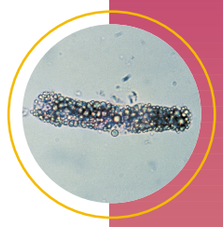
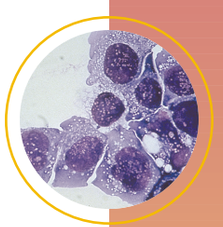
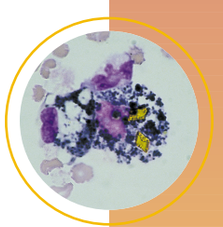
CHAPTER 10







Cerebrospinal Fluid

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

1. State the three major functions of cerebrospinal fluid (CSF).
2. Distribute CSF specimen tubes numbered 1, 2, and 3 to their appropriate laboratory sections and correctly preserve them.
3. Describe the appearance of normal CSF.
4. Define xanthochromia and state its significance.
5. Differentiate between CSF specimens caused by intracranial hemorrhage and a traumatic tap.
6. Calculate CSF total, white blood cell and red blood cell counts when given the number of cells seen, amount of specimen dilution, and the squares counted in the Neubauer chamber.
7. Briefly explain the methods used to correct for WBCs and protein that are artificially introduced during a traumatic tap.
8. Describe the leukocyte content of the CSF in bacterial, viral, tubercular, and fungal meningitis.
9. Describe and give the significance of abnormal macrophages in the CSF.
10. Differentiate between the appearance of normal choroidal cells and malignant cells.
11. State the normal value for CSF total protein.
12. List three pathologic conditions that produce an elevated CSF protein.
13. Discuss the basic principles and advantages and disadvantages of the turbidimetric and the dye-binding methods of CSF protein analysis.
14. Determine whether increased CSF immunoglobulin is the result of damage to the blood-brain barrier or central nervous system production.
15. Discuss the significance of CSF electrophoresis findings in multiple sclerosis and the identification of CSF.
16. State the normal CSF glucose value.
17. Name the possible pathologic significance of a decreased CSF glucose.
18. Briefly discuss the diagnostic value of CSF lactate and glutamine determinations.
19. Name the microorganism associated with a positive India Ink preparation.
20. Briefly discuss the diagnostic value of the bacterial and cryptococcal antigen tests.
21. State the diagnostic value of the limulus lysate test.
22. Determine whether a suspected case of meningitis is most probably of bacterial, viral, fungal, or tubercular origin, when presented with pertinent laboratory data.
23. Describe the role of the Venereal Disease Research Laboratories test and fluorescent treponemal antibody-absorption test for syphilis in CSF testing.
24. Describe quality control procedures and safety precautions related to CSF procedures.

KEY TERMS

arachnoid villi
blood-brain barrier
choroid plexuses
meningitis
oligoclonal bands
pleocytosis
subarachnoid space
traumatic tap
xanthochromia

Formation and Physiology

First recognized by Cotugno in 1764, cerebrospinal fluid (**CSF**) is the third major fluid of the body.[16] The CSF provides a physiologic system to supply nutrients to the nervous tissue, to remove metabolic wastes, and to produce a mechanical barrier to cushion the brain and spinal cord against trauma. As shown in Figure 10–1, the brain and spinal cord are lined by the **meninges**, consisting of three layers: the dura mater, arachnoid mater, and pia mater. The CSF flows through the ***subarachnoid space*** located between the arachnoid mater and the pia mater. Approximately 20 mL of fluid is produced every hour in the ***choroid plexuses*** and reabsorbed by the ***arachnoid villi*** to maintain a total volume of 140 to 170 mL in adults and 10 to 60 mL in neonates.[23]

Production of CSF in the choroid plexuses is by filtration under hydrostatic pressure across the choroidal capillary wall and active transport secretion by the choroidal epithelial cells. Tightly fitting junctions between the endothelial cells of the capillaries and the choroid plexuses restrict entry of macromolecules such as protein, insoluble lipids, and substances bound to serum proteins. The chemical composition of the fluid does not resemble an ultrafiltrate of plasma owing to bidirectional active transport between the CSF, interstitial brain fluid, brain cells, and blood in the brain capillaries. The term ***blood-brain barrier*** is used to represent the control and filtration of blood components to the CSF and then to the brain.[10]

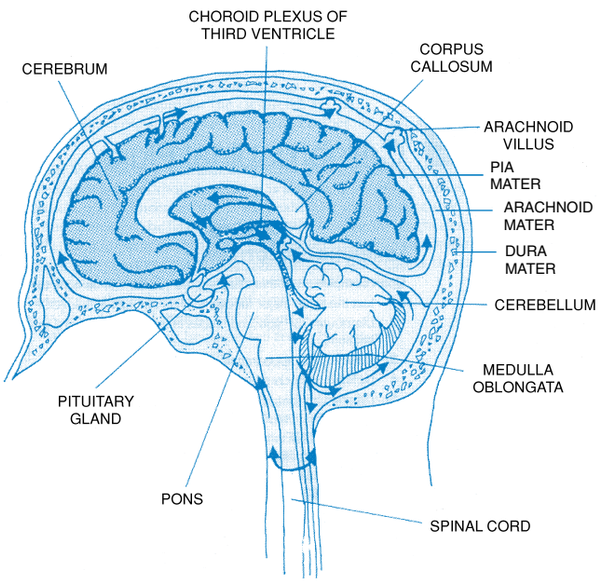


FIGURE 10–1 The flow of CSF through the brain and spinal column.

Specimen Collection and Handling

CSF is routinely collected by lumbar puncture between the third, fourth, or fifth lumbar vertebrae. Although this procedure is not complicated, it does require certain precautions, including measurement of the intracranial pressure and careful technique to prevent the introduction of infection or the damaging of neural tissue. Specimens are usually collected in three sterile tubes, which are labeled 1, 2, and 3 in the order in which they are withdrawn. Tube 1 is used for chemical and serologic tests; tube 2 is usually designated for the microbiology laboratory; and tube 3 is used for the cell count, because it is the least likely to contain cells introduced by the spinal tap procedure. If possible, a fourth tube may be drawn for the microbiology laboratory to provide better exclusion of skin contamination. Supernatant fluid that is left over after each section has performed its tests may be used for additional chemical or serologic tests. Excess fluid should not be discarded until there is no further use for it (Figure 10–2).

Considering the discomfort to the patient and the possible complications that can occur during specimen collection, laboratory personnel should handle CSF specimens carefully. Ideally, tests are performed on a STAT basis. If this is not possible, specimens are maintained in the following manner:

Hematology tubes are refrigerated.
Microbiology tubes remain at room temperature.
Chemistry and serology tubes are frozen.

Appearance

The initial appearance of the normally crystal clear CSF can provide valuable diagnostic information. Examination of the fluid occurs first at the bedside and is also included in the laboratory report. The major terminology used to describe CSF appearance includes crystal clear, cloudy or turbid, milky, xanthochromic, and hemolyzed/bloody (Figure

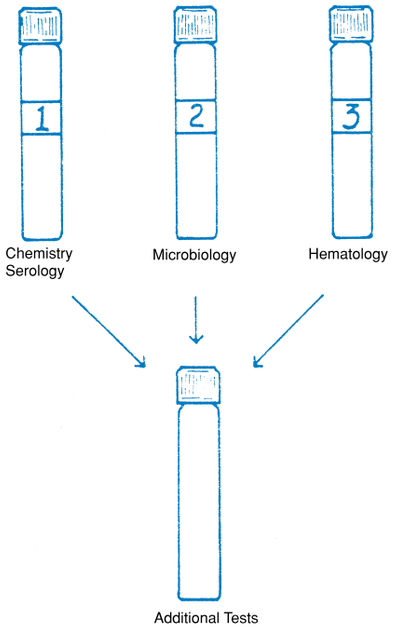


FIGURE 10–2 CSF specimen collection tubes.

10–3). A cloudy, turbid, or milky specimen can be the result of an increased protein or lipid concentration, but it also may be indicative of infection, with the cloudiness being caused by the presence of WBCs. All specimens should be treated with extreme care because they can be highly contagious; gloves must always be worn and face shields or splash guards should be used while preparing specimens for testing. Fluid for centrifugation must be in capped tubes.

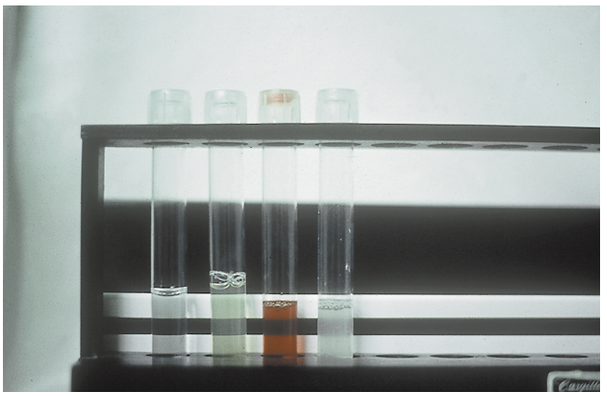


FIGURE 10–3 Tubes of CSF. Appearance left to right is normal, xanthochromic, hemolyzed, and cloudy.

Xanthochromia is a term used to describe CSF supernatant that is pink, orange, or yellow. A variety of factors can cause the appearance of xanthochromia, with the most common being the presence of RBC degradation products. Depending on the amount of blood and the length of time it has been present, the color will vary from pink (very slight amount of oxyhemoglobin) to orange (heavy hemolysis) to yellow (conversion of oxyhemoglobin to unconjugated bilirubin). Other causes of xanthochromia include elevated serum bilirubin, presence of the pigment carotene, markedly increased protein concentrations, and melanoma pigment. Xanthochromia that is due to immature liver function is also commonly seen in infants, particularly in those who are premature. The clinical significance of CSF appearance is summarized in Table 10–1.

TABLE 10–1 **Clinical Significance of Cerebrospinal Fluid Appearance**

Appearance	Cause	Major Significance
Crystal clear		Normal
Hazy, turbid, cloudy, milky	WBCs	Meningitis
	RBCs	Hemorrhage
		Traumatic tap
	Microorganisms	Meningitis
	Protein	Disorders that affect blood-brain barrier
		Production of IgG within the CNS
Oily	Radiographic contrast media	
Bloody	RBCs	Hemorrhage
		Traumatic tap
Xanthochromic	Hemoglobin	Old hemorrhage
		Lysed cells from traumatic tap
	Bilirubin	RBC degradation
		Elevated serum bilirubin level
	Carotene	Increased serum levels
	Protein	SEE ABOVE
	Melanin	Meningeal melanosarcoma
Clotted	Protein	SEE ABOVE
	Clotting factors	Introduced by traumatic tap
Pellicle	Protein	Disorders that affect blood-brain barrier
	Clotting factors	Tubercular meningitis

Traumatic Collection

Grossly bloody CSF can be an indication of intracranial hemorrhage, but it also may be due to the puncture of a blood vessel during the spinal tap procedure. Three visual examinations of the collected specimens can usually determine whether the blood is the result of hemorrhage or a ***traumatic tap***.

UNEVEN DISTRIBUTION OF BLOOD

Blood from a cerebral hemorrhage will be evenly distributed throughout the three CSF specimen tubes, whereas a traumatic tap will have the heaviest concentration of blood in tube 1, with gradually diminishing amounts in tubes 2 and 3. Streaks of blood also may be seen in specimens acquired following a traumatic procedure.

