LEARNING GUIDE

ANALYSIS OF BODY FLUIDS

CORELABORATORY.ABBOTT/HEMATOLOGY

Abbott

CONTENTS

SECTION 1

OVERVIEW OF BODY FLUIDS

LEARNING OBJECTIVES

After completing this section, you will be able to:

1. Identify 3 major categories of body fluids
2. List 3 biochemical tests that may be ordered on body fluids
3. List advantages and disadvantages of automated methods
4. Describe the process for making a cytospin
5. List several types of hematopoietic cells found on the cytospin smear in CSF analysis

OVERVIEW OF BODY FLUIDS

Analysis of body fluids (BFs) in the clinical laboratory plays an important role in diagnosing hemorrhagic, infectious, inflammatory, and malignant processes within body cavities and the central nervous system (CNS). Since these fluids are not derived from blood or urine, these are considered "non-standard" BFs. Body fluids are normally found in the CNS (cerebrospinal fluid), around the heart (pericardial fluid), around the lungs (pleural fluid), surrounding the abdominal organs (peritoneal fluid), and in joints such as the elbow and knee (synovial fluid) (**Figure 1**). Increased quantity or abnormal presence of BFs may be indicative of a disease process. Body fluids can be grouped into three major categories: cerebrospinal fluid (CSF), serous fluids (SEF), and synovial fluid (SYF). The major serous fluids include pleural fluid (PLF), peritoneal fluid (PNF) and pericardial fluid (PCF). In addition, there are other fluids such as peritoneal dialysate, bronchoalveolar lavage, etc.

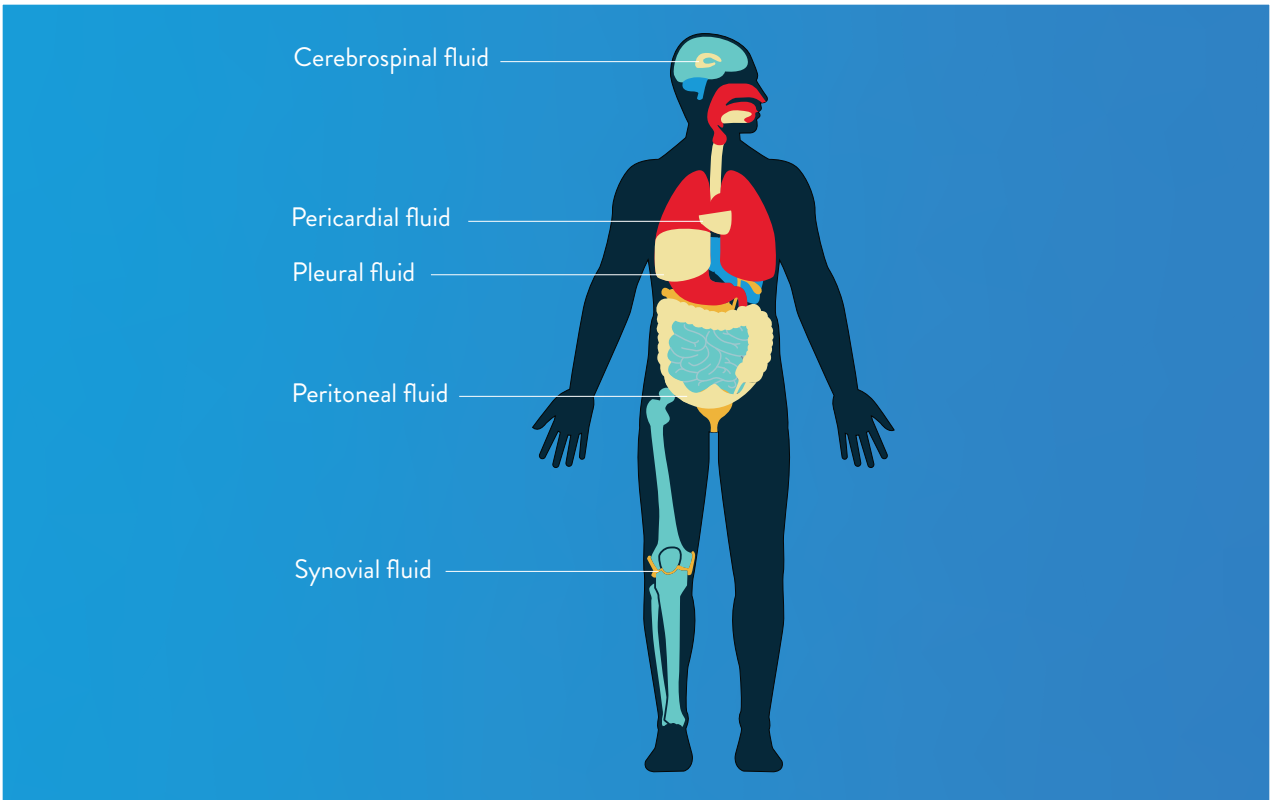


Figure 1. Common areas where body fluids are located

Laboratory evaluation of BFs includes gross examination, biochemical analysis, microbiologic testing, cell count, differential cell count, and cytologic examination.[1] Body fluids are collected aseptically by introducing a needle into the body cavity of interest and gently aspirating the fluid into a syringe. The fluid is then transferred from the syringe into 1 or more (up to 4) sterile tubes. CSF does not require an anticoagulant to be added to the tube, while serous and synovial fluids do require an anticoagulant such as liquid ethylenediaminetetraacetic acid (EDTA) or sodium heparin. Samples for biochemical analysis may or may not require anticoagulation. For cell count and differential counts, 3-5 mL of sample should be added to a tube containing the anticoagulant and mixed gently before analysis. No anticoagulant is required for microbiology studies and cytologic examination. However anticoagulated samples are acceptable for cytology.[1]

BFs are sensitive to the time elapsed between collection of the fluid and the point at which they are analyzed. BFs should be transported to the laboratory and evaluated as quickly as possible at ambient temperature. Most fluids are stable for 2-4 hours at ambient temperature and may be refrigerated up to 24 hours before processing.[1] CSF, however, is the most sensitive to storage time and temperature and should be processed as quickly as possible after collection.

OVERVIEW OF BODY FLUID TESTING

GROSS EXAMINATION

Analysis of body fluids in the laboratory begins with a gross examination of the fluid. The volume, color and appearance are noted. CSF in normal individuals is clear and colorless while normal serous fluids are pale yellow and clear. If a specimen is bloody, a preliminary assessment is made whether the presence of blood is due to the procedure or due to an underlying disease process; for instance, a traumatic tap in contrast to a patient with a subarachnoid hemorrhage or hemorrhagic stroke. In samples that are not bloody, orange color suggests the presence of breakdown products of blood; green color may be associated with meningitis or hyperbilirubinemia; brown color may be due to melanin pigment shed from malignant melanoma or contamination from the disinfectant used to clean the skin. Cloudy or turbid specimens are associated with infection and/or the presence of protein in the fluid.

BIOCHEMICAL ANALYSIS

Laboratories are often asked to perform biochemical analysis on BFs. The BF matrix differs from serum or plasma, so “matrix-effect” must be considered for the results. Only fluid types indicated by the manufacturer or validated by the laboratory should be tested on routine chemistry analyzers. Some common biochemical tests ordered on BFs include: specific gravity, total protein, albumin, lactate dehydrogenase, glucose, C-reactive protein, electrolytes, bilirubin, transferrin, and protein electrophoresis. A variety of pathological conditions can lead to an increased concentration of these analytes in BFs.

MICROBIOLOGICAL ANALYSIS

In suspected infectious diseases, BFs may be sent to the laboratory for culture and/or molecular testing. For microbiologic testing, the BF is added to a culture medium immediately and there is no need for an anticoagulant to be added to the sample. Molecular testing has become the preferred method of testing in many pathologic conditions due to its robust nature and quick turn-around.

CELL COUNTING

Laboratory analysis of BFs also includes a cell count that identifies and quantitates the total nucleated cell count (TNCC) including leukocytes, erythrocytes (RBCs) and non-hematopoietic cells. This can be performed manually, by specialized test systems or by using automated hematology analyzers. The reference method for counting cells in BFs is the manual method that requires a hemocytometer and microscope. A hemocytometer is a counting chamber that consists of a microscopic slide with a grid etched onto the surface (**Figure 2**).[1] While the Neubauer chamber is most commonly used for manual counting, other chambers such as Fuchs-Rosenthal, Burker can also be used.[2] If the BF sample is clear, it is not necessary to make a dilution before counting on the hemocytometer. However, if the BF is turbid and/or grossly bloody, a dilution may be necessary. The WBC count is more accurately reported as the TNCC. Normal tissue lining cells, tissue macrophages, and some malignant cells can be similar in cell size and nuclear content thereby making them impossible to distinguish from WBCs in the counting chamber.[3] RBCs are enumerated separately.

