Examination of Body Fluids:
Preparation of Slides and Cell Morphology

LEILANI COLLINS

ABBREVIATIONS: ALL = acute lymphoblastic leukemia; AML = acute myeloblastic leukemia; CNS = central nervous system; CSF = cerebrospinal fluid; HIV = human immunodeficiency virus; RBC = red blood cell; WBC = white blood cell.

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


LEARNING OBJECTIVES
1. Describe and calculate the proper dilution for preparation of a monolayer cytocentrifuge slide.
2. List cellular findings that may be present in any fluid.
3. Describe lining cells that may be found in CSF, serous, and synovial fluids.
4. Distinguish benign and malignant cells.
5. Compare distinguishing characteristics of significant crystals in synovial fluids.

Leilani Collins MS MT(ASCP)SH CLS(NCA) is associate professor, Clinical Laboratory Science Program, University of Tennessee Health Sciences Center, Memphis TN.

Address for correspondence: Leilani Collins MS MT(ASCP)SH CLS(NCA), associate professor, Clinical Laboratory Science Program, University of Tennessee Health Sciences Center, 930 Madison Avenue, Suite 670, Memphis TN 38163. (901) 448-6299. lcollins@utmem.edu.

Leilani Collins MS MT(ASCP)SH CLS(NCA) is the Focus:

Body Fluids guest editor.
Specimens concentrated by centrifugation or cytocentrifugation are used to perform differential counts and assess cell morphology on body fluids. Using standard centrifugation, the specimen is centrifuged, the supernatant removed, and a slide made from the buffy coat if RBCs or the concentrated cells of the centrifugate are present. In cytocentrifugation the specimen is transferred to a cytofunnel assembly. The specimen is centrifuged at 1000 RPM for 10 minutes allowing fluid to be absorbed into filter paper and cells to be concentrated in a small area of the slide. The slides prepared by either concentration method should be allowed to air dry and are then stained with Wright or Wright-Giemsa stain prior to examination. If it is not possible to prepare cell concentrations to assess morphology of nucleated cells and cells are differentiated while the cell count is performed, a lysing agent that enhances the nucleus of cells must be used to determine the category of the cells. In this article, preparation of slides refers to cytocentrifugation.

To prepare a monolayer cytocentrifuge slide, which is optimal for nucleated cell identification, use the nucleated cell count (not the RBC count) to determine the saline dilution for the cytocentrifuge preparation. A consistent amount of undiluted or diluted fluid should be used to prepare a cytocentrifuge slide—usually 0.25 mL or five drops. A good monolayer preparation can be obtained if the nucleated cell count is less than 200/µL. If the nucleated cell count is greater than 200/µL, divide the cell count by 100 to obtain the dilution factor.

• Example: Nucleated cell count = 1200/µL.
  • Dilution for cytospin = 1200/100 = 12 = 1 drop of fluid + 11 drops of saline. Transfer five drops of the diluted fluid to the cytofunnel.

RBCs in quantities greater than 2000/µL distort a concentrated preparation due to crowding. Dilutions should not be based on the RBC count, however, and any lysing agents used to lyse red cells will also distort the morphology of nucleated cells.

Cell morphology in body fluids
The morphology of nucleated cells is the most important
diagnostic factor in any body fluid analysis. While some cells may be found in all fluids, their significance may be different depending on the fluid being analyzed. Normal cells in all fluids are lymphocytes, macrophages, and lining cells. The appearance of these cells can vary depending on the location. For example, macrophages in CSF appear as monocytes, but in serous and synovial fluids they appear as histiocytes. Once a macrophage has ingested cellular material, it is named for the material ingested. For example, a macrophage that has ingested RBCs is termed an erythrophage; if the RBCs have been ingested for more than 72 hours, the iron in the RBCs is visible and the cell is termed a siderophage. The finding of erythrophages and siderophages has different significance in various fluids. They always indicate the attempt of the body to rid a space of RBCs but in CSF they are indications of a pathologic bleed instead of a traumatic tap because the blood would have to be in the CNS for at least 24 hours. Erythrophages in serous or synovial fluids also indicate bleeding in the space around the organ or joint, but the finding of a significant amount of fluid in these spaces is more indicative of a pathologic condition.