CLOT FORMATION

Fluid collected from a traumatic tap may form clots owing to the introduction of plasma fibrinogen into the specimen. Bloody CSF caused by intracranial hemorrhage will not contain enough fibrinogen to clot. Diseases in which damage to the blood-brain barrier allows increased filtration of protein and coagulation factors will also cause clot formation but do not usually produce a bloody fluid. These conditions include ***meningitis***, Froin's syndrome, and blockage of CSF circulation through the subarachnoid space. A classic weblike pellicle is associated with tubercular meningitis and is frequently seen after overnight refrigeration of the fluid.[27]

XANTHOCHROMIC SUPERNATANT

RBCs must usually remain in the CSF for approximately 2 hours before noticeable hemolysis begins; therefore, a xanthochromic supernatant would be the result of blood that has been present longer than that introduced by the traumatic tap. Care should be taken, however, to consider this examination in conjunction with those previously discussed, because a very recent hemorrhage would produce a clear supernatant, and introduction of serum protein from a traumatic tap could also cause the fluid to appear xanthochromic. To examine a bloody fluid for the presence of xanthochromia, the fluid should be centrifuged in a microhematocrit tube and the supernatant examined against a white background.

Additional testing for differentiation includes microscopic examination and the **D-dimer** test. The microscopic finding of macrophages containing ingested RBCs (**erythrophagocytosis**) or hemosiderin granules is indicative of intracranial hemorrhage. Detection of the fibrin degradation product, D-dimer, by latex agglutination immunoassay indicates the formation of fibrin at a hemorrhage site.

Cell Count

The cell count that is routinely performed on CSF specimens is the leukocyte (WBC) count. As discussed previously, the presence and significance of RBCs can usually be ascertained from the appearance of the specimen. Therefore, RBC counts are usually determined only when a traumatic tap has occurred and a correction for leukocytes or protein is needed. The RBC count can be calculated by performing a total cell count and a WBC count and subtracting the WBC count from the total count, if necessary. Any cell count should be performed immediately, because WBCs (particularly granulocytes) and RBCs will begin to lyse within 1 hour, with 40 percent of the leukocytes disintegrating after 2 hours.[6] Specimens that cannot be analyzed immediately should be refrigerated.

METHODOLOGY

Normal adult CSF contains 0 to 5 WBCs/μL. The number is higher in children, and as many as 30 mononuclear cells/μL can be considered normal in newborns.[32] Specimens that contain up to 200 WBCs or 400 RBCs/μL may appear clear, so it is necessary to examine all specimens microscopically.[12] An improved Neubauer counting chamber (Figure 10–4) is routinely used for performing CSF cell counts. Traditionally, electronic cell counters have not been used for performing CSF cell counts, owing to high background counts and poor reproducibility of low counts. However, newer instrumentation has greatly eliminated background interference and laboratories with documentation of linearity, background, and correlation studies can meet the requirements of accrediting agencies for automated body fluid cell counts.[36]

The standard Neubauer calculation formula used for blood cell counts is also applied to CSF cell counts to determine the number of cells per microliter.

$$\frac{\text{Number of cells counted} \times \text{dilution}}{\text{Number of squares counted} \times \text{volume of 1 square}} = \text{cells}/\mu\text{L}$$

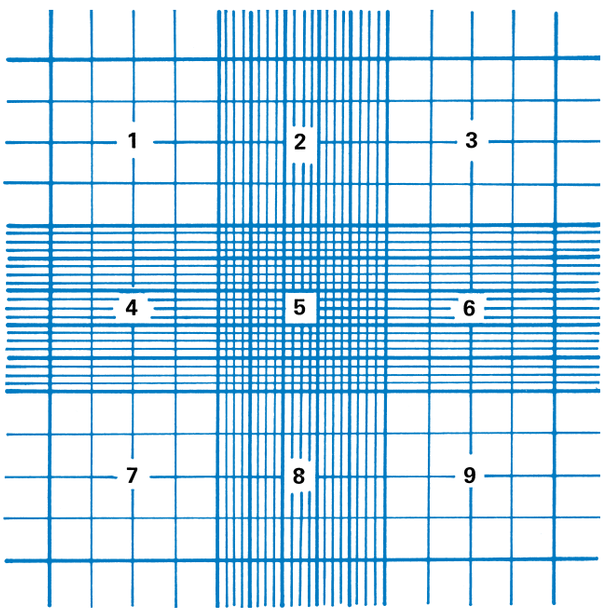


FIGURE 10–4 Neubauer counting chamber depicting the nine large, square counting areas.

This formula can be used for both diluted and undiluted specimens and offers flexibility in the number and size of the squares counted. Many varied calculations are available, including condensations of the formula to provide single factors by which to multiply the cell count. Keep in mind that the purpose of any calculation is to convert the number of cells counted in a specific amount of fluid to the number of cells that would be present in 1 µL of fluid. Therefore, a factor can be used only when the dilution and counting area are specific for that factor.

The methodology presented in this chapter eliminates the need to correct for the volume counted by counting the four large corner squares (0.4 µL) and the large center square (0.1 µL) on each side of the counting chamber.[35]

EXAMPLE:

$$\text{Number of cells counted} \times \text{dilution} \times \frac{1\ \mu L}{\underset{\text{(volume counted)}}{1\ \mu L\ (0.1 \times 10)}} = \text{cells}/\mu L$$

TOTAL CELL COUNT

Clear specimens may be counted undiluted, provided no overlapping of cells is seen during the microscopic examination. When dilutions are required, calibrated automatic pipettes, not mouth pipetting, are used. A sample dilution method is as follows.[35]

Clarity	Dilution	Amount of Sample	Amount of Diluent
Slightly hazy	1:10	30 µL	270 µL
Hazy	1:20	30 µL	570 µL
Slightly cloudy	1:100	30 µL	2970 µL
Slightly bloody	1:200	30 µL	5970 µL
Cloudy Bloody Turbid	1:10,000	0.1 mL of a 1:100 dilution	9.9 mL

Dilutions for total cell counts are made with normal saline, mixed by inversion, and loaded into the hemocytometer with a Pasteur pipette. Cells are counted in the four corner squares and the center square on both sides of the hemocytometer. As shown in the preceding example, the number of cells counted multiplied by the dilution factor equals the number of cells per microliter.

WHITE BLOOD CELL COUNT

Lysis of RBCs must be obtained prior to performing the WBC count on either diluted or undiluted specimens. Specimens requiring dilution can be diluted in the manner described previously, substituting 3 percent acetic acid to lyse the RBCs. Addition of methylene blue to the diluting fluid will stain the WBCs providing better differentiation.

To prepare a clear specimen that does not require dilution for counting, place four drops of mixed specimen in a clean tube. Rinse a Pasteur pipette with glacial acetic acid, draining thoroughly, and draw the four drops of CSF into the rinsed pipette. Allow the pipette to sit for 1 minute, mix the solution in the pipette, discard the first drop, and load the hemocytometer. As in the total cell count, WBCs are counted in the four corner squares, and the center square on both sides of the hemocytometer and the number is multiplied by the dilution factor to obtain the number of WBCs per microliter. If a different number of squares is counted, the standard Neubauer formula should be used to obtain the number of cells per microliter.

CORRECTIONS FOR CONTAMINATION

Calculations are possible to correct for WBCs and protein artificially introduced into the CSF as the result of a traumatic tap. Determination of the CSF RBC count and the blood RBC and WBC counts is necessary to perform the correction. By determining the ratio of WBCs to RBCs in the peripheral blood and comparing this ratio with the number of contaminating RBCs, the number of artificially added WBCs can be calculated using the following formula:

$$\text{WBC (added)} = \frac{\text{WBC (blood)} \times \text{RBC (CSF)}}{\text{RBC (blood)}}$$

An approximate CSF WBC count can then be obtained by subtracting the "added" WBCs from the actual count. When peripheral blood RBC and WBC counts are in the normal range, many laboratories choose to simply subtract 1 WBC for every 700 RBCs present in the CSF.[32] Studies have shown a high percentage of error in the correction of fluids containing a large number of RBCs, indicating correction may be of little value under these circumstances.[28]

QUALITY CONTROL OF CEREBROSPINAL FLUID AND OTHER BODY FLUID CELL COUNTS

In-house controls can be prepared on a daily basis and performed on each shift to ensure the reliability of reagents and technique. This can be done by preparing dilutions of a selected patient sample and comparing manual results with those obtained on an automated cell counter. Results of the manual counts should agree with the automated counts by plus or minus 25 percent. The daily control specimen is refrigerated and manual counts are performed on each shift.[35] Spinalscopics Spinal Fluid Cell Count Controls (Quantimetrix, Redondo Beach, CA), which provides two levels of RBCs and WBCs, are available for purchase.

On a biweekly basis all diluents should be checked for contamination by examining in a counting chamber under 40× magnification. Contaminated diluents should be discarded and new solutions prepared.

On a monthly basis the speed of the cytocentrifuge should be checked with a tachometer and the timing should be checked with a stopwatch.

If nondisposable counting chambers are used, they must be soaked in a bactericidal solution for at least 15 minutes and then thoroughly rinsed with water and cleaned with isopropyl alcohol.

Differential Count on a Cerebrospinal Fluid Specimen

Identifying the type or types of cells present in the CSF is a valuable diagnostic aid. The differential count should be performed on a stained smear and not from the cells in the counting chamber. Poor visualization of the cells as they appear in the counting chamber has led to the laboratory practice of reporting only the percentage of mononuclear and polynuclear cells present, and this can result in the overlooking of abnormal cells with considerable diagnostic importance. To ensure that the maximum number of cells are available for examination, the specimen should be concentrated prior to the preparation of the smear.

Methods available for specimen concentration include sedimentation, filtration, centrifugation, and cytocentrifugation. Automated body fluid microscopy also is available on the IRIS Model 500 (International Remote Imaging Systems, Chatsworth, CA.). Sedimentation and filtration are not routinely used in the clinical laboratory, although they do produce less cellular distortion. Most laboratories that do not have a cytocentrifuge concentrate specimens with routine centrifugation. The specimen is centrifuged for 5 to 10 minutes; supernatant fluid is removed and saved for additional tests; and slides made from the suspended sediment are allowed to air dry and are stained with Wright's stain. When performing the differential count, 100 cells should be counted, classified, and reported in terms of percentage. If the cell count is low and finding 100 cells is not possible, report only the numbers of the cell types seen.

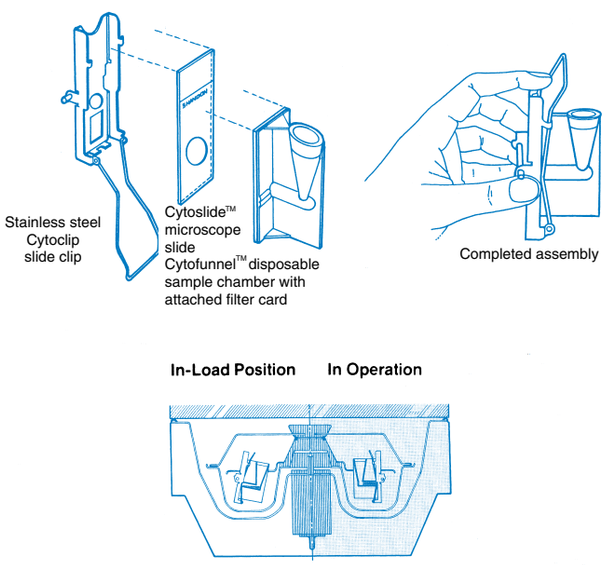


FIGURE 10–5 Cytospin 3 cytocentrifuge specimen processing assembly (Courtesy of Shandon, Inc., Pittsburgh, PA.)