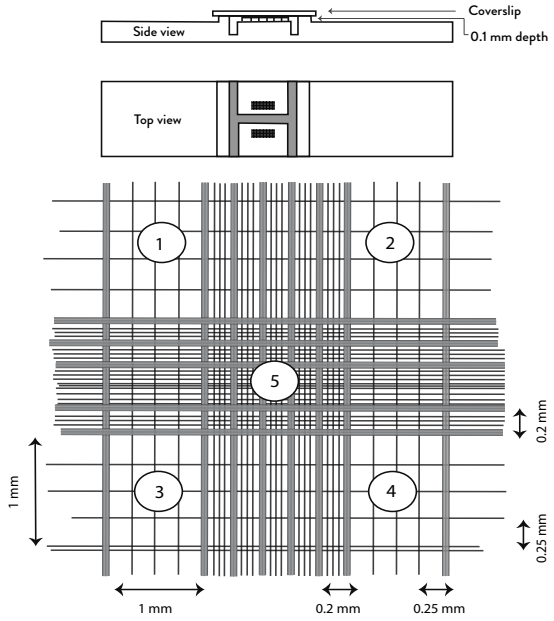


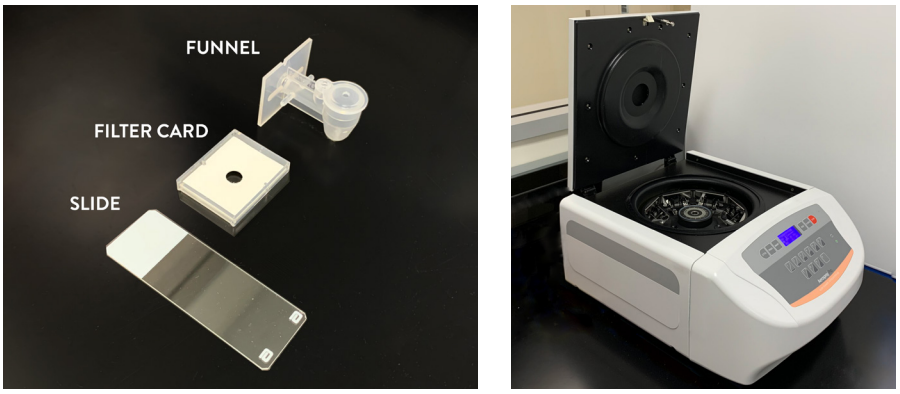
Figure 2. Neubauer chamber used for manual counting of cells in BF

Automated methods have been developed for counting TNCCs and RBCs in body fluids. The methods utilize a wide range of technologies such as impedance, digital imaging, flow cytometry and light scatter.[1] Automated analysis of BFs using hematology analyzers is quick, less labor intensive and can provide accurate testing in a more cost-effective manner as compared to manual counting.[5] In addition, these methods have better precision and while bloody fluids may pose a problem in manual counting, these fluids can be analyzed on most hematology analyzers regardless of the cause of the bloody sample. Although there is good correlation between the automated and manual counting methods, there are some limitations to using automated methods, especially in low count specimens. Due to very low cell counts in body fluids such as CSF and the fact that the distinction between normal and disease states can be narrow, some manufacturers have added dedicated body fluid channels,[2] fluorescent dyes, and extended count cycles to achieve more accurate counts at the lower limits of the analytical measuring range. It is imperative that users follow the manufacturer's recommendations when analyzing BFs on automated hematology analyzers. If performance specifications are not provided by the manufacturer for a specific type of BF, the laboratory should perform their own internal validations for test performance. Guidelines for validation of BFs are available from the Clinical Laboratory Standards Institute.[4,6]

CYTOSPIN AND DIFFERENTIAL CELL COUNT

In addition to counting cells on a hemocytometer, a microscopic analysis of cells is performed to identify the cells present in the BFs. Push smears are not recommended since the smearing process can result in cells not remaining intact. Instead, a cytospin preparation is made to smear the cells on to a glass side followed by appropriate staining (**Figure 3**). Based on the TNCC count from the hemocytometer, a suggested number of drops of the BF are added to a cytofunnel and placed in the cytocentrifuge. The cytocentrifuge concentrates the cells in the sample and automatically applies them on to a small region on a glass slide which is followed by Wright-Giemsa staining and, subsequently microscopic analysis by a trained reader. Preferably, a 100-cell differential count is performed on the stained smear. If fewer than 100 cells are present, the differential count based on the total number of cells available on the smear is recorded.

Hematopoietic cell types observed in BFs include segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils. It is common laboratory practice to group these cells into two broad categories: 1) polymorphonuclear cells (PMN) consisting mostly of segmented neutrophils with occasional band neutrophils and eosinophils, and 2) mononuclear cells (MN) consisting of lymphocytes and monocytes/macrophages. However, the CLSI H56-A guideline states that differentiating monocytes from lymphocytes has diagnostic significance.[6] In addition to hematopoietic cells, other cells such as mesothelial cells, lining cells, malignant cells, and microorganisms such as bacteria, yeast, fungi and parasites is also recorded.



3a 3b

Figure 3. a) Cytofunnel (slide, filter card, and funnel); b) Cytospin centrifuge

CYTOLOGICAL ANALYSIS

BF samples may also be sent for cytological analysis. This is usually performed by preparing a cell block. The BF specimen is centrifuged to concentrate the cellular component. The sediment that contains the cells is fixed and embedded in a paraffin block. Histologic sections are prepared from the block and stained for microscopic examination. If required, immunohistochemical stains can also be applied to these sections. Cell block preparations are usually performed to confirm presence of malignancy.

QUIZ QUESTIONS

1. Which of the following types of body fluids is not considered a serous fluid?

   A. Cerebrospinal fluid

   B. Peritoneal fluid

   C. Pericardial fluid

   D. Pleural fluid

2. Which of the following is considered the reference method for body fluid counting?

   A. Flow cytometry

   B. Automated hematology analyzer counting

   C. Hemocytometry

   D. None of the above

3. Most body fluids are stable at ambient temperature for:

   A. 2-4 hours

   B. 24 hours

   C. 48 hours

   D. 72 hours

SECTION 2

CEREBROSPINAL FLUID (CSF)

LEARNING OBJECTIVES

After completing this section, you will be able to:

1. Identify where CSF is synthesized
2. List at least 3 functions of CSF
3. List indications for CSF analysis
4. Differentiate between traumatic tap and subarachnoid hemorrhage
5. List cells commonly encounterd in normal CSF in healthy adults
6. List conditions where RBCs may be found in CSF

CEREBROSPINAL FLUID

DESCRIPTION AND PHYSIOLOGY

Cerebrospinal fluid (CSF) is the fluid that surrounds the brain and spinal cord. The brain and spinal cord are surrounded by three thin layers of tissues: the dura mater (outermost), the arachnoid mater (middle), and the pia pater (innermost). Collectively, these three layers constitute the meninges and serve as a protective layer for the brain. The capillaries of the pia mater form villi or small projections called choroid plexuses that project into the ventricles (cavities) located in the brain. CSF is produced by the choroid plexus cells by ultrafiltration of the plasma. The ventricles in the brain are lined with a single layer of cells called ependymal cells which also contribute to the production of CSF. CSF flows from the ventricles into the subarachnoid space and down into the spinal column. CSF is produced at a rate of about 20-27 mL/hour in adults and the total volume in adults is 90-155 mL.[7] The function of the CSF is to protect the brain and spinal cord by serving as a cushion, providing nutrients and removing waste products.

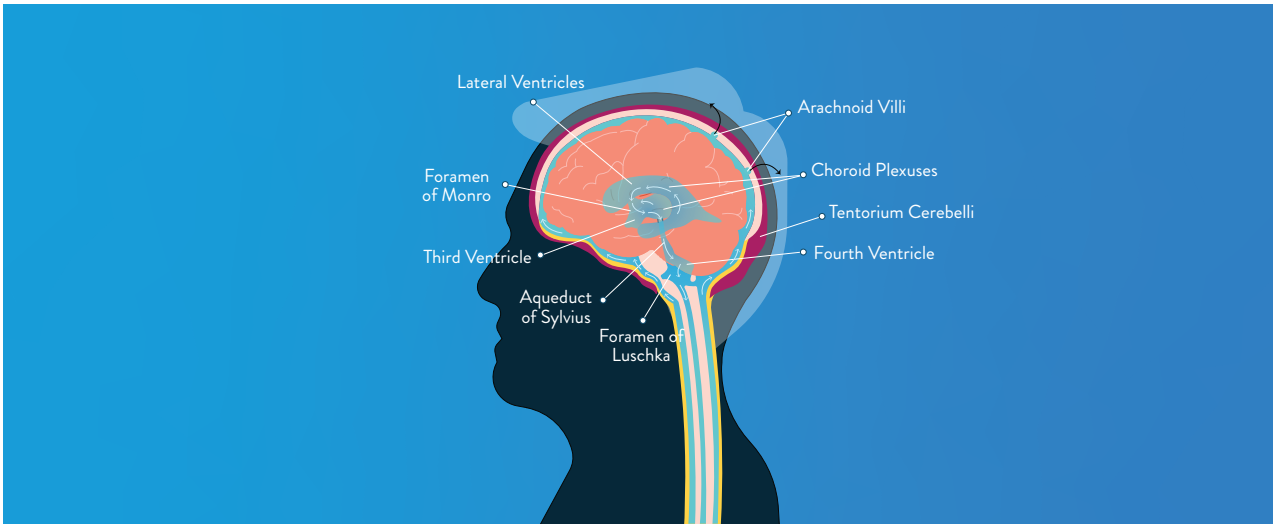


Figure 4. The choroid plexuses and the flow of CSF[7]