Cells found in all fluids
Lymphocytes can be found in any fluid. Usually they appear as normal lymphocytes in peripheral blood. When seen in CSF in viral meningitis, they will demonstrate pleomorphism with variation in size, appearance and basophilia. Macrophages are a normal finding in any fluid. In CSF, they appear as monocytes in peripheral blood unless they have ingested RBCs. In serous and synovial fluids, they are larger with abundant foamy cytoplasm and are known as histiocytes or macrophages.

Neutrophils are not normally present but are present in infection and inflammation. Neutrophils in body fluids are hypersegmented and have prominent filaments.

Eosinophils can be seen in any fluid and usually indicate an allergic response to a foreign substance. Often, basophils are present with eosinophils. Eosinophils are a common finding in CSF from patients with hydrocephalus who have a shunt to drain fluid around the brain to the abdomen.

Bacteria and yeast can be seen in any fluid. When large numbers of neutrophils are seen, a thorough search for bacteria should be made. The finding of intracellular bacteria is significant since that eliminates the possibility of contamination of slides from extraneous sources such as coughs, sneezes, or saliva. All bacteria stain blue or purple with Wright stain and should not be mistaken as Gram positive organisms.

Tumor cells may be seen in any fluid but are rare in synovial fluids. If the presence of tumor cells is suspected, a differential should not be performed on the fluid. Instead, the fluid should be prepared for cytology review and the report should indicate that a cytology report will follow. Tumor cells have some common characteristics though not all tumor cells have the same characteristics. Tumor cells
may be large and dark-staining or unevenly stained. Often they are in clumps that are three dimensional or “stacked”. Mitotic figures may be present within the clumps of cells. The cells may have irregular or bizarre nuclear shapes and often the borders of the nucleus may be indistinct, irregular, and disintegrated making it difficult to determine where the nucleus ends and the cytoplasm begins. Nucleoli may be large and prominent. There can be cytoplasmic and nuclear vacuoles. Tumor cells can demonstrate nuclear and cellular molding where the cells line up together, and even demonstrate cannibalism. Tumor cells can take on many unusual shapes and characteristics (pleomorphic) so if cells are encountered that don’t “fit” into a familiar morphotype, suspect tumor cells and defer to cytology review.

Cerebrospinal fluid
The normal nucleated cell count in CSF is <5/µL. If there are fewer than 100 cells on a cytocentrifuge preparation, the cells present should be differentiated and reported as a whole number, not a percentage. Otherwise, 100 cells should be differentiated and reported as a percentage. The predominant cell type in adults is the lymphocyte with few monocytes. In neonates, the predominant cell is the monocyte.

In the case of a traumatic tap, there will be peripheral blood contamination including RBCs and white blood cells (WBCs). There may also be choroid plexus cells, the lining cells of the CNS. These cells are large with abundant lavender cytoplasm and a well-defined purple nucleus on Wright stained slides. They can occur in clumps but the well defined nucleus identifies them as benign cells. If the cartilage of the vertebrae is punctured during the tap, cartilage cells may be seen. These cells are larger than WBCs and have wine-red cytoplasm with a deeper wine-red nucleus. The vertebrae are sites of bone marrow production so it is possible to have bone marrow contamination as part of a traumatic tap. The best indication of bone marrow contamination is the presence of RBC precursors or nucleated RBCs. Any cell that is found in the bone marrow, including megakaryocytes and blasts, can be seen. A differential should not be reported on CSF contaminated with bone marrow but a note that “bone marrow contamination suspected” should be reported instead.

The cellular findings in a pathologic brain bleed include erythrophages and siderophages. See Figure 1. Some siderophages may contain hematoidin or bilirubin crystals, which are golden-yellow rectangular crystals. If a patient has had severe head trauma or brain surgery, neural tissue may be seen. This tissue will appear as clumps of foamy lavender material containing several small, well-spaced nuclei or dendritic cells containing a nucleus with elongated and branched cytoplasm.

Meningitis is the term used for any condition that causes inflammation to the meninges or covering of the brain. In viral meningitis, there will be an elevated nucleated cell count usually in the hundreds/µL. In bacterial meningitis, the nucleated cell count will be extremely elevated, usually in the thousands/µL. In viral meningitis, the predominant cell is the lymphocyte with marked pleomorphism from normal-appearing to reactive and plasma-like lymphocytes. In bacterial meningitis, the predominant cell is the neutrophil and a careful and thorough search should be made for intracellular bacteria since only a small number of bacteria may be present on the initial spinal tap.