CYTOCENTRIFUGATION

A diagramatic view of the principle of cytocentrifugation is shown in Figure 10–5. Fluid is added to the conical chamber, and as the specimen is centrifuged, cells present in the fluid are forced into a monolayer within a 6-mm diameter circle on the slide. Fluid is absorbed by the filter paper blotter, producing a more concentrated area of cells. As little as 0.1 mL of CSF combined with one drop of 30 percent albumin produces an adequate cell yield when processed with the cytocentrifuge. Addition of albumin increases the cell yield and decreases the cellular distortion frequently seen on cytocentrifuged specimens. Positively charged coated slides to attract cells (Shandon, Inc, Pittsburgh, PA) are also available. Cellular distortion may include cytoplasmic vacuoles, nuclear clefting, prominent nucleoli, and cellular clumping resembling malignancy. Cells from both the center and periphery of the slide should be examined because cellular characteristics may vary between areas of the slide.

A daily control slide for bacteria should also be prepared using 0.2 mL saline and two drops of 30 percent albumin. The slide is stained and examined if bacteria are seen on a patient's slide.[35]

In Table 10–2, a cytocentrifuge recovery chart is provided for comparison with chamber counts. The chamber count should be repeated if too many cells are seen on the slide, and a new slide should be prepared if not enough cells are seen on the slide.[35]

CEREBROSPINAL FLUID CELLULAR CONSTITUENTS

The cells found in normal CSF are primarily lymphocytes and monocytes (Figures 10–6 and 10–7). Adults usually have a predominance of lymphocytes to monocytes (70:30), whereas monocytes are more prevalent in children.[21] Improved concentration methods are also showing occasional neutrophils in normal CSF.[19] The presence of increased numbers of these normal cells (termed ***pleocytosis***) is considered abnormal, as is the finding of immature leukocytes, eosinophils, plasma cells, macrophages, increased tissue cells, and malignant cells.

When pleocytosis involving neutrophils, lymphocytes, or monocytes is present, the CSF differential count is most frequently associated with its role in providing diagnostic information about the type of microorganism that is causing an infection of the meninges (meningitis). A high CSF

TABLE 10–2 **Cytocentrifuge Recovery Chart[35]**

Number of White Blood Cells Counted in Chamber	Number of Cells Counted on Cytocentrifuge Slide
0	0–40
1–5	20–100
6–10	60–150
11–20	150–250
20	250

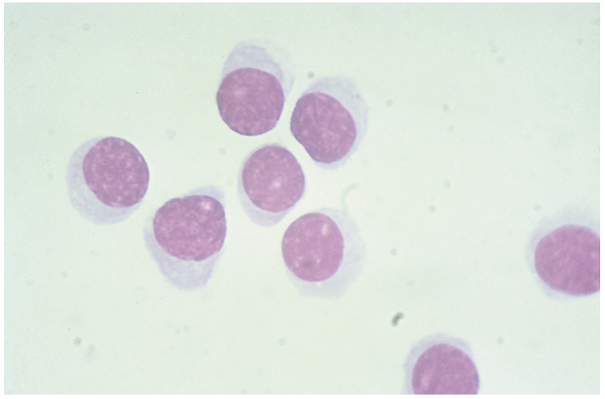


FIGURE 10–6 Normal lymphocytes. Some cytocentrifuge distortion of cytoplasm (×1000).

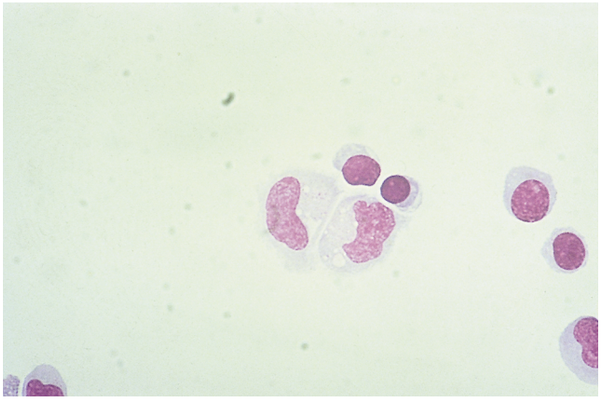


FIGURE 10–7 Normal lymphocytes and monocytes (×500).

WBC count of which the majority of the cells are neutrophils is considered indicative of bacterial meningitis. Likewise, a moderately elevated CSF WBC count with a high percentage of lymphocytes and monocytes suggests meningitis of viral, tubercular, fungal, or parasitic origin.

As seen in Table 10–3, many pathologic conditions other than meningitis can be associated with the finding of abnormal cells in the CSF. Therefore, because laboratory personnel become so accustomed to finding neutrophils, lymphocytes, and monocytes, they should be careful not to overlook other types of cells. Cell forms differing from those found in blood include macrophages, choroid plexus and ependymal cells, spindle-shaped cells, and malignant cells.

Increased neutrophils (Figure 10–8) also are seen in the early stages (1 to 2 days) of viral, fungal, tubercular, and parasitic meningitis. Neutrophils associated with bacterial meningitis may contain phagocytized bacteria (Figure 10–9). Although of little clinical significance, neutrophils may be increased following central nervous system (**CNS**) hemorrhage, repeated lumbar punctures, and injection of medications or radiographic dye.

A mixture of lymphocytes and monocytes is common in cases of viral, tubercular, and fungal meningitis (Figure

TABLE 10–3 **Predominant Cells Seen in Cerebrospinal Fluid**

Type of Cell	Major Clinical Significance	Microscopic Findings
Lymphocytes	Normal Viral, tubercular, and fungal meningitis Multiple sclerosis	All stages of development may be found
Neutrophils	Bacterial meningitis Early cases of viral, tubercular, and fungal meningitis Cerebral hemorrhage	Granules may be less prominent than in blood[19] Cells disintegrate rapidly
Monocytes	Normal Viral, tubercular, and fungal meningitis Multiple sclerosis	Found mixed with lymphocytes
Macrophages	RBCs in spinal fluid Contrast media	May contain phagocytized RBCs appearing as empty vacuoles or ghost cells and hemosiderin granules
Blast forms	Acute leukemia	Lymphoblasts, myeloblasts, or monoblasts
Plasma cells	Multiple sclerosis Lymphocyte reactions	Traditional and classic forms seen
Ependymal, choroidal, and spindle-shaped cells	Diagnostic procedures	Seen in clusters with distinct nuclei and distinct cell walls
Malignant cells	Metastatic carcinomas Primary central nervous system (CNS) carcinoma	Seen in clusters with fusing of cell borders and nuclei

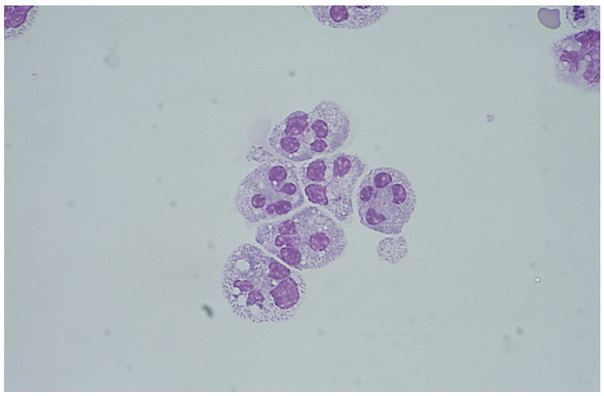


FIGURE 10–8 Neutrophils with cytoplasmic vacuoles resulting from cytocentrifugation (×500).

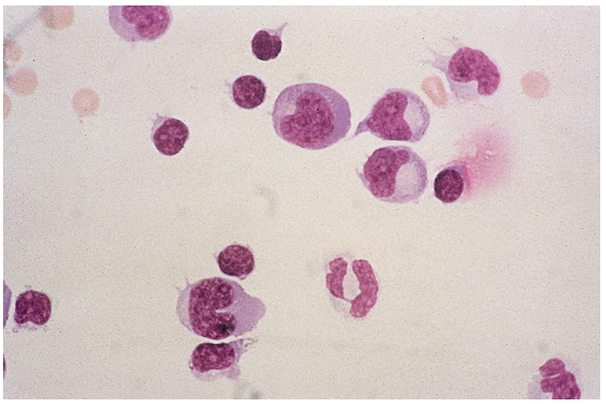


FIGURE 10–10 Broad spectrum of lymphocytes and monocytes in viral meningitis (×100).

10–10). Reactive lymphocytes containing increased dark blue cytoplasm and clumped chromatin are frequently present during viral infections in conjunction with normal cells. Increased lymphocytes are seen in cases of both asymptomatic human immunodeficiency virus (**HIV**) infection and acquired immunodeficiency syndrome (**AIDS**). A moderately elevated WBC count (less than 50 WBCs/μL) with increased normal and reactive lymphocytes and plasma cells may be indicative of multiple sclerosis or other degenerative neurologic disorders.[3]

Increased eosinophils are seen in the CSF in association with parasitic infections, fungal infections (primarily *Coccidioides immitis*), and introduction of foreign material, including medications and shunts into the CNS (Figure 10–11).

Macrophages appear within 2 to 4 hours after RBCs enter into the CSF and are frequently seen following repeated taps (Figure 10–12). The finding of increased macrophages containing RBCs is indicative of a previous hemorrhage (Figures 10–13 and 10–14). The macrophages may also contain hemosiderin granules and hematoidin crystals (Figures 10–15 through 10–17).

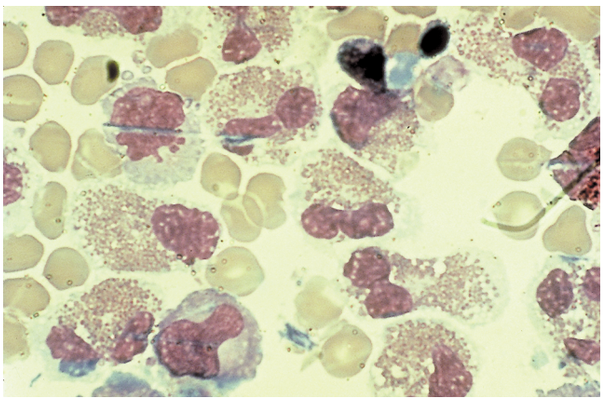


FIGURE 10–11 Eosinophils. Notice cytocentrifuge distortion (×1000).

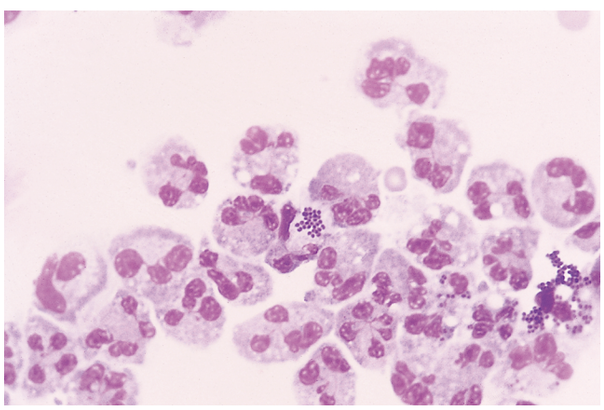


FIGURE 10–9 Neutrophils with intracellular bacteria (×1000).

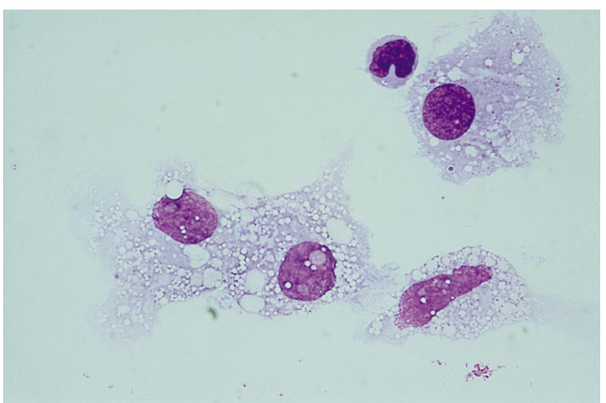


FIGURE 10–12 Macrophages. Notice the presence of large vacuoles (×500).