LABORATORY FINDINGS AND CLINICAL SIGNIFICANCE

CSF is obtained by a procedure referred to as a lumbar puncture also known as a spinal tap. Indications for a lumbar puncture include suspected 1) infection, 2) subarachnoid hemorrhage, 3) malignancy, and 4) demyelinating disease. The CSF is collected by inserting a needle between the third and fourth (L3-L4) or fourth and fifth (L4-L5) lumbar vertebrae. Approximately 10-20 mL of CSF is aspirated and divided into 3-4 tubes for analysis. No anticoagulation is needed. Plastic tubes are preferred, since cells may adhere to the walls of untreated glass tubes and can lead to a false decrease in the cell counts. In the order of which the fluid is collected into the tubes, ideally, tube #1 is used for biochemical analysis; tube #2 for microbiologic analysis; tube #3 for cell count and differential analysis; and tube #4 for cytologic analysis and/or any other ancillary testing. When this sequence if followed, there is low chance of contamination of CSF by peripheral blood for the cell counts and differential analysis.

The hematology laboratory plays a critical role in the cellular analysis of CSF samples. It is often the first lab to receive the sample and is capable of rapid turnaround of results. When CSF samples are received in the laboratory, it is necessary to know the order of collection. Generally, the third tube containing ~1-4 mL of CSF is sent to the hematology lab because this tube has the least probability of contamination with peripheral blood.

Gross examination of the CSF in the tube may help to identify pathological conditions (**Figure 5**). Normal CSF is clear and colorless (**Figure 5a**). A CSF sample that is turbid or cloudy (**Figure 5b**) indicates the presence of infection, proteins or lipids, or increased number of cells.[7] A sample that is bloody might indicate a traumatic tap or subarachnoid hemorrhage (SAH). SAH is potentially fatal and must be differentiated from a traumatic tap. In case of a traumatic tap, there is a progressive clearing of the sample from tube #1 to tube #4 (**Figure 5c**). If all tubes are uniformly bloody, a SAH should be suspected (**Figure 5d**).[8] In addition, clot formation in the CSF sample is associated with a traumatic tap rather than SAH. In some labs, an aliquot of bloody CSF is centrifuged for gross examination of the supernatant. If the supernatant is clear, the bloody appearance in the original tube is most likely due to contamination. If the supernatant has a yellowish or pinkish yellow tinge, referred to as xanthochromia, it may indicate SAH (**Figure 5e**). CLSI document H56-A recommends avoiding use of the term xanthochromia and reporting the actual color of the sample.[6]

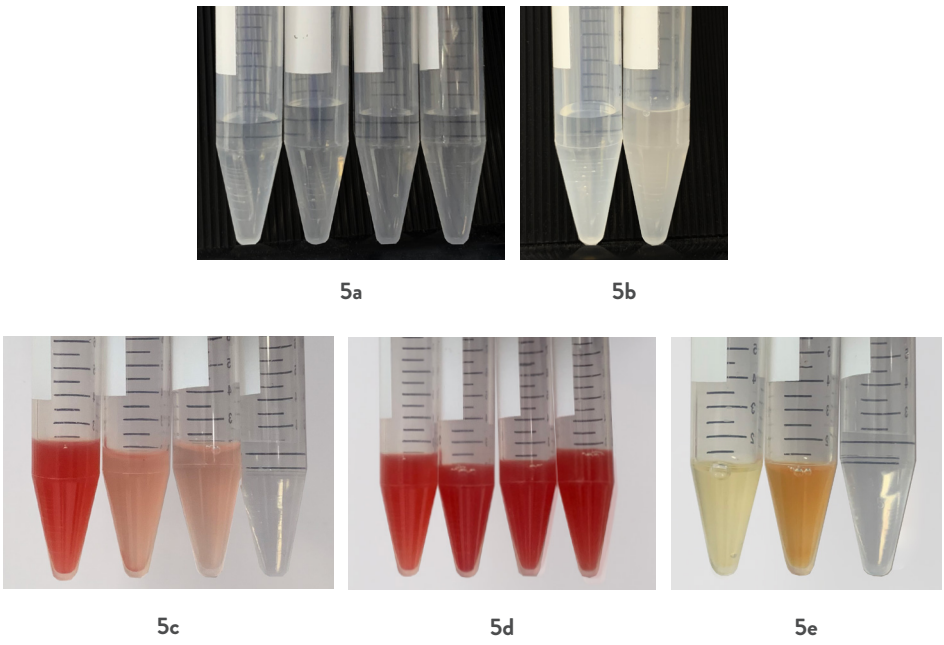


Figure 5. Gross appearance of CSF. a) Normal; b) Cloudy; c) Traumatic tap; d) Subarachnoid hemorrhage (SAH); e) Xanthochromic and xanthochromic/bloody

Following the gross examination of CSF, cell counting is performed for TNCCs and RBCs as described in Section 1, either manually or by automated hematology analyzers. Cell counts and differential counts should be performed a soon as possible (within 1-2 hours) of collection due to rapid deterioration of the sample. Normal CSF is almost completely free of cells. Reference intervals for TNCCs and RBCs in CSF in adults and in neonates are listed in **Table 1.**

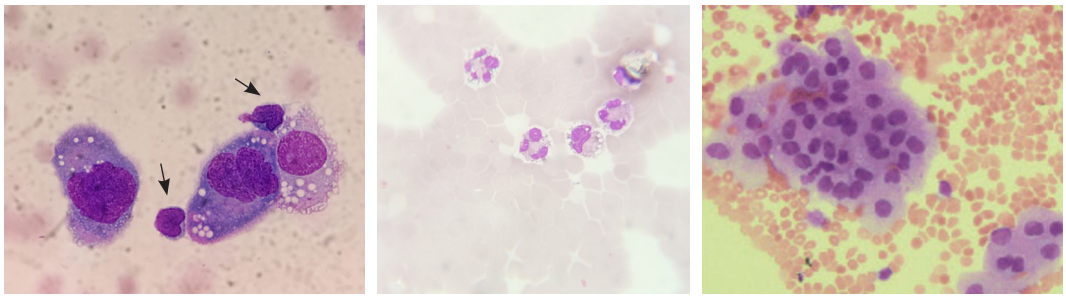
Table 1. Reference ranges for different cell types found in normal CSF

Cell type		Adults	Neonates
Total Nucleated Cell Count (TNCC)		0 - 5/µL	0 - 30/µL
Mononuclear cell (MN)	Lymphocytes	40 - 80%	5 - 35%
	Monocytes	15 - 45%	50 - 90%
Neutrophils (PMN)	Neutrophils	0 – 6%	0 - 8%
	Eosinophils	Rare	Rare
Lining cells	Ependymal cells	Very rare	Rare
RBCs	Normally not present		

Adapted from Body Fluid Analysis, Kjeldsberg and Hussong, 2015[1]

Microscopic analysis using a stained cytospin smear, including a differential cell count, yields important information that aids the diagnosis of the pathology.

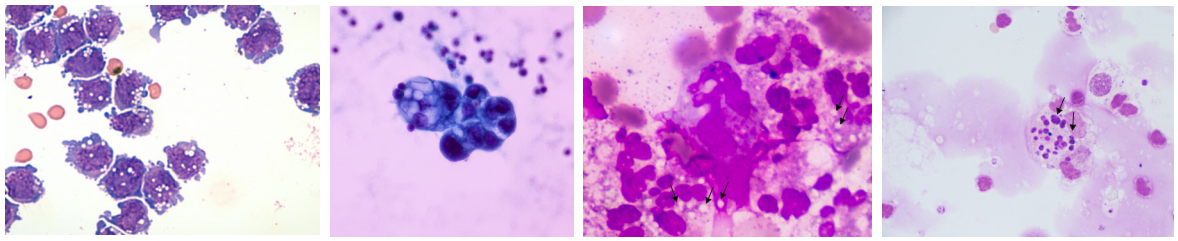
In adults, most cells encountered on the stained smear are mononuclear cells consisting primarily of lymphocytes (40 - 80%) and monocytes (15 – 45%). Neutrophils are rare in the CSF (0 - 6%) and an increased presence indicates an underlying pathologic process. Lining cells, which are non-hematopoietic cells, may also be present on the stained smear. These cells are normal and their presence results from adhering to the needle during aspiration of the CSF. These cell types are shown in **Figure 6.**



6a **6b** **6c**

Figure 6. Types of cells present in normal CSF. a) Lymphocyte (arrow), monocytes/macrophages , b) Neutrophils, c) Lining cells (ependymal/choroid plexus). Image 6c was originally published in ASH Image Bank. Paul Yeh; David A. Westerman. Ependymal cells in cerebrospinal fluid: a traumatic occurrence. ASH Image Bank. 2016; image number-00060670. © The American Society of Hematology.

