CEREBROSPINAL FLUID (CSF)

CSF is produced in the choroid plexus cells of the brain by ultra filtration of the plasma. CSF protects the brain and spinal cord from sudden changes in pressure, maintains a stable chemical environment for these structures, and removes the waste products of cerebral metabolism. Total CSF in adults is 85-150 ml and 10-60 ml in neonates.

Collection:
CSF is obtained by lumbar puncture (between 3rd & 4th lumbar vertebrae) for suspected meningitis, brain abcess, encephalitis, treatment of benign intracranial hypertension, or introduction of anesthetic drugs.

It is routine practice to collect three tubes of CSF (1-5 ml per tube) for analysis. The first tube is designated for chemical and immunological studies, the second for microbiological gram stain/culture, and the third tube for cell counts and differential.

Exam: Must be done immediately; STAT due to critical importance of results and to reduce cell deterioration.

A. Color - normal is colorless

1. Red, bloody - could indicate a recent hemorrhage in the brain or a traumatic puncture at collection. If a traumatic tap, the blood in the 1st tube is greater than the last tube and the supernatant fluid is usually colorless after centrifugation. Tubes are equally bloody if due to a recent pathologic bleed.

Note: DO NOT centrifuge CSF specimen before cell count is done.

2. Xanthochromic (yellowish) color of the supernatant fluid after centrifugation - denotes past bleeding and occurs as a result of hemoglobin degradation to bilirubin.

B. Turbidity - normal is clear

1. CSF can be hazy or cloudy due to increased protein, increased WBC's, increased RBC's, or the presence of bacteria.

2. Occasionally, the presence of fibrinogen will cause a specimen from a traumatic tap to clot. If clotted, the cell count is not accurate because the cells will be caught in the fibrin clot. In this case, consult pathologist and/or doctor.

C. Cellular elements - normal CSF is acellular

1. Normal CSF WBC:
   a. Adults: 0-5 WBC/µL, predominantly mononuclear WBCs with mainly lymphocytes; a critical CSF WBC is >10/µL. The RBC count is only significant if intracranial hemorrhage is suspected.

   b. Neonates: 0-30 WBC/µL with mainly monocytes; a critical CSF WBC is >40/µL.

2. Increased CSF WBC with predominantly polymorphonuclears (neutrophils) indicates bacterial (purulent) meningitis,...commonly due to pneumococci, strep, staph, hemophilus, neisseria.

3. Increased CSF WBC with predominantly mononuclears (lymphocytes and monocytes) indicates viral (aseptic) meningitis, fungal meningitis, TB infection or possibly multiple sclerosis.
D. Chemistry – the CSF specimen is centrifuged

1. Protein (normal 15-45 mg/dL) - elevated CSF protein is a non-specific, but reliable, indicator of CNS pathology. Increased CSF protein and lactate levels indicate a bacterial meningitis.

2. Glucose (normal ~70% of plasma glucose) - low glucose values usually signal an infection such as bacterial, TB, or fungal meningitis (due to increased glucose utilization by leukocytes or bacteria). CSF glucose is usually normal in viral meningitis. An increased lactate suggests bacterial meningitis.

E. Procedure for manual CSF cell count….CSF is not counted by automated methods (currently).

1. Macroscopic exam - note fluid color and turbidity.

2. Mix fluid; use a separate aliquot for dilutions if indicated.
   a. Cell counts are performed on undiluted fluid if the fluid is clear and colorless. Plate straight on hemocytometer and count all 18 squares.
   b. If cells are numerous, make an appropriate dilution of CSF with 0.9% saline, plate and count the number of squares on hemocytometer that is appropriate for the cell number. The dilution to make is guided by the fluid appearance and the area counted depends on the number of cells present on the hemocytometer.

3. Standard cell count formula:
   a. \[ \frac{\text{Total } \# \text{cells} \times \text{Dilution} \times \text{Depth}}{\text{Area counted}} = \text{cell type/uL} \]
   **Dilution and area will vary.
   b. Example: Clear and colorless CSF plated straight and all 18 squares counted.
      \[ \frac{\text{Total } \# \text{cells} \times 1 \times 10}{18} = \text{RBC/uL or WBC/uL} \]

F. Differential

1. All CSF’s have a cytospin smear made for a differential. Addition of albumin helps prevent cell breakage/distortion. The cytocentrifuge concentrates the fluid cells onto the slide that is then Wright’s stained. A 100 cell diff is done separating cells into polys (neutrophils), lymphocytes, monocytes/histiocytes and others***.

2. For extremely low WBC counts, it may not be possible to obtain a 100 cell differential. In this case, use the calculation below to obtain the % of each WBC type.
   a. \[ \text{Differential } \% = \frac{\# \text{ WBC type}}{\text{Total } \# \text{ WBC’s counted}} \times 100 \]
   b. Example: 0 polys, 42 lymphs, 18 monos are counted for a total of 60 white cells
      \[ \frac{42}{60} \times 100 = 70\% \text{ lymphs} \quad \frac{18}{60} \times 100 = 30\% \text{ monos} \]

***Review list of cells/findings that may be present in CSF and other body fluids.
SEROUS & SYNOVIAL FLUIDS

A. Types - normally only a small amount of clear, pale yellow fluid is present as production and reabsorption take place at a constant rate.
1. Pleural fluid - fluid within the pleural lining of the lungs to allow free movement of lungs.
2. Pericardial fluid - fluid within the pericardial lining of the heart to allow free movement of heart.
3. Peritoneal fluid - fluid within the peritoneal lining of the abdominal cavity to allow free movement of the viscera. In disease, fluid build-up in the peritoneal cavity = ascites.
4. Synovial fluid - fluid surrounding joints to minimize friction between bones.