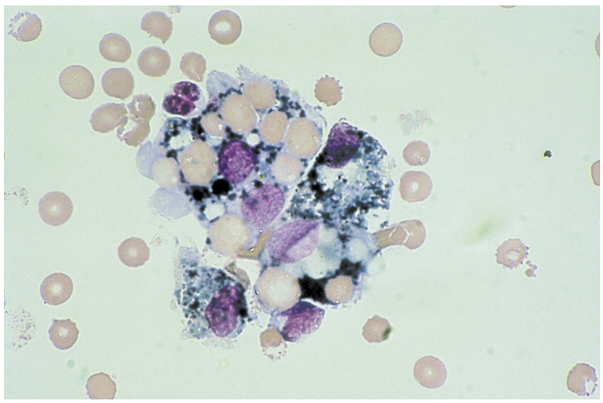


FIGURE 10–13 Macrophages showing erythrophagocytosis (×500).

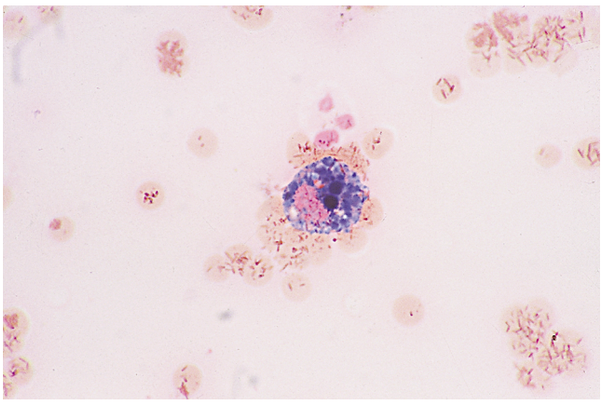


FIGURE 10–16 Macrophage containing hemosiderin stained with Prussian blue (×250).

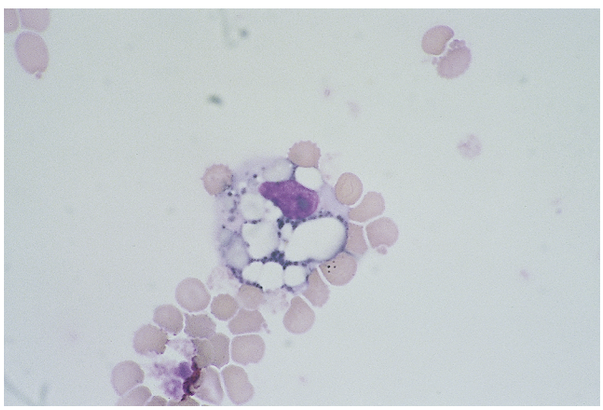


FIGURE 10–14 Macrophage with RBC remnants (×500).

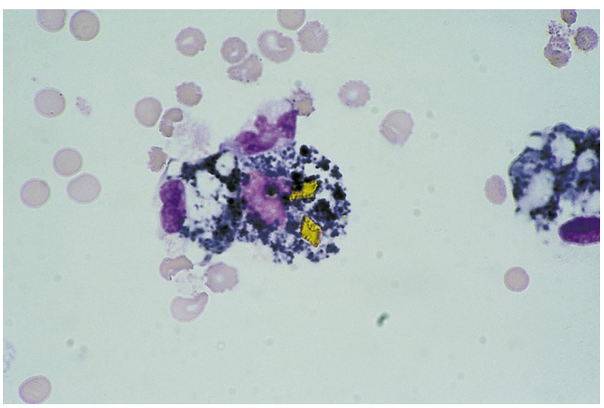


FIGURE 10–17 Macrophage containing hemosiderin and hematoidin crystals (×500).

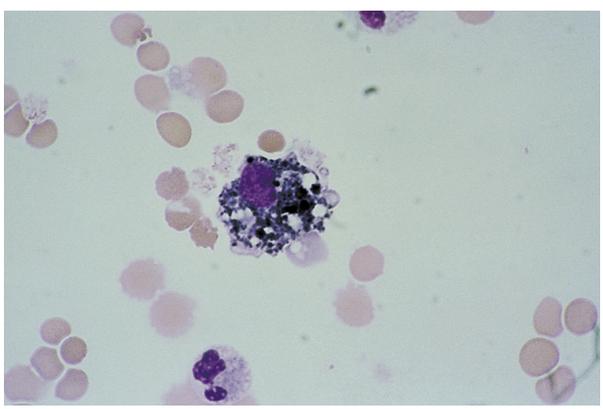


FIGURE 10–15 Macrophage with aggregated hemosiderin granules (×500).

Ependymal cells and choroid plexus cells (Figures 10–18 and 10–19) from the lining of the ventricles and spindle-shaped cells from the arachnoid lining (Figure 10–20) are not considered clinically significant. They are most frequently seen following diagnostic procedures such as pneumoencephalography and in fluid obtained from ventricular taps or during neurosurgery. These cells often appear as clusters and can be distinguished from malignant cells by their uniform appearance.

Nucleated RBCs are seen as a result of bone marrow contamination during the spinal tap (Figures 10–21 and 10–22). This is found in approximately 1 percent of specimens.[1] Capillary structures and endothelial cells may be seen following a traumatic tap (Figure 10–23).

Lymphoblasts, myeloblasts, and monoblasts (Figures 10–24 through 10–26) in the CSF are frequently seen as a complication of acute leukemias. Nucleoli are often more

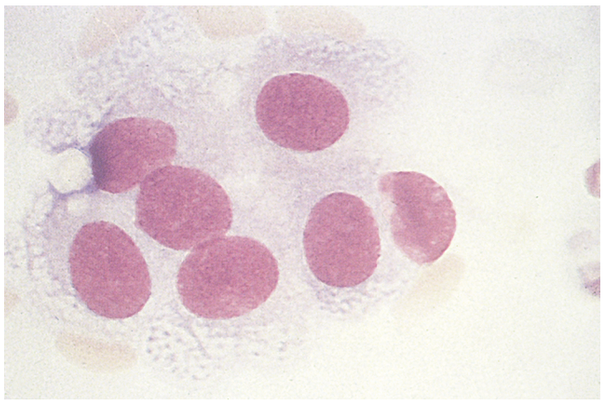


FIGURE 10–18 Ependymal cells (×1000).

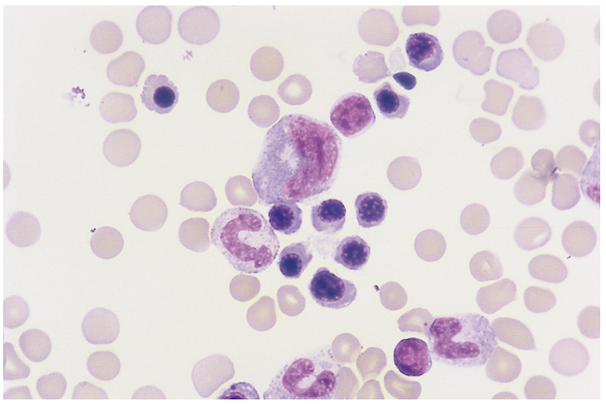


FIGURE 10–21 Nucleated RBCs seen with bone marrow contamination (×1000).

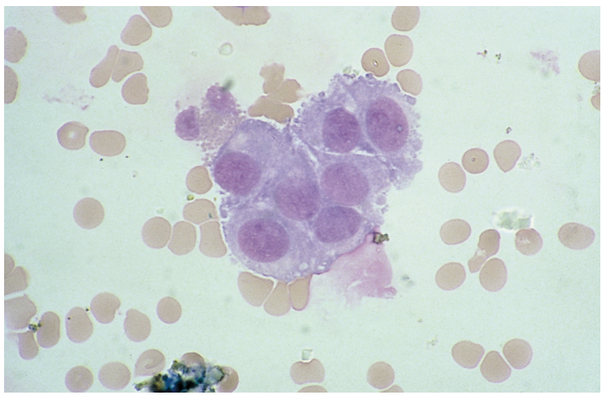


FIGURE 10–19 Choroid plexus cells showing distinct cell borders and nuclear uniformity (×500).

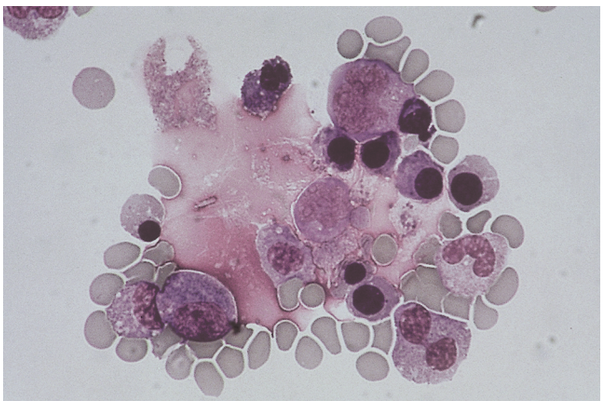


FIGURE 10–22 CSF bone marrow contamination (×500).

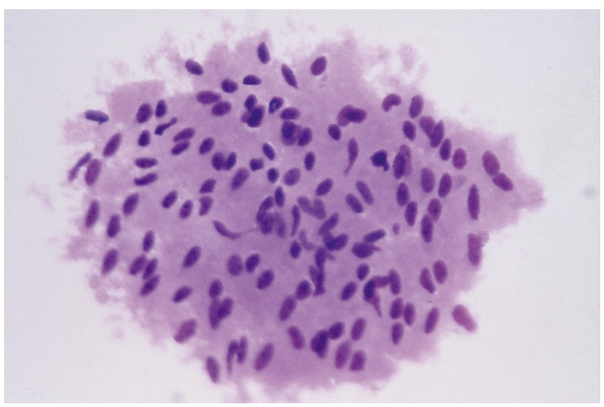


FIGURE 10–20 Cluster of spindle-shaped cells (×500).

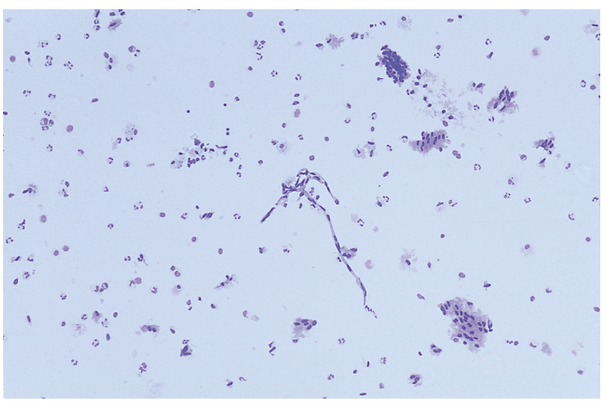


FIGURE 10–23 Capillary and RBCs seen with traumatic tap (×100).

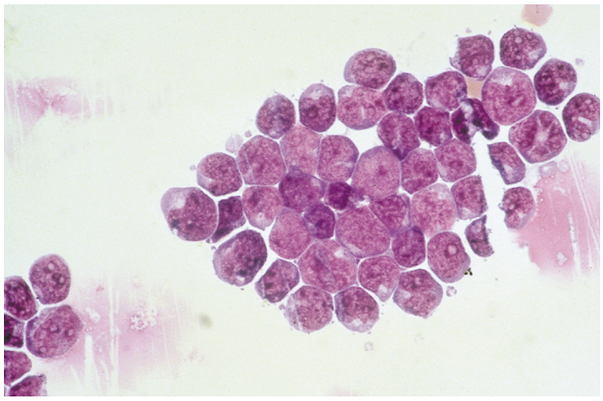


FIGURE 10–24 Lymphoblasts from acute lymphocytic leukemia (×500).