An increase in WBCs, referred to as pleocytosis, is usually associated with disease, such as infection, inflammation, or malignancy. Pleocytosis with a preponderance of neutrophils may suggest bacterial meningitis, which is a life-threatening situation. Pleocytosis with an increase in lymphocytes and monocytes may suggest viral, fungal or tubercular meningitis.[7] Bacteria and fungi may also be seen in CSF. It is important to determine whether the bacteria represent a true infection or is a contaminant. Intracellular bacteria observed in neutrophils is a reliable indicator of a true infection. Malignant cells, both hematopoietic and non-hematopoietic, may also be seen in CSF. Hematopoietic tumors that metastasize in the meningeal space include acute lymphoblastic leukemia (ALL), acute non-lymphocytic leukemias (ANLL), and lymphomas. Non-hematopoietic tumors include tumor cells from solid tumors such as breast, colon, lung and prostate that have spread to the CNS. RBCs are usually not present in normal CSF. The presence of RBCs in CSF indicates SAH or traumatic tap. Examples of abnormal cell types found in the CSF are shown in **Figure 7.**



7a 7b 7c 7d

Figure 7. Types of abnormal cells found in CSF. a) Abnormal hematopoietic cells (lymphoma cells/blasts), b) Nonhematopoietic metastatic tumor cells, c) Intracellular bacteria, d) Intracellular fungi

QUIZ QUESTIONS

1. CSF is produced by which of the following?

   A. Ependyma
   B. Choroid plexus
   C. Dura mater
   D. Arachnoid mater

2. Pleocytosis refers to which of the following?

   A. Increase in erythrocytes
   B. Increase in CSF pressure
   C. Increase in leukocytes
   D. Increase in lining cells

3. An increase in which of the following cell types is indicative of bacterial meningitis?

   A. Lymphocytes
   B. Monocytes
   C. Erythrocytes
   D. Neutrophils

4. A decreasing RBC count from the 1st tube to the 4th tube is suggestive of which of the following?

   A. Subarachnoid hemorrhage
   B. Traumatic tap
   C. Xanthochromia
   D. Viremia

5. Which of the following is associated with SAH?

   A. Clot formation in the sample
   B. Fat globules
   C. Consistent amount of visible blood in tubes 1-4.
   D. Elevated bilirubin

6. Which of the cells below is the most prevalent cell type observed in normal CSF?

   A. Neutrophil
   B. Monocyte
   C. Eosinophil
   D. Lymphocyte

SECTION 3

SEROUS FLUIDS

LEARNING OBJECTIVES

After completing this section, you will be able to:

1. Name 3 types of serous fluids
2. Define the location of pleural fluid
3. Differentiate between exudate and transudate
4. List indications for pleural fluid analysis
5. Describe the appearance of mesothelial cells on the cytospin
6. Identify the location of peritoneal fluid
7. List indications for pericardiocentesis
8. Differentiate between peritoneal dialysate and peritoneal lavage

DESCRIPTION OF SEROUS FLUIDS

Serous body fluids are secreted by serous membranes that line body cavities. These membranes comprise the outer (parietal) layer that lines the wall of the body cavity and an inner layer (visceral) layer that surrounds the internal organs: lung, heart, and abdominal organs. Both of these membranes are lined by a single layer of mesothelial cells, and the space between the two layers contains a small amount of fluid (1-10 mL) that is referred to as serous fluid. The main function of the serous fluid is to provide lubrication. There are 3 main types of serous fluids present in the body; 1) pleural fluid surrounding the lungs, 2) pericardial fluid surrounding the heart and 3) peritoneal fluid that is present in the abdominal cavity.[1,6,9]

In abnormal conditions excess fluid may accumulate within the body cavities, called an effusion. Effusions may be divided into transudates and exudates (**Figure 8**), although the differentiation is not always straightforward. Transudates usually have low protein and cell concentration, and indicate systemic disease, while exudates are usually associated with localized inflammatory/infectious conditions or neoplasms, and have high protein and cell concentration.[9] The characteristics that distinguish transudates from exudates are shown in **Table 2**.

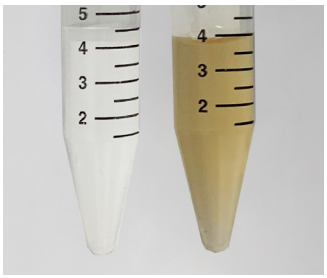


Figure 8. Gross appearance of transudate (left) and exudate (right)

Table 2. Characteristics of transudates and exudates[10]

	Transudate	Exudate
Appearance	Clear, pale yellow	Cloudy, turbid, purulent or bloody
Specific gravity	< 1.015	>1.015
Pleural fluid/serum protein ratio	≤ 0.5	>0.5
Pleural fluid/serum LDH ratio	≤ 0.6	>0.6
WBC	< 1,000 /µL	> 1,000 /µL
Total protein	< 3.0 gm/dL	> 3.0 gm/dL

PLEURAL FLUID

DESCRIPTION AND PHYSIOLOGY

Pleural fluid is located in the space between the parietal membrane lining the chest wall and the visceral membrane surrounding the lungs. Normally, this space between the two membranes is very small and contains a small amount of fluid that facilitates the movement of the two membranes against each other.[11] In normal adults, approximately 15 mL is produced daily by the mesothelial cells.[11,12] In specific disease states excess fluid accumulates in the pleural cavity producing an effusion. Transudates can be due to increased hydrostatic pressure forcing increased fluid across the membrane, as seen in congestive heart failure, or due to decreased oncotic pressure associated with hypoproteinemia in renal disease. In contrast, exudates are due to localized inflammation and infection (pleuritis, pneumonia), pulmonary infarction and malignancies, which damage the pleural lining and impair the filtration process.[12,13,14]

LABORATORY FINDINGS AND CLINICAL SIGNIFICANCE

When fluid accumulates in the pleural cavity, it may be removed to relieve pressure in the lung and also for biochemical and cellular analysis. The fluid is collected by a procedure called thoracentesis. An 18-guage IV needle is inserted above the rib and into the pleural space and an inner catheter is advanced gently to avoid puncturing the lung, then the needle is removed. The fluid is drained or collected via a syringe and aliquoted into tubes containing EDTA for cell counts and heparin for additional studies (**Figure 9**).[11]

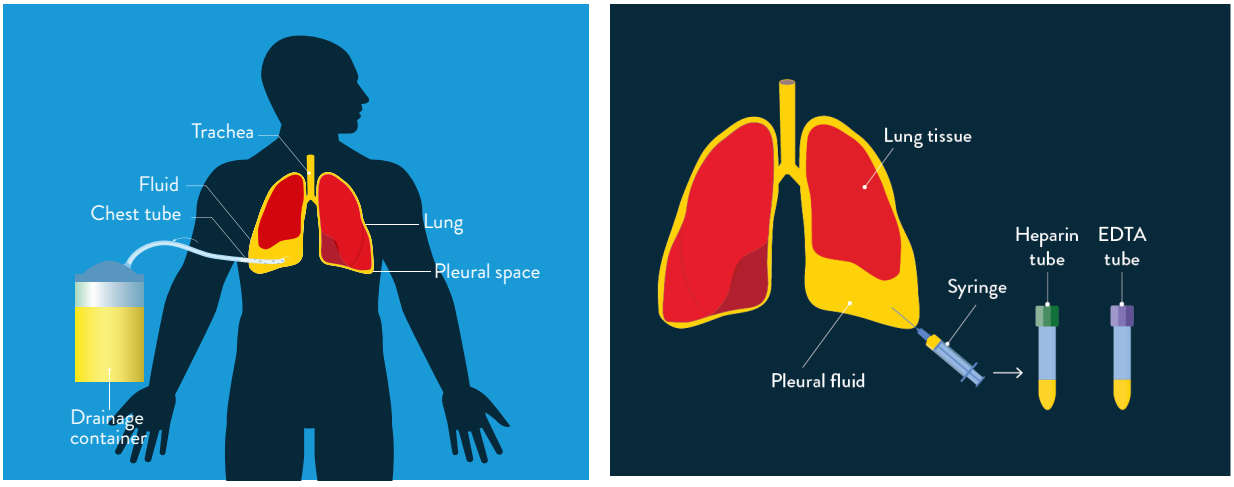


Figure 9. Pleural fluid collection from the pleural space into a drainage container (left) or syringe (right)

The specimen should be transported and processed as soon as possible because of limited specimen stability. A gross examination of the fluid for color and turbidity is useful in differentiating between transudates and exudates. Serous fluids are usually pale yellow or straw-colored and clear. Cloudy, turbid fluids suggest infection; bloody fluids suggest a traumatic tap, chest trauma or malignancy; milky appearance suggests a chylous effusion (contamination of the pleural fluid with chyle).[11,14]

The WBC count and differential are diagnostically significant in pleural fluid analysis. In addition to hematopoietic cells, mesothelial cells may also be present in pleural fluid. References ranges for each cell type in pleural fluid are shown in **Table 3**.