B. Collection procedure:
Under pathologic conditions, excessive fluid can accumulate. The effusion is collected by paracentesis of a body cavity (thoracentesis, arthrocentesis). For cell counts, fluids are anticoagulated with heparin or EDTA to prevent clotting.

C. Serous fluids: pleural, pericardial, or peritoneal - the effusion can be a transudate or an exudate.
1. A transudate is a fluid that consists mostly of water that moves out of the blood vessel into the body cavity. It is due to an imbalance in hydrostatic and osmotic forces. It is usually low in protein with rare cells, and may be the result of congestive heart failure, cirrhosis of the liver, or nephrotic syndrome, i.e., non-inflammatory. *See comparison chart.
2. An exudate is a fluid that exuded out of direct tissues because of injury or inflammation. It can also be the result of an increase in permeability of the blood vessel to plasma proteins. It is usually high in protein and leukocytes, and caused by infection and malignancy or decreased absorption by the lymphatic system. *See comparison chart on page 4.

D. Procedure for cell count - mix and use separate aliquot for dilutions.
1. Mix and plate undiluted/straight or make appropriate dilution of body fluid with 0.9% saline.....1:10 dilution or greater.
2. Count 18 squares** and calculate # WBC’s/uL and # RBC’s/uL using standard formula. **Higher dilutions and/or fewer squares counted may be required as warranted by cell #.
3. If quantity is sufficient, cell counts on serous fluids (NOT CSF) or synovial fluid (with hyaluronidase added) can be done using an automated cell counter after a wet prep assures that fibrin clots, bacteria or clumps of cells are not present. See site procedure.
4. For serous fluids, a WBC >1000/uL is considered elevated. For synovial fluid, a WBC >50,000/uL is classified as septic (normal <200/uL).

E. Differential
All body fluids must have a differential done on a Wright’s stained cytospin slide. Lymphocytes, macrophages/monocytes/histiocytes, and mesothelial/synovial lining cells are normally seen. **See list of other cells/findings that may be present in body fluids.

F. Reminders:
1. A clotted specimen will not give an accurate cell count.
2. Never use a diluting fluid other than saline for body fluids - the protein will precipitate and WBC’s will clump.
### Comparison of transudates and exudates

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<thead>
<tr>
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<th>Transudates</th>
<th>Exudates</th>
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<tbody>
<tr>
<td><strong>Turbidity</strong></td>
<td>Clear, thin, watery, pale yellow</td>
<td>Cloudy, thick, creamy (green - bile present)</td>
</tr>
<tr>
<td><strong>WBC count/mm$^3$</strong></td>
<td>$&lt;1000$/uL (few cells)</td>
<td>Usually $&gt;1000$/uL (many cells)</td>
</tr>
<tr>
<td><strong>Total protein</strong></td>
<td>$&lt;3$ gm/dL (protein poor)</td>
<td>$&gt;3$ gm/dL (protein rich)</td>
</tr>
<tr>
<td><strong>Fluid to serum</strong></td>
<td>$&lt;0.5$</td>
<td>$&gt;0.5$</td>
</tr>
<tr>
<td><strong>LD ratio</strong></td>
<td>$&lt;0.6$</td>
<td>$&gt;0.6$</td>
</tr>
<tr>
<td><strong>Clots</strong></td>
<td>Usually absent</td>
<td>Often present (↑ fibrinogen)</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td>None</td>
<td>May be present</td>
</tr>
<tr>
<td><strong>Associated with</strong></td>
<td><strong>No</strong> (rare)</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>inflammation</strong></td>
<td>Caused by disease outside the body cavity</td>
<td>Caused by disease directly involving the organs</td>
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### Cells/Findings that may be present in CSF and other body fluids

- segmented neutrophils (look for bacteria if large numbers)
- lymphocytes (also reactive)
- macrophages/monocytes/histiocytes (also signet ring cells)
- plasma cells
- eosinophils and basophils
- phagocytized red cells by macrophages (erythrophages)
- hemosiderin laden macrophages (siderophages)
- choroidal cells - cells lining the choroid plexus, CSF
- ependymal cells - cells lining the ventricles, CSF
- mesothelial cells - cells lining body cavities, serous fluids
- synovial cells - lining cells, synovial fluid
- infectious agents (bacteria, Cryptococcus, yeast)
- LE cells
- pyknotic cells (necrotic neutrophils can be confused with nucleated red cells)
- may see crystals (MSU/CPPD) in synovial fluids, inflammatory type (II)
- may see nucleated red cells (can indicate possible bone marrow puncture during CSF collection)
- malignant cells - leukemic blast cells, lymphoma cells, metastatic cancer cells, primary brain tumors, signet ring carcinoma cells

**Notes:**
- Cell clumping can be a sign of malignancy
- Always scan cytospin slide on low power initially - use red cells in the field to help you judge cell size
- Some cell distortion does occur in cytospin preps