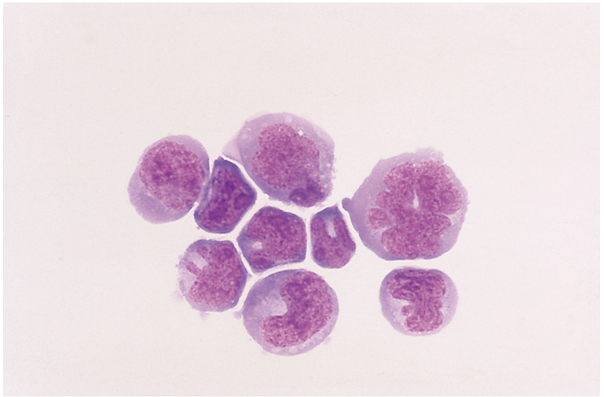


FIGURE 10–27 Noncleaved lymphoma cells (×1000).

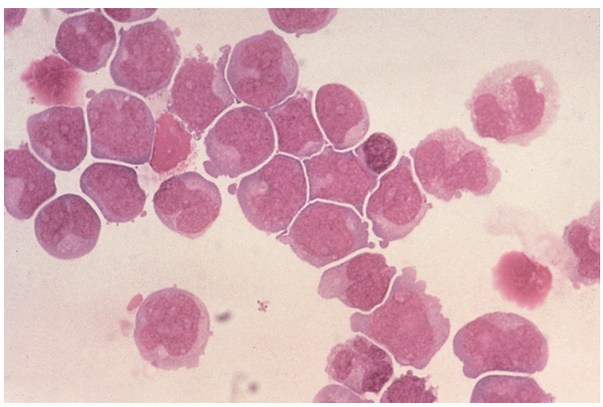


FIGURE 10–25 Myeloblasts from acute myelocytic leukemia (×500).

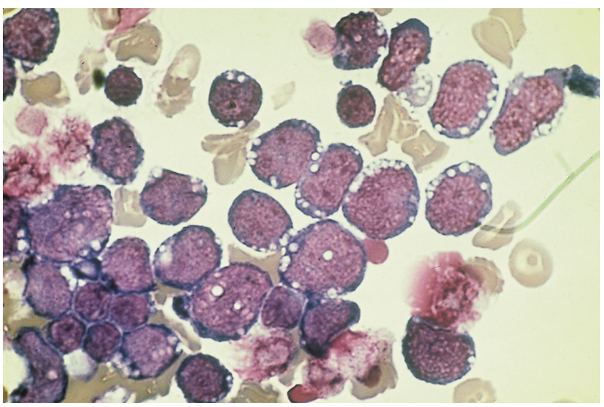


FIGURE 10–28 Burkitt's lymphoma. Notice characteristic vacuoles (×500).

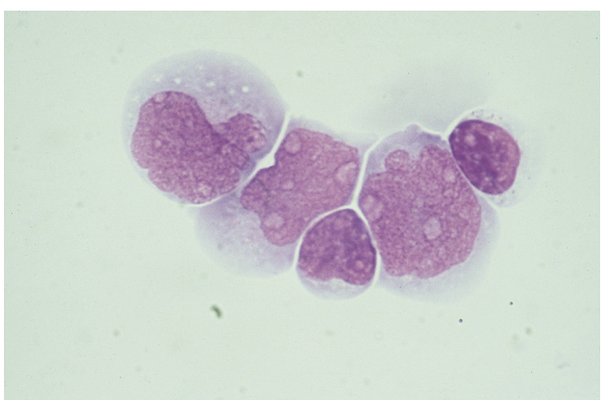


FIGURE 10–26 Monoblasts and two lymphocytes. Notice the prominent nucleoli (×1000).

prominent than in blood smears. Both cleaved and noncleaved lymphoma cells are also seen in the CSF (Figures 10–27 and 10–28).

Metastatic carcinoma cells are primarily from lung, breast, renal, and gastrointestinal malignancies and melanoma (Figure 10–29). Cells from primary CNS tumors include the astrocytomas and medulloblastomas, frequently occurring in children (Figure 10–30). They usually appear in clusters and must be distinguished from normal clusters of ependymal, choroid plexus, and leukemia cells. Fusing of cell walls and nuclear irregularities and hyperchromatic nucleoli are seen in clusters of malignant cells. Slides containing abnormal cells must be referred to pathology.

Chemistry Tests

Because CSF is formed by filtration of the plasma, one would expect to find the same low-molecular-weight chemicals in the CSF that are found in the plasma. This is essen-

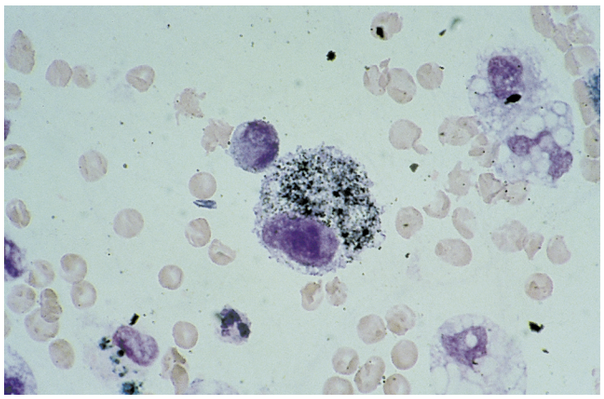


FIGURE 10–29 Malignant melanoma cell containing dustlike granules that are much finer than hemosiderin granules (×500).

tially true; however, because the filtration process is selective and the chemical composition is controlled by the blood-brain barrier, normal values for CSF chemicals are not the same as the plasma values. Abnormal values result from alterations in the permeability of the blood-brain barrier or increased production or metabolism by the neural cells in response to a pathologic condition, and they seldom have the same diagnostic significance as plasma abnormalities. The clinically important CSF chemicals are few in number, although under certain conditions, it may be necessary to measure a larger variety. Many CSF metabolites are currently under investigation to determine their possible diagnostic significance.

CEREBROSPINAL PROTEIN

The most frequently performed chemical test on CSF is the protein determination. Normal CSF contains a very small amount of protein. Normal values for total CSF protein are usually listed as 15 to 45 mg/dL, but are somewhat method dependent, and higher values are found in infants and older persons.[4] This value is reported in milligrams per deciliter and not grams per deciliter, as are plasma protein concentrations.

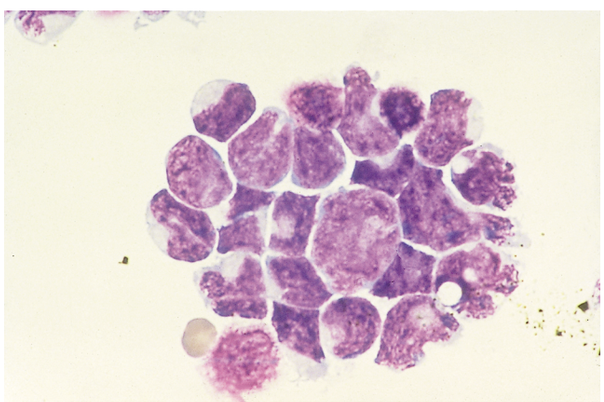


FIGURE 10–30 Medulloblastoma. Notice cellular clustering, nuclear irregularities, and rosette formation (×1000).

TABLE 10–4 **Cerebrospinal Fluid and Serum Protein Correlations**

	Cerebrospinal Fluid (mg/dL)	Serum (mg/dL)	Ratio
Prealbumin	1.7	23.8	14
Albumin	15.5	3600	236
Ceruloplasmin	0.1	36.6	366
Transferrin	1.4	204	142
Immunoglobulin G	1.2	987	802
Immunoglobulin A	0.13	175	1346

Adapted from Fishman.[10]

In general, the CSF contains protein fractions similar to those found in serum; however, as can be seen in Table 10–4, the ratio of CSF proteins to serum proteins varies among the fractions. As in serum, albumin comprises the majority of CSF protein. But in contrast to serum, prealbumin is the second most prevalent fraction in CSF. The alpha globulins include primarily haptoglobin and ceruloplasmin. Transferrin is the major beta globulin present; also, a separate carbohydrate-deficient transferrin fraction, referred to as "tau," is seen in CSF and not in serum. CSF gamma globulin is primarily immunoglobulin G (IgG), with only a small amount of IgA. IgM, fibrinogen, and beta lipoprotein are not found in normal CSF.[10]

Clinical Significance of Elevated Protein Values

Elevated total protein values are most frequently seen in pathologic conditions. Abnormally low values will be present when fluid is leaking from the CNS. The causes of elevated CSF protein include damage to the blood-brain barrier, production of immunoglobulins within the CNS, decreased clearance of normal protein from the fluid, and degeneration of neural tissue. Meningitis and hemorrhage conditions that damage the blood-brain barrier are the most common causes of elevated CSF protein. Many other neurologic disorders can elevate the CSF protein, and finding an abnormal result on a clear fluid with a low cell count is not unusual.

In the same manner as blood cells can be artificially introduced into a specimen by a traumatic tap, so can plasma protein. A correction calculation similar to that used in cell counts is available for protein measurements; however, if the correction is to be used, both the cell count and the protein determination must be done on the same tube.[12] When the blood hematocrit and serum protein values are normal, subtracting 1 mg/dL of protein for every 1200 RBCs counted is acceptable.[32]

$$\text{mg/dL protein added} = \frac{[\text{serum protein mg/dL} \times (1.00 - \text{Hct})] \times \text{CSF RBCs}/\mu\text{L}\ (\text{plasma volume})}{\text{blood RBCs}/\mu\text{L}}$$

Methodology

The two most routinely used techniques for measuring total CSF protein use the principles of turbidity production or dye-binding ability. Turbidimetric methods have been available for many years and rely on the precipitation of protein by either sulfosalicylic acid (SSA) or trichloroacetic acid. The reagent of choice is trichloroacetic acid because it will precipitate both albumin and globulin equally. Unless SSA is combined with sodium sulfate, albumin will contribute more to the turbidity than globulin. Standards should be prepared using a mixture of albumin and globulin and not just from albumin.

Dye-binding techniques offer the advantages of smaller sample size and less interference from external sources. The development of dye-binding procedures, which are almost as rapid and easy to perform as the turbidity methods, has greatly increased their acceptance in laboratories. This method uses the dye Coomassie brilliant blue G250 and the principle of "protein error of indicators" discussed in Chapter 5. Coomassie brilliant blue dye is used because it will bind to a variety of proteins rather than just to albumin. The color change of the pH-stabilized dye reagent from red to blue occurs when protein binds to the dye.[13] The concentration of protein present will determine the amount of blue color produced, thereby allowing a mathematic conversion of the intensity of the blue color present to the concentration of protein present (Beer's law).

Methods for the measurement of CSF protein are available for most automated chemistry analyzers.

Protein Fractions

Routine CSF protein procedures are designed to measure total protein concentration. However, diagnosis of neurologic disorders associated with abnormal CSF protein often requires measurement of the individual protein fractions. Protein that appears in the CSF as a result of damage to the integrity of the blood-brain barrier will contain fractions proportional to those in plasma, with albumin present in the highest concentration. Diseases, including multiple sclerosis, that stimulate the immunocompetent cells in the CNS will show a higher proportion of IgG.[34]

To accurately determine whether IgG is increased because it is being produced within the CNS or is elevated as the result of a defect in the blood-brain barrier, comparisons between serum and CSF levels of albumin and IgG must be made. Methods include the CSF/serum albumin index to evaluate the integrity of the blood-brain barrier and the CSF IgG index to measure IgG synthesis within the CNS.