Table 3. Reference ranges for Pleural Fluid[6]

Cell type		Reference range
Total Nucleated Cell Count (TNCC)	1935 – 3734 /µL	
Mononuclear cells (MN)	Macrophages	64 – 80%*
	Lymphocytes	18 – 36%*
Neutrophils (PMN)		0 – 1%*
Mesothelial cells		0 – 2%*

*Results expressed as interquartile range.

Elevated neutrophil counts are usually associated with a bacterial infection such as pneumonia or pulmonary abscess; elevated lymphocyte counts may be associated with tuberculosis and malignancy. Macrophages are often found in pleural fluids and may contain various amounts of phagocytized particles (**Figure 10a**). Many laboratories do not find it necessary to differentiate between monocytes and macrophages.

Mesothelial cells are normal non-hematopoietic lining cells that may be present as they get dragged off from the lining of the pleura when a needle passes during aspiration. These cells have abundant cytoplasm that appears light gray to deep blue and have a fried-egg appearance (**Figure 10b**). They may appear as a single cell or in loose aggregates or clumps. In the loose aggregates, the cytoplasm of one cell may appear to embrace the adjacent cell.[11] The presence of mesothelial cells (normal and reactive) have no clinical significance. In addition to mesothelial cells, malignant cells may also be found in pleural fluids; those are associated with lung cancer or metastatic carcinoma.[15] It is sometimes challenging to differentiate mesothelial cells from malignant cells. One of the differentiating features is the fact that clusters of mesothelial cells have a uniform appearance whereas malignant cells are non-uniform with bizarre shapes and staining intensity (**Figure 10c**).[16] RBCs have little diagnostic value in serous fluids.

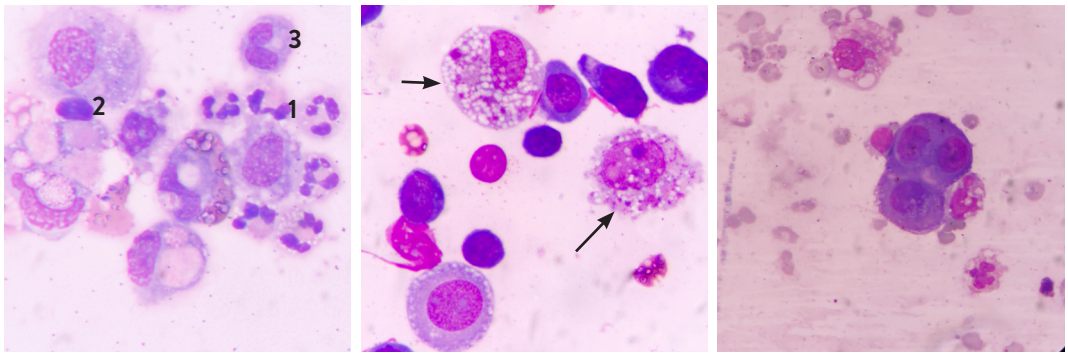


Figure 10. Different cell types found in pleural fluids. a) PMN (1), lymphocyte (2), and monocyte (3); b) mesothelial cells (arrows); c) malignant cells

PERITONEAL FLUID

DESCRIPTION AND PHYSIOLOGY

Peritoneal fluid is present inside the peritoneal sac within the abdominal cavity. In healthy individuals, less than 50 mL of peritoneal fluid is present, and it serves as a lubricant to reduce friction between the membranes. In certain pathological conditions, the fluid may accumulate and is sometimes referred to as ascites. Common causes of a transudative effusion include heart failure, cirrhosis, and hypoalbuminemia (usually associated with renal disease).[17] Exudative effusions are associated with infections (bacterial, tubercular, fungal, and parasitic), neoplastic diseases (hepatocellular carcinoma, mesothelioma, lymphoma), and other gastrointestinal diseases (pancreatitis, bile peritonitis).[18]

LABORATORY FINDINGS AND CLINICAL SIGNIFICANCE

Common indications for analyzing peritoneal fluid are to diagnose or rule out bacterial peritonitis and suspected neoplastic disease. The procedure for removing fluid from the peritoneal cavity is called paracentesis. A needle or catheter is inserted into the peritoneal cavity to obtain the fluid for diagnostic purposes (**Figure 11**).

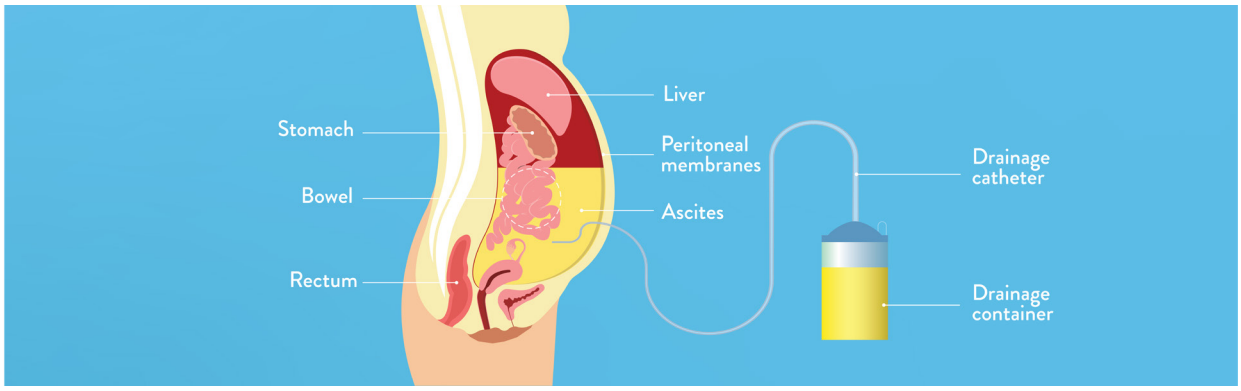


Figure 11. Collection of peritoneal fluid by paracentesis

The gross appearance of the peritoneal fluid provides information on the probable etiology. Cloudy and purulent fluids are often seen in cases of peritonitis; a green color may indicate injury to the gallbladder or small intestine; grossly hemorrhagic fluids suggest trauma; milky colored (chylous) fluids is suggestive of leakage of lymphatic vessels that may occur from trauma or malignancy.[18]

The cell counts and differential counts are key for differentiating between transudates and exudates. For instance, a neutrophil count >250 cells/μL suggests the presence of Spontaneous Bacterial Peritonitis (SBP), which is a frequent complication observed in cirrhotic patients with ascites.[18]

Mesothelial cells may also be encountered in peritoneal fluids. Increased numbers of mesothelial cells are sometimes seen in inflammatory and infectious conditions. Reference ranges for each of these cell types in peritoneal fluid is shown in **Table 4**. In addition, malignant cells may be present in neoplastic conditions. Malignant cells are usually large cells with a high nuclear-cytoplasmic ratio, irregular chromatin pattern, and visible nucleoli.[18]

Table 4. Reference ranges for Peritoneal Fluid[6]

Cell type		Reference range
Total Nucleated Cell Count (TNCC)	1395 – 3743/μL	
Mononuclear cells (MN)	Macrophages	64 – 80%**
	Lymphocytes	48 – 36%*
Neutrophils (PMN)		0 – 2%*
Mesothelial cells		Not present

*Results expressed as interquartile range.

PERICARDIAL FLUID

DESCRIPTION AND PHYSIOLOGY

The pericardium is a two-layered sac that surrounds the heart. These membranes produce the pericardial fluid that acts as a lubricant to reduce friction as the heart pumps blood. If there is an accumulation of fluid within the sac (pericardial effusion), it may interfere with the function of the heart and it becomes necessary to remove it to relieve the increased pressure in the pericardial cavity, and for analysis. Pericardial transudate is observed in patients with hypoproteinemia and/or increased hydrostatic pressure of the circulatory system.[19] Pericardial exudates are associated with infection, inflammatory conditions and malignancy. Bacterial, fungal, or viral infections may originate in the pericardial space or spread from other areas in the body into the pericardial space. Bleeding disorders and trauma may result in accumulation of blood into the pericardial space, resulting in hemopericardium. Inflammatory conditions associated with pericardial exudates include post-cardiac injury syndrome in myocardial infarction, systemic autoimmune diseases such as lupus or rheumatoid arthritis and radiation therapy. In addition, malignant conditions such as mesothelioma, a tumor originating from mesothelial cells, and metastatic spread of cancer may also result in a pericardial exudate.[19, 20]

LABORATORY FINDINGS AND CLINICAL SIGNIFICANCE

The procedure to collect pericardial fluid is called pericardiocentesis. It is performed by inserting a needle through the space between the fifth and sixth ribs on the left side of the chest into the pericardial sac. An ultrasound may be used to help guide the needle to the pericardial cavity (**Figure 12**).[19] Due to the invasive nature of the procedure, especially around the heart, the treating physician has to decide if it is necessary to perform a pericardiocentesis. If a transudate is suspected, a pericardiocentesis is not required, provided the amount of fluid is small and does not interfere with heart function. However, if an exudate is suspected, pericardiocentesis may be required to help diagnose the specific etiology. Normal pericardial fluid has very few WBCs and no RBCs. In many laboratories, only a cytospin is made and microscopic examination is performed. Additional analysis such as culture or cytology may be required to differentiate between an infectious, inflammatory or malignant condition.

