Examination of Body Fluids: Evaluating Gross Appearance; Performing Cell Counts

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ABBREVIATIONS: CNS = central nervous system; CSF = cerebrospinal fluid; RBC = red blood cell; WBC = white blood cell.

INDEX TERMS: body fluids; cerebrospinal; serous; synovial.

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LEARNING OBJECTIVES

1. Describe the normal appearance of cerebrospinal fluid, serous fluids, and synovial fluid.
2. Define gross findings that distinguish a traumatic tap from a pathologic bleed when a bloody CSF is encountered.
3. Name laboratory findings that aid in identifying a serous fluid as a transudate or exudate.
4. Describe how the appearance of a fluid can be used to determine the correct dilution for accurate cell counts.

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Evaluating gross appearance: CSF

Spinal fluid is the only fluid that is normally available in quantities sufficient to sample. Adults have 90-150 mL of cerebrospinal fluid (CSF) and neonates 60-90 mL.[1] The spinal fluid bathes the brain and spinal column providing nutrients to, removing waste products from, and acting as a protective cushion for the central nervous system (CNS). Since this fluid circulates around the brain and spinal column, it is an excellent source of information for the condition of the CNS. CSF is collected by lumbar puncture and, ideally, is placed into three or four sterile tubes labeled 1, 2, 3, and 4 in the order in which it is collected. If multiple tubes are received and multiple tests are ordered, tube #1 should be used for chemistry and immunology testing, tube #2 for microbiology, tube #3 for cell counts and differential, and tube #4 for any other tests.[1]

The normal appearance of CSF is colorless and crystal clear, resembling water. A cloudy or turbid fluid indicates increased WBCs or protein. A bloody fluid can indicate a traumatic tap in which a blood vessel is inadvertently punctured in the process of collection or blood can mean an intracranial hemorrhage or pathologic bleed. If multiple tubes are collected, there is clearing of the blood from the first tube to the last tube collected in the case of a traumatic tap but a consistent amount of blood in all tubes in the case of a pathologic bleed. If only one tube containing bloody fluid is obtained, an aliquot of the fluid should be centrifuged and the appearance of the supernatant examined. The supernatant will be colorless and crystal clear in the case of traumatic tap and xanthochromic (yellow) to hemolyzed (red) if a pathologic bleed has taken place.

Serous fluid

The heart, lungs, and intestine—organs that move—are each enclosed in a sac. Between the sac and the organ there is a single layer of cells called mesothelial cells. Serous fluid flows through the "space" between the organ and its sac, allowing the organ to move smoothly against the sac. Serous fluid does not normally exist in quantities sufficient to sample so when it arrives in the laboratory to be analyzed—usually in very large quantities—it indicates an abnormal condition and is termed an effusion. An effusion is determined to be

a transudate or an exudate using several visual and chemical findings (Table 1).[2] Transudates indicate accumulation of fluid due to a benign systemic process such as chronic heart failure or cirrhosis. Transudates are created when fluid is filtered through vessel walls. Transudates contain few chemical and cellular elements and have a low specific gravity.

Exudates indicate a localized condition that affects the surfaces of organs directly such as malignancies, infections, or trauma. Because these conditions cause fluid release due to vessel damage, exudates contain a high concentration of protein and cellular elements and have a high specific gravity.

Normal serous fluid is pale yellow and clear. Transudates are pale yellow and hazy to cloudy depending on the number of cells present. Exudates are cloudy and may be bloody. In pleural fluids, chylous effusions are milky white or yellow-bloody and indicate damage or obstruction to the thoracic duct secondary to trauma, malignancy, or congenital abnormality; pseudochylous effusions are also milky or green with a metallic sheen, contain cholesterol crystals and indicate chronic inflammation.[3]

Synovial fluid

Synovial (joint) fluid is normally not present in amounts sufficient to sample, but volume increases in inflammation. When it does not contain significant numbers of cells or crystals, synovial fluid is pale yellow and clear. Because this fluid contains hyaluronic acid, it is viscous and is difficult to work with unless it is liquefied with hyaluronidase, an enzyme that breaks down the hyaluronic acid. Synovial fluid that contains large numbers of crystals may appear "milky".

Table 1. Transudates vs. exudates in serous fluids[2]

Characteristic	Transudates	Exudates
Color	clear/straw	cloudy/ yellow, amber, or grossly bloody
Specific gravity	<1.016	>1.016
Protein	<3 g/dL	>3 g/dL
LDH	<200 IU	>200 IU
Nucleated cell count	<1000/ µL	>1000/ µL

Performing cell counts

All samples should be thoroughly mixed prior to performing cell counts or preparing slides. If the specimen contains clots, tissue particles, or other bodies, that finding should be noted on the report. If those "objects" interfere with the performance of counts, they can be removed prior to performing cell counts or preparing slides.

Cell counts on body fluids are usually performed on the hemacytometer. When performed on cell counting instruments, linearity must be confirmed since very low cell counts can be significant in CSF. Hyaluronidase should be added to synovial fluids before cell counts are performed or cytocentrifuge slides are prepared. When using the hemacytometer to perform cell counts, the dilution and number of squares counted should be adjusted for the number of nucleated cells present, which may be estimated from the specimen's clarity.

$$\text{Cells}/\mu\text{L} \quad \frac{\text{Cells counted X 10 (depth factor) X dilution factor}}{\text{Area counted in mm}^2}$$

While the presence of RBCs in fluids is significant and should be reported in the gross appearance description, the RBC *count* has no clinical significance. The clinically significant cells are the nucleated cells. When performing cell counts, fluids with low nucleated cell counts may be counted without dilution. If RBCs are present, it is helpful to use a diluting fluid such as acetic acid or Türk's solution that will lyse the RBCs and enhance the nucleus of nucleated cells. Türk's solution preparation: Dilute 3 mL glacial acetic acid to 100 mL total volume with deionized water and add two to three drops methylene blue or Giemsa stain. Using calibrated pipettes, dilutions of 1:2, 1:3, and so on can be made to provide accurate cell counts on fluids containing low numbers of nucleated cells. Acetic acid-based diluting fluids should not be used when counting cells in synovial fluids since acetic acid causes the formation of mucin clots.[1]

The dilution needed to perform cell counts can be estimated by the appearance of the fluid using the following criteria:

- Clear: Nucleated cell count will be less than 200/µL; RBC count will be less than 2000/ µL. No dilution is necessary; the number of squares counted depends upon

the number of cells seen.

- Hazy or cloudy: Nucleated cell count will be greater than 200/µL or RBC count will be greater than 2000/µL. The standard WBC dilution used for blood, 1:20, should yield an accurate nucleated cell count.
- Bloody: RBC count will be greater than 5000/µL and there will be distortion of nucleated cells on cytocentrifuge preparations. The standard WBC dilution used for blood, 1:20, should yield an accurate nucleated cell count.
- Grossly bloody: RBC count will be ≥1,000,000/µL. The standard WBC dilution used for blood, 1:20, should yield an accurate nucleated cell count. To prepare a slide for cellular examination, make a "push" slide and perform a differential on cells pushed out to the end of the smear or on the edges of the smear, not in the body of the smear as on a peripheral blood smear. The larger and more significant cells (including tumor cells) will be pushed out to the end or edges of the smear.

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