The CSF to serum albumin index is calculated after determining the concentration of CSF albumin in milligrams per deciliter and the serum concentration in grams per deciliter. The formula used is as follows:

$$\text{CSF/serum albumin index} = \frac{\text{CSF albumin (mg/dL)}}{\text{Serum albumin (g/dL)}}$$

An index value less than 9 represents an intact blood-brain barrier. The index increases relative to the degree of damage to the barrier.

Calculation of an IgG index, which is actually a comparison of the CSF/serum albumin index with the CSF/serum IgG index, will compensate for any IgG entering the CSF via the blood-brain barrier.[17] It is performed by dividing the CSF/serum IgG index by the CSF/serum albumin index as follows:

$$\text{IgG index} = \frac{\text{CSF IgG (mg/dL)/serum IgG (g/dL)}}{\text{CSF albumin (mg/dL)/serum albumin (g/dL)}}$$

Normal IgG index values vary slightly among laboratories; however, in general values greater than 0.77 are indicative of IgG production within the CNS.

Techniques for the measurement of CSF albumin and globulin include electrophoresis, radial immunodiffusion, and nephelometry. Electrophoresis will provide an overall picture of all proteins present, and radial immunodiffusion and nephelometry measure individual fractions.

Electrophoresis

The primary purpose for performing CSF protein electrophoresis is for the detection of ***oligoclonal bands*** representing inflammation within the CNS. The bands are located in the gamma region of the protein electrophoresis, indicating Ig production. To ensure that the oligoclonal bands are present as the result of neurologic inflammation, simultaneous serum electrophoresis must be performed. Disorders, including leukemia, lymphoma, and viral infections, may produce serum banding, which can appear in the CSF as a result of blood-brain barrier leakage or traumatic introduction of blood into the CSF specimen. Banding representing both systemic and neurologic involvement is seen in the serum and CSF with HIV infection.[14]

The presence of two or more oligoclonal bands in the CSF that are not present in the serum can be a valuable tool in the diagnosis of multiple sclerosis, particularly when accompanied by an increased IgG index. Other neurologic disorders including encephalitis, neurosyphilis, Guillain-Barré syndrome, and neoplastic disorders also produce oligoclonal banding that may not be present in the serum. Therefore, the presence of oligoclonal banding must be considered in conjunction with clinical symptoms. Oligoclonal banding remains positive during remission of multiple sclerosis, but disappears in other disorders.[3]

Low protein levels in the CSF make concentration of the fluid prior to performing electrophoresis essential for most electrophoretic techniques. Agarose gel electrophoresis followed by Coomassie brilliant blue staining is most frequently performed in the clinical laboratory. Better resolution can be obtained using immunofixation electrophoresis (**IFE**) and isoelectric focusing (**IEF**) followed by silver staining. Specimen concentration is not required by the more sensitive IEF procedure.

Electrophoresis is also the method of choice when determining if a fluid is actually CSF. Identification can be made

based on the appearance of the previously mentioned extra isoform of transferrin, tau, that is found only in CSF.[30]

Myelin Basic Protein

The presence of myelin basic protein (**MBP**) in the CSF is indicative of recent destruction of the myelin sheath that protects the axons of the neurons (**demyelination**). Measurement of the amount of MBP in the CSF can be used to monitor the course of multiple sclerosis.[15] It may also provide a valuable measure of the effectiveness of current and future treatments.[38]

CEREBROSPINAL FLUID GLUCOSE

Glucose enters the CSF by selective transport across the blood-brain barrier, which results in a normal value that is approximately 60 to 70 percent that of the plasma glucose. If the plasma glucose is 100 mg/dL, then a normal CSF glucose would be approximately 65 mg/dL. For an accurate evaluation of CSF glucose, a blood glucose test must be run for comparison. The blood glucose should be drawn about 2 hours prior to the spinal tap to allow time for equilibration between the blood and fluid. CSF glucose is analyzed using the same procedures employed for blood glucose. Specimens should be tested immediately because glycolysis occurs rapidly in the CSF.

The diagnostic significance of CSF glucose is confined to the finding of values that are decreased in relation to plasma values. Elevated CSF glucose values are always a result of plasma elevations. Low CSF glucose values can be of considerable diagnostic value in determining the causative agents in meningitis. The finding of a markedly decreased CSF glucose accompanied by an increased WBC count and a large percentage of neutrophils is indicative of bacterial meningitis. If the WBCs are lymphocytes instead of neutrophils, tubercular meningitis is suspected. Likewise, if a normal CSF glucose value is found with an increased number of lymphocytes, the diagnosis would favor viral meningitis. Classic laboratory patterns such as those just described may not be found in all cases of meningitis, but they can be helpful when they are present.

Decreased CSF glucose values are caused primarily by alterations in the mechanisms of glucose transport across the blood-brain barrier and by increased use of glucose by the brain cells. The common tendency to associate the decreased glucose totally with its use by microorganisms and leukocytes cannot account for the variations in glucose concentrations seen in different types of meningitis and the decreased levels seen in other disorders producing damage to the CNS.[24]

CEREBROSPINAL FLUID LACTATE

The determination of CSF lactate levels can be a valuable aid in the diagnosis and management of meningitis cases. In bacterial, tubercular, and fungal meningitis, the elevation of CSF lactate to levels greater than 25 mg/dL occurs much more consistently than does the depression of glucose and provides more reliable information when the initial diagnosis is difficult. Levels greater than 35 mg/dL are frequently seen with bacterial meningitis, whereas in viral meningitis lactate levels remain lower than 25 mg/dL. CSF lactate levels remain elevated during initial treatment but fall rapidly when treatment is successful, thus offering a sensitive method for evaluating the effectiveness of antibiotic therapy.

Destruction of tissue within the CNS owing to oxygen deprivation (**hypoxia**) causes the production of increased CSF lactic acid levels. Therefore, elevated CSF lactate is not limited to meningitis and can result from any condition that decreases the flow of oxygen to the tissues. CSF lactate levels are frequently used to monitor severe head injuries. RBCs contain high concentrations of lactate, and falsely elevated results may be obtained on xanthochromic or hemolyzed fluid.[19]

CEREBROSPINAL FLUID GLUTAMINE

Glutamine is produced in the CNS by the brain cells from ammonia and α-ketoglutarate. This process serves to remove the toxic metabolic waste product ammonia from the CNS. The normal concentration of glutamine in the CSF is 8 to 18 mg/dL.[18] Elevated levels are found in association with liver disorders that result in increased blood and CSF ammonia. Increased synthesis of glutamine is caused by the excess ammonia that is present in the CNS; therefore, the determination of CSF glutamine provides an indirect test for the presence of excess ammonia in the CSF. Several methods of assaying glutamine are available and are based on the measurement of ammonia liberated from the glutamine. This is preferred over the direct measurement of CSF ammonia because the concentration of glutamine remains more stable than that of the volatile ammonia in the collected specimen. The CSF glutamine level also correlates with clinical symptoms much better than does the blood ammonia.[18]

As the concentration of ammonia in the CSF increases, the supply of α-ketoglutarate becomes depleted; glutamine can no longer be produced to remove the toxic ammonia, and coma ensues. Some disturbance of consciousness is almost always seen when glutamine levels are more than 35 mg/dL.[10] Therefore, the CSF glutamine test is a frequently requested procedure for patients with coma of unknown origin. Approximately 75 percent of children with Reye's syndrome have elevated CSF glutamine levels.[11]

CEREBROSPINAL FLUID ENZYMES

Throughout the years, many enzymes in the CSF have been studied, but little clinical application of CSF enzyme tests has resulted. Measurement of lactate dehydrogenase (**LD**) and/or its isoenzymes LD1, LD2, LD3, LD4, and LD5 continues to be studied.

Measurement of the creatine kinase isoenzyme CK-BB in CSF after resuscitation from cardiac arrest has been shown to reliably predict recovery when levels are less than 17 mg/mL.[29]

Summary of Cerebrospinal Fluid Chemistry Tests

Protein

1. Normal concentration is 15–45 mg/dL.
2. Elevated values are most frequently seen in patients with meningitis, hemorrhage, and multiple sclerosis.

Glucose

1. Normal value is 60–70% of the plasma concentration.
2. Decreased levels are seen in patients with bacterial, tubercular, and fungal meningitis.

Lactate

1. Levels >35 mg/dL are seen in patients with bacterial meningitis.
2. Levels >25 mg/dL are found in patients with tubercular and fungal meningitis.
3. Lower levels are seen in patients with viral meningitis.

Glutamine

1. Normal concentration is 8–18 mg/dL.
2. Levels >35 mg/dL are associated with some disturbance of consciousness.

Creatine Kinase CK-BB Isoenzyme

1. Elevated levels in patients post cardiac arrest indicate a poor prognosis.

Microbiology Tests

The role of the microbiology laboratory in the analysis of CSF lies in the identification of the causative agent in meningitis. For positive identification, the microorganism must be recovered from the fluid by growing it on the appropriate culture medium. This can take anywhere from 24 hours in cases of bacterial meningitis to 6 weeks for tubercular meningitis. Consequently, in many instances, the CSF culture is actually a confirmatory rather than a diagnostic procedure. However, the microbiology laboratory does have several methods available to provide information for a preliminary diagnosis. These methods include the Gram stain, acid-fast stain, India Ink preparation, limulus lysate test, and latex agglutination tests. In Table 10–5, the laboratory tests used in the differential diagnosis of meningitis are compared.

GRAM STAIN

The Gram stain is routinely performed on CSF from all suspected cases of meningitis, although its value lies in the detection of bacterial and fungal organisms. All smears and cultures should be performed on concentrated specimens because often only a few organisms are present at the onset of the disease. The CSF should be centrifuged at 1500 g for 15 minutes, and slides and cultures should be prepared from the sediment.[25] Use of the cytocentrifuge will provide a highly concentrated specimen. Even when concentrated specimens are used, at least a 10 percent chance exists that Gram stains and cultures will be negative. Thus, blood cultures should be taken, because the causative organism will often be present in both the CSF and the blood.[19] A CSF Gram stain is one of the most difficult slides to interpret because the number of organisms present is usually small, and they can easily be overlooked, resulting in a false-negative report. Also, false-positive reports can occur if precipitated stain or debris is mistaken for microorganisms. Therefore, considerable care should be taken when interpreting a Gram stain. Organisms most frequently encountered include *Streptococcus pneumoniae* (gram-positive cocci), *Hemophilus influenzae* (pleomorphic gram-negative rods), *Escherichia coli* (gram-negative rods), and *Neisseria meningitidis* (gram-negative cocci). The gram-positive cocci, *Streptococcus agalactiae* and the gram-positive rods *Listeria monocytogenes* may be encountered in newborns.