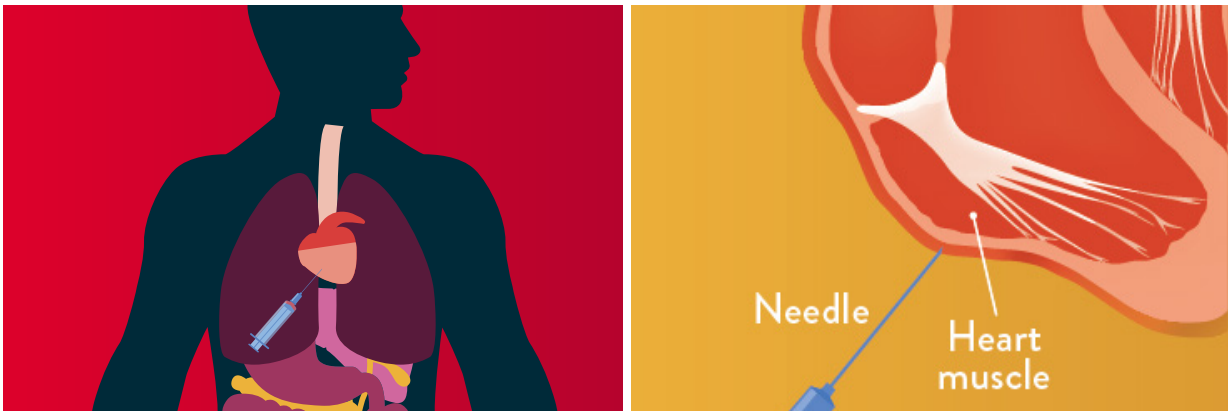


Figure 12. Pericardial fluid collection by pericardiocentesis

OTHER FLUIDS

There are other fluids that may be introduced in the serous cavity for diagnostic purposes. These include peritoneal dialysate, peritoneal lavage, and bronchoalveolar lavage.[6,7,9]

PERITONEAL DIALYSATE

Peritoneal dialysate refers to a specific type of fluid used in a therapeutic procedure called continuous ambulatory peritoneal dialysis (CAPD). Peritoneal dialysis is an alternate form of treatment to hemodialysis for patients with kidney failure. It involves the introduction of exogenous fluid into the peritoneum. The peritoneum serves as the filter in this procedure and the exogenous fluid absorbs the waste and fluid from the blood. The fluid that is drained into the waste bag is referred to as the peritoneal dialysate (**Figure 13**).[21] Peritonitis (infection) is the major complication of this procedure and should be suspected if the WBC count is greater than 100/µL and neutrophils constitute at least 50% of the TNCC in the peritoneal dialysate. In such circumstances, further testing, including culture and Gram stain, is indicated.[22]

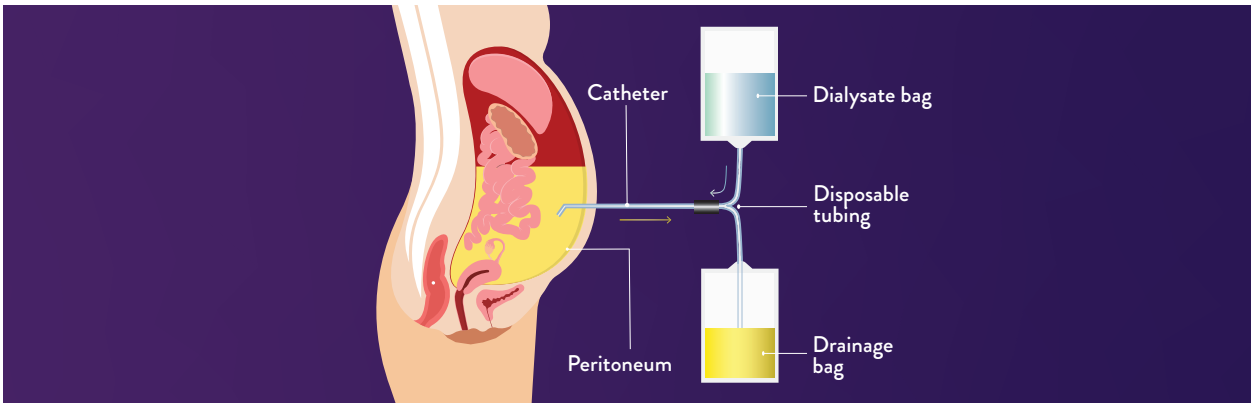


Figure 13. Peritoneal dialysate fluid collection by peritoneal dialysis

PERITONEAL LAVAGE

Peritoneal lavage is a process by which a sterile physiologic fluid such as saline is introduced into the peritoneal cavity and aspirated for cellular analysis. This procedure is usually performed to diagnose intra-abdominal bleeding from a ruptured organ in cases of abdominal trauma to decide whether an exploratory laparotomy is further required.[23] While the red cell count is relevant in assessing intra-abdominal bleeding, evaluation of the total nucleated cell count may also provide information about intestinal perforation.[6] Nowadays, this procedure has largely been replaced by radiological techniques such as ultrasound.

BRONCHOALVEOLAR LAVAGE FLUID

Bronchoalveolar lavage (BAL) is a diagnostic procedure that removes cells lining the mucosal surface of as well as any non-adherent cells in the bronchioloalveolar system by rinsing with a sterile solution.[24] BAL specimens are a result from the introduction and removal of warm saline into the lungs via a bronchoscope (**Figure 14**), an invasive procedure performed under general anesthesia.[8] Indications for BAL include: 1) interstitial infiltrates such as sarcoidosis; 2) alveolar infiltrates such as pneumonia of unknown origin; 3) pulmonary infiltrates seen in immunocompromised patients; and 4) occupational dust exposure such as asbestos-related disorders, silicosis, and coal worker's pneumoconiosis.[24] Analysis of the BAL fluid involves cell and differential count. Reference ranges are difficult to determine because the concentration depends on the volume of fluid infused and the return yield. Nucleated cell counts in healthy individuals range from 100-400/µL.[3] The majority of cells present are macrophages (>80%). The presence of other hematopoietic cells such as lymphocytes is considered a contaminant caused by the procedure.[3] Nonhematopoietic cells observed in the BAL fluid are mainly pneumocytes and ciliated epithelial lining cells. It is common to see bacteria and or yeast because the sample may contain airborne organisms.

Many hematology labs refer the sample to the microbiology and flow cytometry laboratories for further analysis.

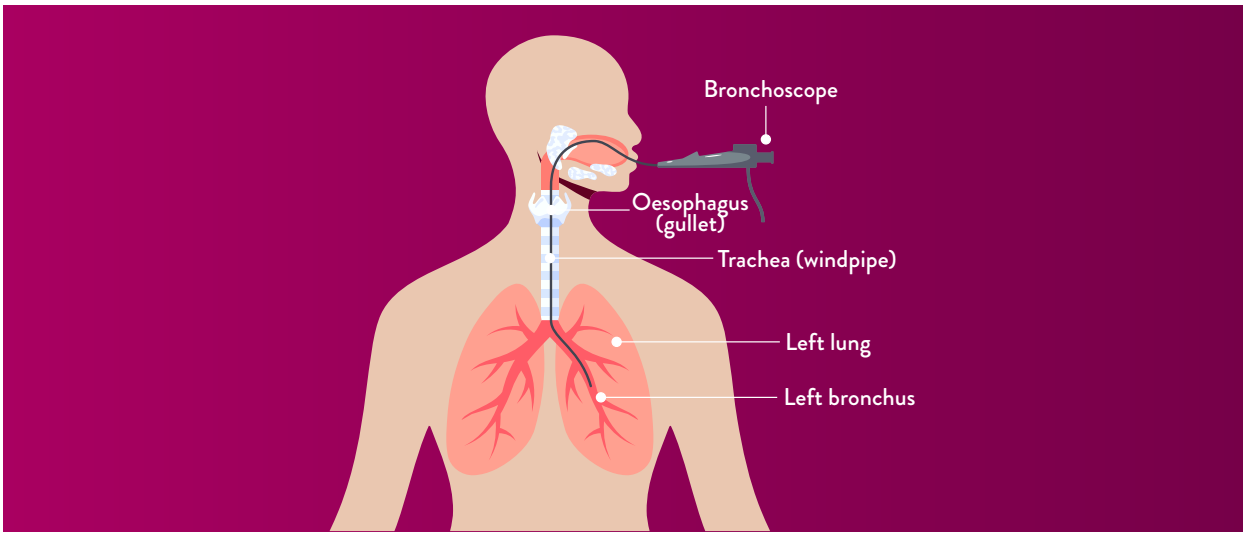


Figure 14. Bronchoalveolar fluid (BAL) collected using bronchoscopy

QUIZ QUESTIONS

1. Which of the following is not considered a serous fluid?

   A. Synovial fluid
   B. Peritoneal fluid
   C. Pericardial fluid
   D. Pleural fluid

2. The procedure used to collect pleural fluid is called?

   A. Paracentesis
   B. Thoracentesis
   C. Bronchial lavage
   D. Ascites

3. Which of the following cells line the pleural cavity?

   A. Polymorphonuclear cells
   B. Monocytes
   C. Mesothelial cells
   D. Synoviocytes

4. Cloudy or purulent body fluids are associated with which of the following?

   A. Metastatic tumor
   B. Hemorrhage
   C. Bile
   D. Infection

5. Which of the following cells has a fried-egg appearance?

   A. Mesothelial cells
   B. Histiocytes
   C. Tumor cells
   D. Synoviocytes

6. Changes in hydrostatic pressure due to increased capillary permeability and/or decreased lymphatic resorption lead to the development of an effusion.

