of granules, and blurring of the nuclei. • In long-standing effusions, the granules may be decreased in number or lost. The nuclei may appear as densely stained spherical fragments and may be mistaken for nucleated RBCs (pyknotic).
Eosinophils	• Appear nearly identical to those seen in blood. More than 10% is significant.
Chondrocytes	• Cartilage cells, have a central small round pyknotic nucleus surrounded by a clear zone and a distinct burgundy cytoplasm.
Neuro-ectodermal cells	• It is difficult to distinguish ependymal from choroid plexus cells • May occur singly or more often in papillary clusters of sheets. • Nuclei are round to oval, the size of small lymphocytes. The cytoplasm is moderate to abundant and gray-blue. The nuclear chromatin is delicate and finely granular with evenly distributed chromatin. Nucleoli are not present. The cytoplasmic borders may contain vacuoles, and occasionally cilia may be present.

h. Record the differential on the body fluid worksheet and enter into the LIS.

PROCEDURAL NOTES

- If examination of the Wright's stained preparation shows the cells to be too concentrated to identify, repeat the cytocentrifuge procedure using a more appropriate dilution with saline.
- Disinfect the hemacytometer with an approved laboratory disinfectant, then rinse well with water and then with alcohol prep and dry with lens paper. Alternatively, you may use a disposable

hemacytometer chamber with cover slip which can be discarded directly into a sharps biohazard container.

REFERENCE RANGES

- CSF fluid should be colorless and clear.
- Count: WBC = 0-5 (mostly mononuclear cells)
  RBC = None seen

LIMITATIONS

- If the specimen is grossly bloody, the comment "too numerous to count" (TNTC) may be entered for the RBC count result.
- Since CSF specimens sent for analysis often contain pathogenic organisms, they must be handled carefully and equipment (hemacytometer) should be thoroughly disinfected after use.
- Organisms such as yeast and bacteria may be found, especially during acute phases of infection. Both intracellular and extracellular form may be seen.
- Reject any unlabeled or mislabeled specimens. Perform the cell count and differential, but do NOT report results until the specimen is positively identified and correctly labeled.

REFERENCES

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