TABLE 10–5 **Major Laboratory Results for the Differential Diagnosis of Meningitis**

Bacterial	Viral	Tubercular	Fungal
Elevated WBC count Neutrophils present	Elevated WBC count Lymphocytes present	Elevated WBC count Lymphocytes and monocytes present	Elevated WBC count Lymphocytes and monocytes present
Marked protein elevation	Moderate protein elevation	Moderate to marked protein elevation	Moderate to marked protein elevation
Markedly decreased glucose level	Normal glucose level	Decreased glucose level	Normal to decreased glucose level
Lactate level >35 mg/dL	Normal lactate level	Lactate level >25 mg/dL	Lactate level >25 mg/dL
Positive limulus lysate test result with gram-negative organisms		Pellicle formation	Positive India Ink with *Cryptococcus neoformans*
Positive Gram stain and bacterial antigen tests			Positive immunologic test for *C. neoformans*

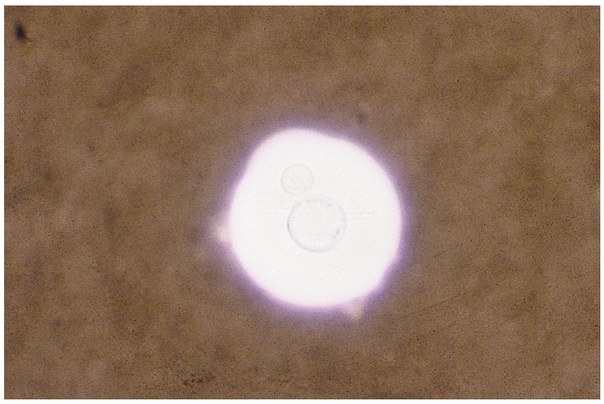


FIGURE 10–31 India Ink preparation of *C. neoformans*. Notice budding yeast form (×400). (Courtesy of Ann K. Fulenwider, MD.)

Acid-fast or fluorescent antibody stains are not routinely performed on specimens, unless tubercular meningitis is suspected. Considering the length of time required to culture mycobacteria, a positive report from this smear is extremely valuable.

Specimens from possible cases of fungal meningitis are Gram stained and often have an India Ink preparation performed on them. The India Ink preparation is performed to detect the presence of thickly encapsulated *Cryptococcus neoformans* (Figure 10–31). As one of the more frequently occurring complications of AIDS, cryptococcal meningitis is now commonly encountered in the clinical laboratory. Particular attention should be paid to the Gram stain for the classic starburst pattern produced by *Cryptococcus*, as this may be seen more often than a positive India Ink (Figure 10–32).[31]

Latex agglutination and enzyme-linked immunosorbent assay (**ELISA**) methods provide a rapid means for detecting and identifying microorganisms in CSF. Test kits are available to detect *Streptococcus* group B, *H. influenza type b*, *S. pneumoniae*, *N. meningitidis* A, B, C, Y, W135, and *E. coli K1* antigens. The bacterial antigen test (**BAT**) does not appear to be as sensitive to detection of *N. meningitidis* as it is to the other organisms.[5] The BAT should be used in combination with results from the hematology and clinical chemistry laboratories for diagnosing meningitis.[37] The Gram stain is still the recommended method for detection of organisms.[7]

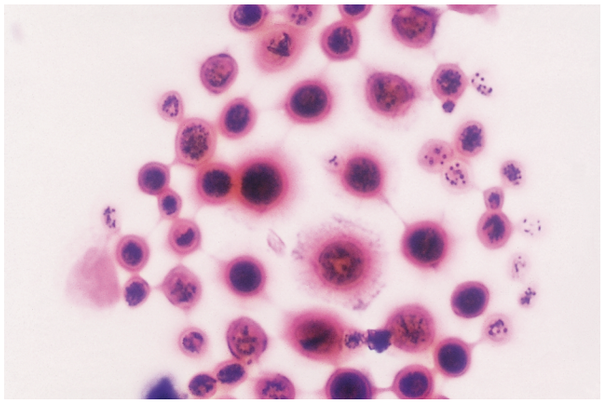


FIGURE 10–32 Gram stain of *C. neoformans* showing starburst pattern (×1000). (Courtesy of Ann K. Fulenwider, MD.)

Latex agglutination tests to detect the presence of *C. neoformans* antigen in serum and CSF provide a more sensitive method than the India Ink preparation. However, immunologic testing results should be confirmed by culture and demonstration of the organisms by India Ink, because false-positive reactions do occur. Interference by rheumatoid factor is the most common cause of false-positive reactions. Several commercial kits with pretreatment techniques are available and include incubation with dithiothreitol or pronase and boiling with ethylenediaminetetraacetic acid.[9,33] An enzyme immunoassay technique has been shown to produce fewer false-positive results.[20]

The limulus lysate test can be useful in the diagnosis of meningitis caused by gram-negative bacteria.[26] The reagent for this test is prepared from the blood cells of the horseshoe crab (*Limulus polyphemus*). These cells, termed amebocytes, contain a copper complex that gives them a blue color, thereby making the horseshoe crab a true "blue blood." Endotoxin found in the cell walls of gram-negative bacteria coagulates the amebocyte lysate within 1 hour if incubated at 37°C. The test is sensitive to minute amounts of endotoxin and will detect all gram-negative bacteria. The procedure must be performed using sterile technique to prevent false-positive results caused by contamination of specimens or tubes with endotoxin. Considerable amounts of endotoxin can be found in tap water.

Serologic Testing

In addition to the serologic procedures performed for identification of microorganisms, serologic testing of the CSF is performed to detect the presence of neurosyphilis. The use of penicillin in the early stages of syphilis has greatly reduced the number of cases of neurosyphilis. Consequently, the number of requests for serologic tests for syphilis on CSF is currently low. However, detection of the antibodies associated with syphilis in the CSF still remains a necessary diagnostic procedure.

Although many different serologic tests for syphilis are available when testing blood, the procedure recommended by the Centers for Disease Control and Prevention to diagnose neurosyphilis is the Venereal Disease Research Laboratories (VDRL) even though it is not as sensitive as the fluorescent treponemal antibody-absorption (FTA-ABS) test for syphilis. If the FTA-ABS is used, care must be taken to prevent contamination with blood, because the FTA-ABS remains positive in the serum of treated cases of syphilis.

The purpose of performing a test for syphilis on the CSF is to detect active cases of syphilis within the CNS. Therefore, the less sensitive VDRL procedure, the blood levels for which decrease in the later stages of syphilis, will be

PROCEDURE

Simulated Spinal Fluid Procedure

EQUIPMENT AND REAGENTS

1. Whole blood is collected the same day in EDTA. The "ideal" blood specimen for preparing SSF has a white count around 10×10^9 per liter, a low platelet count, and a normal-appearing differential with at least 20% lymphocytes. To prepare 50 mL of SSF, 5–7 mL of blood are needed.
2. Hanks' balanced salt solution (10×) without phenol red, sodium bicarbonate, calcium, or magnesium (Grand Island Biological Company, Grand Island, NY). Dilute 1:10 with deionized water.
3. 30% bovine serum albumin.
4. Macrohematocrit tubes.
5. Capillary (Pasteur type) pipettes, both standard and 9-inch lengths.
6. Horizontal head centrifuge. A Beckman TJ6 model (Beckman Instruments, Inc, Palo Alto, CA) was used for this study.

STEPS

1. For each SSF sample, dispense 50-mL diluted balanced salt solution into a 125-mL Erlenmeyer flask. (The amount of balanced salt solution may be varied; 50 mL will make approximately 30 aliquots of SSF.)
2. Centrifuge the blood in the original collection tube at 300 g for 5 min. A gray-pink buffy coat layer should be visible at the interface between the plasma and the RBCs.
3. Aspirate off as much plasma as possible with a capillary pipette. Do not disturb the top (buffy coat) layer. Discard the plasma.
4. With a 9-inch capillary pipette and a circular motion, aspirate off the remaining plasma and the entire buffy coat layer. A small amount of the RBC layer will be aspirated into the pipette at the same time. This is acceptable.
5. Fill a macrohematocrit tube with this buffy coat mixture. Do not mix blood specimens from more than one source in one tube (they may agglutinate).
6. Centrifuge the macrohematocrit tube at 900 g for 10 min.
7. Pipette off as much of the plasma as possible and discard it. If a definite white layer (platelets) is visible above the gray buffy coat, carefully remove as much of it as possible without disturbing the gray layer.
8. Using a clean 9-inch capillary pipette, aspirate off the buffy coat (and as little of the red cell layer as possible) and add it to the flask containing diluted balanced salt solution. Rinse the pipette several times.

PROCEDURE

Simulated Spinal Fluid Procedure (continued)

9. Mix well and check the concentration of RBCs and WBCs by examining the SSF in a hemocytometer.
10. Adjust the concentration of cells as needed; add more balanced salt solution to decrease the number of RBCs and WBCs. The number of RBCs may be increased by adding more cells from the red cell layer. Since the entire buffy coat has been used, increasing the number of WBCs is not possible.
11. Add one drop (approximately 0.05 mL) of 30% bovine serum albumin to each 50 mL of SSF for each 30 mg/dL total protein desired.
12. Mix well and dispense aliquots of approximately 1.5 mL SSF into appropriate tightly stoppered containers.

From Lofsness and Jensen,[22] with permission

more specific for infection of the CNS.[8] The rapid plasma reagin (**RPR**) test is not recommended for use on CSF, because it is less sensitive and specific than the VDRL. To prevent unnecessary testing of the CSF in suspected cases of neurosyphilis, a positive serum test should be obtained using the FTA-ABS. Fluid can be frozen until serum results are available.[2]

Teaching Cerebrospinal Fluid Analysis

Many of the problems that occur in the analysis of CSF are the result of inadequate training of the personnel performing the tests. This is understandable when one considers that not only is CSF difficult to collect, but also there is often very little fluid left for student practice after the required tests have been run. Preparation of simulated fluids by adding blood cells to saline has met with limited success owing to the instability of the cells in saline and the inability to perform routine chemical analyses for glucose and protein. More satisfactory results can be achieved using the simulated spinal fluid procedure presented in this chapter, which provides the teaching laboratory with a specimen suitable for all types of cell analyses and glucose and protein determinations. The advantages of this procedure over others include the absence of bicarbonate, which may cause bubbling with acidic diluting fluids; the absence of calcium, which prevents clot formation when blood is added; stability for 48 hours under refrigeration; no distortion of cellular morphology; and the presence of glucose and protein.[22]