   A. True
   B. False

7. Spontaneous bacterial peritonitis is associated with which of the following findings?

   A. >250 PMNs /µL
   B. 100 – 200 PMNs/µL
   C. >250 MNs/µL
   D. 100-200 MNs/µL

8. Peritoneal dialysis may be performed in which of the following disorders?

   A. Pleural effusion
   B. Pericardial effusion
   C. End-stage renal disease
   D. Spontaneous bacterial peritonitis

SECTION 4

SYNOVIAL FLUID

LEARNING OBJECTIVES

After completing this section, you will be able to:

1. Describe the functions of synovial fluid
2. List indications for synovial fluid analysis
3. List advantages and disadvantages of anticoagulants used of synovial fluid analysis
4. List types of crystals seen in gout and pseudogout
5. Differentiate between MSU and CPPD crystals

SYNOVIAL FLUID

DESCRIPTION AND PHYSIOLOGY

Synovial fluid (SYF) is present in the cavities of movable joints such as the knee, elbow, and wrist. The synovial membrane lines the surface of the joint cavity and has a single layer of flat lining cells called synoviocytes. The synoviocytes help in phagocytosis, absorption and secretion, as well as hyaluronan synthesis.[25] Synovial fluid is secreted by the cells lining the synovial membrane and by filtration through the pores in the synovial capillaries. In healthy individuals, it is present in small amounts, but a large joint may contain up to 3.5 mL of fluid. The main function of SYF is to lubricate the joint space, transport nutrients to the joint structures and remove waste products from the joint space. The examination of SYF is useful for the diagnosis of joint diseases, which can be categorized into five groups: 1) noninflammatory disorders; 2) inflammatory disorders; 3) septic disorders; 4) crystal associated arthropathies; and 5) hemorrhagic arthropathies.[26,27] The most important reasons for SYF analysis are to diagnose joint infection by Gram stain and/or microbiological culture and to diagnose crystal induced arthritis by polarized light microscopy.

LABORATORY FINDINGS AND CLINICAL SIGNIFICANCE

Analysis of synovial fluid requires withdrawal of the excess fluid from the joint cavity by a procedure called arthrocentesis (**Figure 15**). In this procedure, a needle is inserted into the joint space and the excess fluid is withdrawn. Normal SYF does not clot, but in certain disease states, specifically in inflammatory conditions, the fluid may contain fibrinogen and may clot. To prevent clotting, fluids may be collected in a syringe that is coated with heparin or EDTA.[26] Some investigators suggest that lithium heparin and powdered EDTA may produce crystalline material which can be confused with pathologic crystals.[6] Oxalate should not be used as an anticoagulant since it may be confused with calcium and oxalate crystals that are present in SYF in pathological states.[6] If samples cannot be processed immediately, they may be refrigerated to prevent deterioration. Due the presence of hyaluronic acid which causes SYF to have a viscous consistency, samples can be pre-treated with hyaluronidase enzyme to reduce the viscosity prior to processing. SYF is analyzed for total WBC count, WBC differential, Gram stain and culture, and crystal examination.[26] RBC counts are usually performed along with the WBC count.

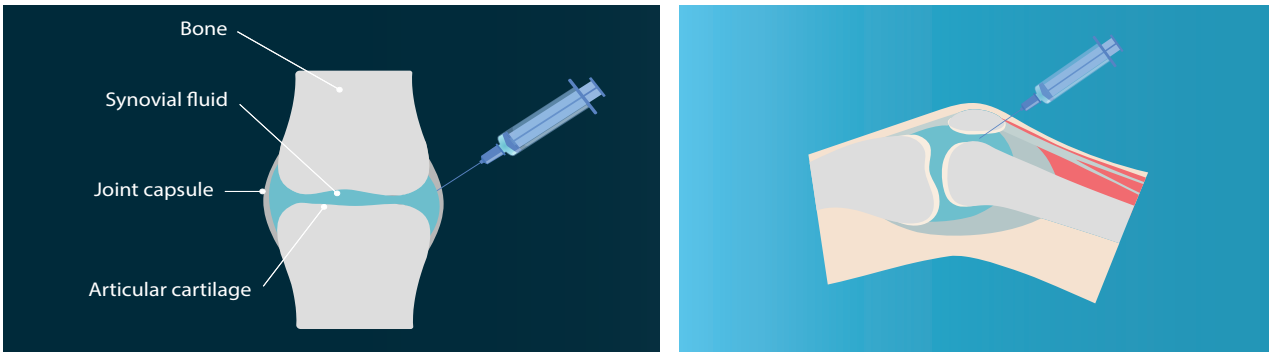


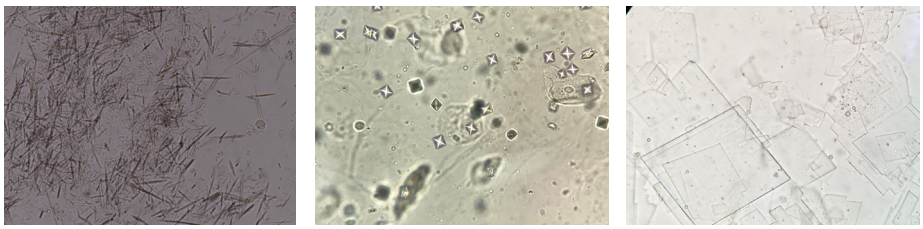
Figure 15. Synovial fluid collection by arthrocentesis

Normal SYF appears pale yellow to colorless and is usually clear.[26] Adult reference values for SYF are shown in the table below (**Table 5**).

Table 5. Reference ranges for Synovial Fluid[27]

Cell type			Reference range
Total Nucleated Cell Count (TNCC)		0-150/ µL	
Differential (%)			
	PMN	Neutrophils	0-25
	MN	Lymphocytes	0-78
		Monocytes	0-71
		Histiocytes	0-26
		Synoviocytes	0-12
Erythrocytes			0-2000/µL
Crystals			None

WBC differential counts are performed on cytocentrifuged preparations. Mononuclear cells, including monocytes, macrophages, and synoviocytes are the primary cells observed in normal SYF. In infectious arthritis, the WBC count is greatly increased, as high as 50,000/µL, with a predominance of polymorphonuclear cells.[25] Malignant cells are usually not seen in SYF; however, when they are seen, it suggests metastatic spread of disease to the cavity.[27] Microscopic examination for crystals in SYF is important when evaluating arthritis. This can be performed using an unstained wet preparation and viewing the slide with a regular brightfield microscope or using polarized light.[26,28] Monosodium urate (MSU) crystals are present in patients with gout who have elevated levels of serum uric acid. These crystals appear as thin needle-like structures with pointed ends under brightfield microscopy (**Figure 16a**) and exhibit positive birefringence under polarized microscopy. Calcium pyrophosphate (CPPD) crystals are seen in patients with pseudogout who have elevated levels of serum calcium. These crystals appear rhomboid or square under a bright field microscope and show negative birefringence using polarized microscopy (**Figure 16b**). Cholesterol crystals are associated with chronic rheumatic diseases; those appear as large flat plates with notched corners under a brightfield microscope (**Figure 16c**), and show weak birefringence when observed under polarized light.[28]



16a **16b** **16c**

Figure 16. a) Monosodium urate; b) Calcium pyrophosphate; and c) Cholesterol crystals in synovial fluid

QUIZ QUESTIONS

1. The procedure to collect synovial fluid is called _____?

   A. Arthrocentesis
   B. Paracentesis
   C. Gram stain
   D. Synoviocentesis

2. Which one of the following anticoagulants should not be used when collecting synovial fluid?

   A. Oxalate
   B. Heparin
   C. EDTA
   D. Normal saline

3. Which one of the following is not a function associated with synovial fluid?

   A. Transport nutrients to the joint structures
   B. Lubrication for the joints
   C. Removal of waste products from the joints
   D. Generation of Synoviocytes

4. Which one of the following types of crystals is present in synovial fluid in patients with gout?

   A. Monosodium urate crystals
   B. Calcium pyrophosphate crystals
   C. Cholesterol crystals
   D. Oxalate crystals

5. Which cell type is elevated in synovial fluid during an acute bacterial infection?

   A. Monocytes
   B. Lymphocytes
   C. Neutrophils
   D. Erythrocytes

GLOSSARY, APPENDIX, AND REFERENCES

GLOSSARY[29]

