KAISER PERMANENTE

LABORATORY
CEREBROSPINAL FLUID EXAMINATION
(CELL COUNT AND DIFFERENTIAL)

PRINCIPLE:
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. This procedure describes the examination of cerebrospinal fluid (CSF) in the Medical Office Laboratories

SCOPE:
All Medical Technologists and Medical Laboratory Technicians working at Franklin, Lakewood, Rock Creek & Lone Tree 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.

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, etc). 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. Disposable Hemacytometer 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% Ammonium oxalate – Available from Rocky Mountain reagents
12. Sterile Saline
13. 5% bovine serum albumin. Store at 2-8 ^{0}C. Expiration date on the bottle.
14. 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 manual body fluid worksheet unless using the XT-4000i analyzer (See 6b below).

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

3. APPEARANCE
    a. 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 RBCs)

4. COLOR
    a. Spin an aliquot of the CSF fluid if the specimen color is anything but colorless. This will assist in determining whether it's a traumatic tap or prolonged subarachnoid hemorrhage.
    b. Record the color of the spun CSF supernatant as:
        - Colorless
        - Xanthochromic (Supernatant appears faint pink, yellow or orange)
        - Other (Supernatant color other than xanthochromic described above) – When selecting this option, add a comment to indicate the color of the supernatant.

5. WBC CLUMPING
    a. Before performing the cell count on the hemacytometer, check for presence of WBC clumping while on the 10X dry objective.
    b. Record if clumping is present or absent on the worksheet. This is not reported in the LIS.

6. CELL COUNT
    a. WBCs and RBCs are counted and reported on CSF fluids.
    b. Specimens that are bloody can be run through the BF channel of the Sysmex XT-4000i analyzer for the WBC and RBC count as long as the WBC count >1 thou / cmm and the RBC count >3000 thou / cmm. If the automated RBC count <3000 thou / cmm, a manual count must be performed for the RBCs.
        i. When using the XT-4000i analyzer for the WBC and RBC counts, use the Automated Body Fluid worksheet.
        ii. Follow the Sysmex XT-4000i Body Fluid Cell Count Automated procedure.
    c. The Sysmex XT1800i analyzer has not been validated for CSF fluids.
    d. Counts must be performed in duplicate and must agree within 10% - record results on the worksheet.
    e. Use the following chart to determine the type of dilution.
    f. A separate dilution may need to be made for Counting WBCs if the specimen is bloody. Use 1% ammonium oxalate to lyse the RBCs using the table below.

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
Hazy to Cloudy	1:20	25 ul	475 ul	• Normal Saline
Cloudy to Bloody	1:100	20 ul	1980 ul	• Normal Saline

    g. Fill a non-heparinized plastic coated hematocrit tube with well mixed CSF
    h. Charge both sides of the disposable hemacytomer with fluid. Be sure to discard the 1st drop before charging the counting chambers.

i. Place the counting chamber in a Petri dish with moist gauze for 2-5 minutes to allow the cells to settle.

j. Count both WBC and RBC
   - Use the table below for the minimum number of large squares to count.

# 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

   - 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:

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

k. 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.

7. DIFFERENTIAL

a. Prepare a cytocentrifuge smear using a frosted slide and add one drop of 5% albumin and a few drops of CSF.
   - The amount of CSF used in the cup is dependent on the type of cytocentrifuge you have.
   - See chart posted on your cytocentrifuge for the correct pad to use, time and speed.
   - With a pencil, write the patient's name and accession on the frosted slide

b. Stain the cytocentrifuged slide on the automated slide stainer.

c. A differential is always performed on CSF fluid whenever WBCs are present.
   - Scan the slide using the 10 X dry objective and note the distribution of cell types and look for clumping of cells.
   - Switch to the appropriate oil-immersion objective 50X or 100X to verify the abnormal findings noted on scan and to perform the differential count.
   - Count 100 cells or all cells present and report the percentage of each cell type.
   - Manually calculate the % of each type of WBC seen if the manual differential is <100 using the following formula:

$$\frac{\#\text{ of WBC type}}{\text{Total \# of WBC}} \text{ X } 100$$

Example: Total # of WBC seen = 40, # of lymphocyte seen = 32

$$\frac{32}{40} \text{ x } 100 = 80\%$$

d. Record the differential on the worksheet and enter in the LIS.

e. If there are no WBCs seen on the count, a differential is not performed. Enter a result of "See comment" in one of the WBC differential parameter
   i. Access the "ARE" application of CERNER
   ii. Click in the result field, right click and select freetext
   iii. Type "See comment"
   iv. Click on the comment icon
   v. Click on Edit
   vi. Type the following information in the comment field: "Within reference range – Differential not performed"
   vii. Click on OK

f. 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.

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.

REFERENCE RANGES:

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

LIMITATIONS:

- 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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2. Clinical Diagnosis and Management, 18th ED., U.B. Saunders CO., Philadelphia, 1991.
3. Urinalysis and Body Fluids – A Color Text and Atlas, 1st edition, 1995. Karen Munson Rigsrud and Jean Jorgenson Linne.
4. Body Fluids: Laboratory Examination of Cerebrospinal, Seminal, Serous & Synovial Fluids, 3rd edition, 1993.