Cryptococcal meningitis can be seen in immunocompromised patients. Cryptococcus neoformans is an encapsulated yeast. On Wright stain, it may appear as individual organisms appearing separated from each other by unstained capsules or the capsules can take up the stain producing a reddish-purple “starburst” pattern. See Figure 2.

Leukemic meningitis occurs in patients with acute leukemia when leukemic cells are present in the meninges. The blood-brain barrier prevents chemotherapeutic drugs from entering the central nervous system so these patients must receive intrathecal therapy: chemotherapy injected directly into the
spinal column. This is especially common in acute lymphoblastic leukemia (ALL) but may be seen in acute myelogenous leukemia (AML) and lymphoma. If evidence of a traumatic tap is present in a specimen from a leukemic patient, great caution should be taken in reporting blasts in the spinal fluid since blasts seen can be the result of peripheral blood contamination if there are blasts in the patient's peripheral blood. The spinal tap should be repeated in approximately three days to ascertain the presence of blasts in the CNS.

Carcinomatous meningitis occurs when tumor cells are seen in CSF. These may be the result of primary brain tumors or metastatic carcinoma. The tumor cells seen will have characteristics mentioned earlier in this article.

**Serous fluid**
The presence of serous fluid is indicative of a pathologic condition. Normal cells in serous fluids include lymphocytes, macrophages, and mesothelial cells. Neutrophils are commonly seen in these fluids since an inflammatory or infectious condition is the likely cause of the effusion.

Mesothelial cells are the lining cells found in the pleural, pericardial, and peritoneal cavities. The mesothelium is composed of a single layer of cells and these cells can be seen in clumps or clusters that are only one cell thick. They are large cells and have a “fried egg” appearance with one or more distinct, well-defined oval nucleus or nuclei. They may become very large with many nuclei and abundant cytoplasm. The cytoplasm may contain vacuoles. See Figure 3.

Malignant cells due to either primary or metastatic carcinomas can be found in serous fluids, especially pleural fluids. These cells will have characteristics described earlier. If tumor cells are seen, a differential count should not be performed but the specimen should be prepared for cytological evaluation. See Figure 4.

In the case of a pseudochylous effusion of pleural fluid, cholesterol crystals may be seen. These are large extracellular, plate-like crystals with notched edges. They indicate an inflammatory process.

![Figure 4. Tumor in pleural fluid](image)

Cells are large (compare with normal lymphocytes and RBC in picture), dark staining with undistinguishable nuclear borders, and in clumps.

![Figure 5. Calcium pyrophosphate crystals (pseudgout)](image)

![Figure 6. Monosodium urate crystals seen in gout](image)
Synovial fluid
A small amount of hyaluronidase should be added to synovial fluid prior to performing cell counts or preparing cytocentrifuge slides to liquefy the fluid.

Normal cells in synovial fluid include lymphocytes, macrophages, and synovial cells. Neutrophils are often seen since inflammation or infection is a common cause of the effusion. Synovial cells are the lining cells of the synovium, the sac around the joint. These cells resemble mesothelial cells in that they are large with well-defined oval nuclei. The lining cells usually are not as abundant in synovial fluid as serous fluid. It is not unusual to see cartilage cells in these fluids. Synovial fluid is often sent to the lab to evaluate for crystals. Cholesterol crystals are indicative of inflammation. They are large, extracellular, plate-like, colorless crystals with notched edges.

Clinically significant crystals include calcium pyrophosphate crystals that are seen in pseudogout and monosodium urate crystals that are seen in gout. The morphology of the crystals is helpful in identifying the category of the crystals and polarizing with red compensation will provide definitive information. Calcium pyrophosphate crystals are colorless, intracellular crystals that may be rhomboid, square, or thin and elongated. They often look like intracellular crushed glass and there may be abundant crystals in a single cell. Monosodium urate crystals are colorless, needle-like crystals that may be intracellular or extracellular. When these crystals are viewed under polarization with red compensation, calcium pyrophosphate crystals appear blue when the long side of the crystal is parallel to the slow or y-axis and monosodium urate crystals appear yellow when the long side of the crystal is parallel to the y-axis. See Figures 5 and 6.

Clin Lab Sci encourages readers to respond with thoughts, questions, or comments regarding this Focus section. Email responses to george@fritsmafactor.com. In the subject line, please type “CLIN LAB SCI 22(1) FO BODY FLUIDS”. Selected responses will appear in the Dialogue and Discussion section in a future issue. Responses may be edited for length and clarity. We look forward to hearing from you.

WORKS CITED