Ascites: an abnormal accumulation of fluid in the abdomen

Body fluid: any fluid in the body including blood, urine, CSF, serous fluids, synovial fluid, saliva, sputum, tears, semen, milk, or vaginal secretions

Bronchial lavage: procedure that involves repeatedly washing the inside of the bronchial tubes of the lung

Cerebrospinal fluid: fluid within the subarachnoid space, spinal cord, and ventricles of the brain

Chyle: a milky fluid taken up by the lacteals from the intestine during digestion, consisting of lymph and triglyceride fat (chylomicrons) in a stable emulsion

Effusion: the escape of blood or other fluid into a body cavity or tissue

Ethylenediaminetetraacetic acid (EDTA): crystalline acid capable of chelating a variety of divalent metal cations, used as an anticoagulant

Exudate: a fluid with a high content of protein and cellular debris that has escaped from blood vessels and has been deposited in tissues or on tissue surfaces, usually as a result of inflammation

Hemocytometer: a device for estimating the number of blood cells in a quantitatively measured volume of blood or body fluid

Paracentesis: procedure to remove fluid from the peritoneal cavity

Pericardiocentesis: procedure to remove pericardial fluid by needle from the sac surrounding the heart for diagnostic or therapeutic purposes

Peritoneal dialysate: fluid and solutes from the peritoneal cavity that pass through a dialyzing membrane

Peritoneal fluid: a clear straw-colored serous fluid secreted by the cells of the peritoneum

Peritoneal lavage: procedure that involves irrigation of the peritoneal cavity

Pleocytosis: presence of more cells than normal, often denoting leukocytosis

Pleural fluid: fluid secreted by serous membranes in the pleurae that reduces friction during respiratory movement of the lungs

Pneumocytes: any of the cells lining the alveoli of the lung

Serous fluids: fluid secreted by serous membranes that reduces friction in the serous cavities (pleural, pericardial, and peritoneal)

Synoviocytes: fibroblast-like cells located in the synovial membrane of joins, believed to contribute proteoglycans and hyaluronate to the synovial fluid

Synovial fluid: a viscous fluid contained within a membrane enclosing moveable joints such as the elbow and knee

Thoracentesis: procedure that removes fluid or air from the chest through a needle or tube

Transudate: any fluid without a high protein content that passes through a membrane, especially through the wall of a capillary

APPENDIX: QUIZ ANSWERS

SECTION 1 OVERVIEW OF BODY FLUIDS

1. A

2. C

3. A

SECTION 2 CEREBROSPINAL FLUID

1. B.

2. C

3. D

4. B

5. C

6. D

SECTION 3 SEROUS FLUIDS

1. A

2. B

3. C

4. D

5. A

6. A

7. A

8. C

SECTION 4 SYNOVIAL FLUID

1. A

2. A

3. D

4. A

5. C

REFERENCES

1. Hussong, JW, Sorensen, E, Perkins, SL, Couturier, MR, Grenache, DG, Lamb, AN, Straseki, JA, Cohen, MB. Laboratory Methods. In: Kjeldsberg's Body Fluid Analysis. Chapter 2. ASCP Press, 2015.
2. Fleming, C, Russcher, H, Lindemans, J, de Jonge, R. Clinical relevance and contemporary methods for counting blood cells in body fluids suspected of inflammatory disease. Clin Chem Lab Med 2015;53(11):1689-1706.
3. Walters, J, Clare, CN. Morphologic Analysis of Body Fluids in the Hematology Laboratory. In: Clinical Hematology, McKenzie, SB, Williams JL, 3rd edition, 2015. Pearson, Upper Saddle River, NJ.
4. Block, DR, Ouverson, LJ, Wittwer, CA, Saenger, AK, Baumann, NA. An approach to analytical validation and testing of body fluid assays for the automated clinical laboratory. Clinical biochemistry 2018;58:44-52.
5. Zimmermann, M, et al. Automated vs. manual cerebrospinal fluid cell counts: a work and cost analysis comparing the Sysmex XE-5000 and the Fuchs-Rosenthal manual counting chamber. Int. Jnl. Lab. Hem. 2011;33:629-637
6. Body Fluid Analysis for Cellular Composition; Approved Guideline, H56-A, Vol 26, No. 26. Clinical and Laboratory Standards Institute, 2006, Wayne, PA.
7. Perkins, SL, Couturier, MR, Grenache, DG, Kjeldsberg, CR. Cerebrospinal Fluid, Ch. 4. In: Kjeldsberg's Body Fluid Analysis. Chapter 2. ASCP Press, 2015.
8. Rodak, BF. Body Fluid Analysis in the Hematology Laboratory. In: Rodak's Hematology: Clinical Principles and Applications, Keohane, EM, Smith, LJ, Walenga, JM, 5th Edition, Elsevier, St. Louis, MO.
9. Karcher DS, McPherson, RA. Cerebrospinal, Synovial, Serous Body Fluids, and Alternative Specimens. In: Henry's Clinical Diagnosis. 23rd edition, 2017. St. Louis, Mo.
10. Galagan, KA, et al. Transudates vs Exudates. In: Color Atlas of Body Fluids. CAP Press, 2006, Northfield, Il.
11. Kjeldsberg, CR, Grenache, DG, Couturier, MR, Cohen, MB. Pleural & Pericardial Fluid, Ch. 5. In: Kjeldsberg's Body Fluid Analysis. Chapter 2. ASCP Press, 2015.
12. BMJ Best Medical Practice. Pleural effusion. https://bestpractice-bmj-com.ez03.infotrieve.com/topics/en-us/287/epidemiology, (last accessed 10/15/19).
13. Walters, J, Clare, CN. Morphologic Analysis of Body Fluids in the Hematology Laboratory. In: Clinical Hematology, McKenzie, SB, Williams JL, 3rd edition, 2015. Pearson, Upper Saddle River, NJ.
14. Galagan, KA, Blomberg, D, Cornbleet, PJ, Glassy, E. Pleural, Peritoneal, Pericardial Fluids. Color Atlas of Body Fluids: An Illustrated Field Guide Based on Proficiency Testing. College of American Pathologists. 2006, CAP, Northfield, IL.
15. Strasinger, SK, Di Lorenzo, MS. Serous Fluid. In: Urinalysis and Body Fluids, 5th Edition, Chapter 13, F. A. Davis, Philadelphia, 2008.
16. Walters, J. Analysis of Serous Fluids (by LabCE), PACE Program No: 015-1186-15. Educational Materials for Health Professionals Inc.
17. Light, RW. Pleural Effusion. https://www.merckmanuals.com/professional/pulmonary-disorders/mediastinal-and-pleural-disorders/pleural-effusion#, (last accessed 03/03/2020).
18. Kjeldsberg, CR, Grenache, DG, Couturier, MR, Cohen, MB. Peritoneal fluid, Ch. 6. In: Kjeldsberg's Body Fluid Analysis. Chapter 2. ASCP Press, 2015.
19. Pericardial Fluid Analysis. In: The Merck Manual. https://www.merckmanuals.com/professional/SearchResults?query=pericardial+fluid#, (last accessed 10/15/2019).

REFERENCES (CONTINUED)

20. Pericardial Fluid Analysis. https://labtestsonline.org/tests/pericardial-fluid-analysis, (last accessed 10/15/2019).

21. Peritoneal dialysis. https://www.kidneyfund.org/kidney-disease/kidney-failure/treatment-of-kidney-failure/peritoneal-dialysis-pd.html (last accessed 11/01/2019).

22. Hechanova, LA. Peritoneal Dialysis. https://www.merckmanuals.com/professional/genitourinary-disorders/renal-replacement-therapy/peritoneal-dialysis, (last accessed 03/03/2020).

23. Hockberger, RS, Walls, RM, Rosen, P, Marx, JA. Peritoneal Lavage. In: Rosen's Emergency Medicine: Concepts and Clinical Practice. Mosby, St. Louis, MO., 2010.

24. Collins, AM, Tylance, J, Wootton, DG, Wright, AKA, Fullerton, DG, Gordon, SB. Bronchoalveolar Lavage (BAL) for Research; Obtaining Adequate Sample Yield. J. Vis. Exp. (85), e4345, 2014.

25. Tercic, D, Bozic, B. The Basis of the Synovial Fluid Analysis. Clin Chem Lab Med 2001;39(12):1221-1226.

26. Synovial Fluid, Strasinger, SK, Di Lorenzo, MS. In: Urinalysis and Body Fluids, 5th Edition, Chapter 12, F. A. Davis, Philadelphia, 2008.

27. Couturier, MR, Straseski, JA, Kjeldsberg, CR. Synovial fluid, Ch7. In: Kjeldsberg's Body Fluid Analysis. ASCP Press, 2015.

28. Educational Commentary – Synovial Fluid Crystals: Monosodium Urate, Calcium Pyrophosphate, and Cholesterol. http://www.api-pt.com/Reference/Commentary/2007Cmicroscopy.pdf (last accessed 01/19/2020).

29. Thefreedictionary.com (last accessed 12/04/19).

CORELABORATORY.ABBOTT/HEMATOLOGY

Abbott