REFERENCES

1. Abrams, J, and Schumacher, HR: Bone marrow in cerebrospinal fluid and possible confusion with malignancy. Arch Pathol Lab Med 110:366–369, 1986.
2. Albright, RE, et al: Issues in cerebrospinal fluid management. Am J Clin Pathol 95(3):397–401, 1991.
3. Bentz, JS: Laboratory investigation of multiple sclerosis. Lab Med 26(6):393–399, 1995.
4. Biou, D, et al: Cerebrospinal fluid protein concentrations in children: Age-related values in patients without disorders of the central nervous system. Clin Chem 46(3):399–403, 2000.
5. Buck, GE: Nonculture methods for detection and identification of microorganisms in clinical specimens. Pediatr Clin North Am 36(1):95–100, 1989.
6. Chow, G, and Schmidley, JW: Lysis of erythrocytes and leukocytes in traumatic lumbar punctures. Arch Neurol 41:1084–1085, 1984.
7. Coovadia, YM, and Soliva, Z: Three latex agglutination tests compared with Gram staining for the detection of bacteria in cerebrospinal fluid. S Afr Med J 71(7):442, 1987.
8. Davis, LE, and Schmitt, JW: Clinical significance of cerebrospinal fluid tests for neurosyphilis. Ann Neurol 25:50–53, 1989.
9. Eng, RHK, and Person, A: Serum cryptococcal antigen determination in the presence of rheumatoid factor. J Clin Microbiol 14: 700–702, 1981.
10. Fishman, RA: Cerebrospinal Fluid in Diseases of the Nervous System, ed. 2. WB Saunders, Philadelphia, 1992.
11. Glasgow, AM, and Dhiensiri, K: Improved assay for spinal fluid glutamine and values for children with Reye's syndrome. Clin Chem 20(6):642–644, 1974.
12. Glasser, L: Tapping the wealth of information in CSF. Diagn Med 4(1):23–33, 1981.
13. Godd, K: Protein estimation in spinal fluid using Coomassie blue reagent. Med Lab Sci 38:61–63, 1981.
14. Grimaldi, LME, et al: Oligoclonal IgG bands in cerebrospinal fluid and serum during asymptomatic human immunodeficiency virus infection. Ann Neurol 24:277–279, 1988.
15. Gupta, MK, et al: Measurement of immunoreactive myelin basic protein in cerebrospinal fluid. Ann Neurol 23:274–276, 1988.
16. Hammock, M, and Milhorat, T: The cerebrospinal fluid: Current concepts of its formation. Ann Clin Lab Sci 6(1):22–28, 1976.
17. Hershey, LA, and Trotter, JL: The use and abuse of the cerebrospinal fluid IgG profile in the adult: A practical evaluation. Ann Neurol 8(4):426–434, 1980.
18. Hourani, BT, Hamlin, EM, and Reynolds, TB: Cerebrospinal fluid glutamine as a measure of hepatic encephalopathy. Arch Intern Med 127:1033–1036, 1971.
19. Kjeldsberg, CR, and Knight, JA: Body Fluids: Laboratory Examination of Amniotic, Cerebrospinal, Seminal, Serous and Synovial Fluids: A Textbook Atlas. ASCP, Chicago, 1993.
20. Knight, FR: New enzyme immunoassay for detecting cryptococcal antigen. J Clin Pathol 45(9):836–837, 1992.
21. Kolmel, HW: Atlas of Cerebrospinal Fluid Cells. Springer-Verlag, New York, 1976.
22. Lofsness, KG, and Jensen, TL: The preparation of simulated spinal fluid for teaching purposes. Am J Med Technol 49(7):493–496, 1983.
23. McComb, JG: Recent research into the nature of cerebrospinal fluid formation and absorption. J Neurosurg 59:369–383, 1983.
24. Menkes, J: The causes of low spinal fluid sugar in bacterial meningitis: Another look Pediatrics 44(1):1–3, 1969.
25. Murray, PR, and Hampton, CM: Recovery of pathogenic bacteria from cerebrospinal fluid. J Clin Microbiol 12:554–557, 1980.
26. Nachum, R, and Neely, M: Clinical diagnostic usefulness of the limulus amebocyte lysate assay. Lab Med 13(2):112–117, 1982.
27. Nagda, KK: Procoagulant activity of cerebrospinal fluid in health and disease. Indian J Med Res 74:107–110, 1981.
28. Novak, RW: Lack of validity of standard corrections for white blood cell counts of blood contaminated cerebrospinal fluid in infants. Am J Clin Pathol 82:95–97, 1984.
29. Roine, RO, et al: Neurological outcome after out-of-hospital cardiac arrest. Predictions by cerebrospinal fluid enzyme analysis. Arch Neurol 46:753–756, 1989.
30. Rouah, E, Rogers, BB, and Buffone, GJ: Transferrin analysis by immunofixation as an aid in the diagnosis of cerebrospinal fluid otorrhea. Arch Pathol Lab Med 111:756–757, 1987.
31. Sato, Y, et al: Rapid diagnosis of cryptococcal meningitis by microscopic examination of centrifuged cerebrospinal fluid sediment. J Neurol Sci 164(1):72–75, 1999.
32. Smith, GP, and Kjeldsberg, CR: Cerebrospinal, synovial, and serous body fluids, ed. 19. In Henry, JB (ed): Clinical Diagnosis and Management by Laboratory Methods. WB Saunders, Philadelphia, 1996.
33. Stockman, L, and Roberts, GD: Specificity of the latex test for cryptococcal antigen: A rapid simple method for eliminating interference. J Clin Microbiol 17(5):945–947, 1983.
34. Tourtellotte, WW: Cerebrospinal fluid in multiple sclerosis. In Vinken, PJ, and Bruyn, GW (eds): Handbook of Clinical Neurology. Elsevier, New York, 1970.
35. University of Virginia Health Sciences Center: Clinical Laboratory Procedure Manual. Charlottesville, VA, 1993.
36. Walter, J: Hematology and the analysis of body fluids. Advance for Medical Laboratory Professional 4:10–19, 1996.
37. Werner, V, and Kruger, RL: Value of the bacterial antigen test in the absence of CSF fluid leukocytosis. Lab Med 22(11):787–789, 1991.
38. Whitaker, JN: Myelin basic protein in cerebrospinal fluid and other body fluids. Mult Scler 4(1):16–21, 1998.

STUDY QUESTIONS

1. List three functions of the CSF.
2. State the function of the following structures with regard to the CSF: choroid plexuses, subarachnoid space, and arachnoid villi.
3. State a possible discrepancy associated with the following actions:
   a. CSF tube 1 is sent to the microbiology laboratory.
   b. CSF tube 2 is sent to the hematology laboratory.
   c. CSF tube 1 remains at room temperature for 4 hours.
   d. CSF tube 3 remains at room temperature for 4 hours.
4. How does pink xanthochromia differ clinically from yellow xanthochromia?
5. Indicate whether each of the following statements represents a hemorrhage or a traumatic tap by placing an "H" or a "T" in the blank.
   _____ Clot formation
   _____ Erythrophagocytosis
   _____ Positive D-dimer test
   _____ Clear supernatant
   _____ Even distribution of blood
6. What is the clinical significance of a weblike pellicle in the CSF?
7. Should a cell count be performed on a clear CSF? Why or why not?
8. Given the following information, calculate the total CSF cell count.
   Cells counted = 50
   Dilution = 1:10
   Large Neubauer squares counted = 10
9. What diluting fluid is used for a total CSF cell count? A CSF WBC count?
10. What is the purpose of the WBC and protein correction calculation?

11. How must the CSF be prepared prior to performing a cell differential count?

12. What is the purpose of adding albumin to the cytocentrifuge preparation?

13. Name the primary clinical concern when a pleocytosis of neutrophils or lymphocytes is present.

14. Name the type of cell(s) that predominate in each of the following conditions: multiple sclerosis, following pneumoencephalography, malfunctioning shunts, AIDS, and cerebral hemorrhage.

15. What is the significance of nucleated RBCs in the CSF?

16. How do clusters of choroid plexus and malignant cells differ in appearance?

17. Name four sources of malignant cells found in CSF.

18. Why does the chemical composition of the CSF differ from that of plasma?

19. True or False? The normal range of CSF protein is 15 to 45 g/dL.

20. What is the primary difference in the concentration of protein fractions between CSF and plasma?

21. Name four causes of elevated CSF protein levels.

22. Why is trichloroacetic acid recommended over SSA in turbidometric methods measuring CSF protein? Coomassie brilliant blue in dye-binding methods?

23. How does the clinical significance of the CSF/serum albumin index differ from that of the IgG index?

24. Why must electrophoresis to detect oligoclonal banding be performed on both CSF and serum? In the diagnosis of what disorder is this most important?

25. How can an unidentified fluid be determined to be CSF?

26. What is the significance of MBP in the CSF?

27. Why are serum and CSF samples for glucose analyzed simultaneously?

28. What is the cause of decreased CSF glucose levels in patients with bacterial meningitis?

29. Explain the relationship of increased blood and CSF ammonia to increased levels of CSF glutamine.

30. Prior to performing Gram staining and culture of CSF, what must be done to the fluid?

31. State a cause of a false-negative CSF Gram stain report and a cause of a false-positive report.

32. Why is the laboratory currently receiving increased requests for India Ink preparations?

33. Will the limulus lysate test detect the presence of *S. pneumoniae* or *E. coli*?

34. What is the most probable cause of a false-positive Cryptococcal antigen test?

35. What is the significance of a positive serum FTA-ABS with a negative CSF VDRL test? With a positive CSF VDRL test?

CASE STUDIES AND CLINICAL SITUATIONS

1. Three tubes of CSF containing evenly distributed visible blood are drawn from a 75-year-old disoriented patient and delivered to the laboratory. Initial test results are as follows:

WBC COUNT: 250 µL PROTEIN: 150 mg/dL
GLUCOSE: 70 mg/dL GRAM STAIN: No organisms seen
DIFFERENTIAL: Neutrophils, 68%; monocytes, 3%; lymphocytes, 28%; eosinophils, 1%
Many macrophages containing ingested RBCs

a. What is the most probable condition indicated by these results? State two reasons for your answer.
b. Are the elevated WBC count and protein of significance? Explain your answer.
c. Are the percentages of the cells in the differential of any significance? Explain your answer.
d. If this patient had recently experienced a cardiac arrest, what additional test performed on the CSF might be of value?
e. If the blood was unevenly distributed and nucleated RBCs and capillary structures were seen instead of macrophages, what would this indicate?
f. Combining the patient's condition and the information in "e," what additional chemical test might be requested?

2. A patient with AIDS is hospitalized with symptoms of high fever and rigidity of the neck. Routine laboratory tests on the CSF show a WBC count of 100/µL with a predominance of lymphocytes and monocytes, glucose of 55 mg/dL (plasma: 85 mg/dL), and a protein of 70 mg/dL. The Gram stain shows a questionable starburst pattern.

a. What additional microscopic examination should be performed?
b. If the test is positive, what is the patient's diagnosis?
c. If the results of the test are questionable, what additional testing can be performed?
d. What could cause a false positive reaction in this test?
e. If the tests named in "a" and "c" are negative, the glucose level is 35 mg/dL and a pellicle is observed in the fluid, what additional testing should be performed?
f. If CSF and serum IFE was performed on this patient, what unusual findings might be present?

3. A 35-year-old woman is admitted to the hospital with symptoms of intermittent blurred vision, weakness, and loss of sensation in her legs. A lumbar puncture is performed with the following results:

APPEARANCE: Colorless, clear
WBC COUNT: 35 cells/µL (90% lymphocytes)
GLUCOSE: 60 mg/dL (plasma: 100 mg/dL)

PROTEIN: 50 mg/dL (serum: 7 g/dL)
ALBUMIN: 30 mg/dL (serum: 5 g/dL)
IgG GLOBULIN: 15 mg/dL (serum: 2 g/dL)

a. Name and perform the calculation to determine the integrity of the patient's blood-brain barrier.
b. Does the patient have an intact barrier?
c. Name and perform the calculation used to determine if IgG is being synthesized within the CNS.
d. What does this result indicate?
e. Considering the patient's clinical symptoms and the calculation results, what diagnosis is suggested?
f. If immunofixation electrophoresis is performed on the patient's serum and CSF, what findings would be expected?
g. What substance in the CSF can be measured to monitor this patient?

4. Mary Howard, age 5, is admitted to the pediatrics ward with a temperature of 105°F, lethargy, and cervical rigidity. A lumbar spinal tap is performed, and three tubes of cloudy CSF are delivered to the laboratory. Preliminary tests results are as follows:

APPEARANCE: White and cloudy
WBC COUNT: 6,000 cells/μL
DIFFERENTIAL: 90% neutrophils, 10% lymphocytes
PROTEIN: 150 mg/dL
GLUCOSE: 10 mg/dL
GRAM STAIN: No organisms seen

a. From these results, what preliminary diagnosis could the physician consider?
b. Is the Gram stain result of particular significance?
c. What additional rapid test could be performed to supplement the Gram stain?
d. Would a CSF lactate test be of any value for the diagnosis? Why or why not?

5. State possible technical errors that could result in the following discrepancies:

a. An unusual number of Gram stains reported as gram-positive cocci fail to be confirmed by positive cultures.
b. A physician complains that CSF differentials are being reported only as polynuclear and mononuclear cells.
c. Bacteria observed on the cytospin differential cannot be confirmed by Gram stain or culture.
d. The majority of CSF specimens sent to the laboratory from the neurology clinic have glucose readings less than 50 percent of the corresponding blood glucose results performed in the clinic.