Anatomy and Formation

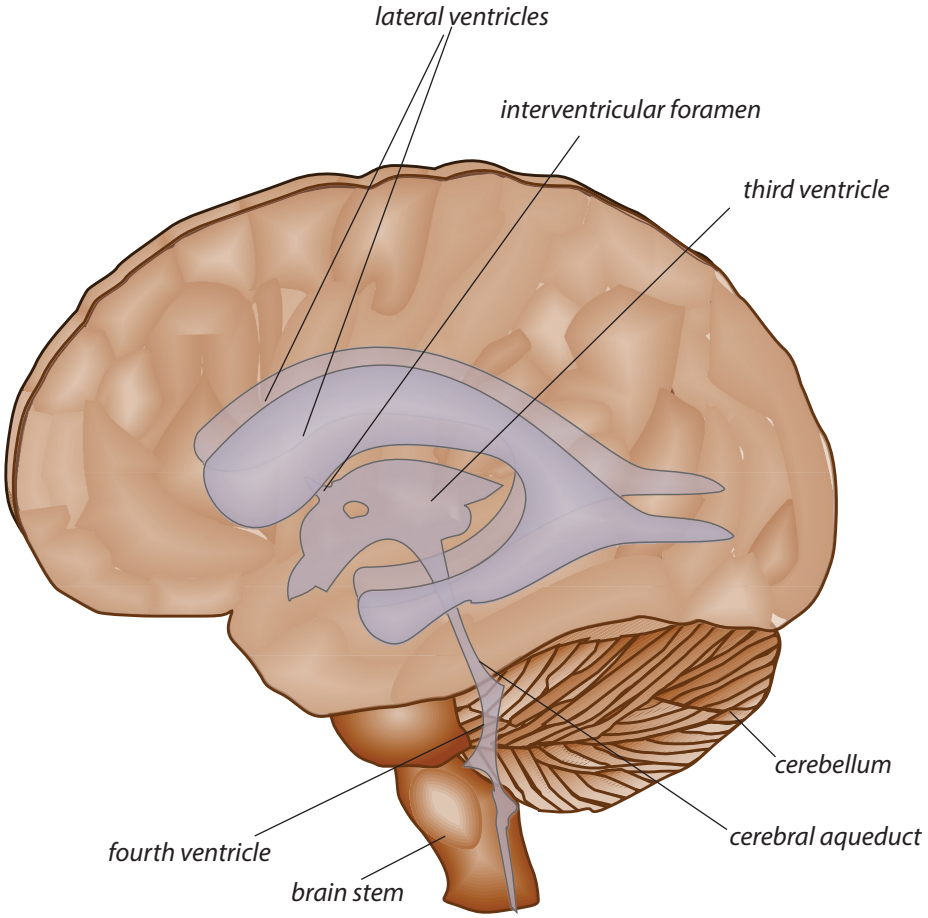
The cerebrospinal fluid (CSF) is an ultrafiltrate of plasma that protects the nervous system from abrupt changes in pressure, provides a homeostatic chemical environment, and is a vehicle for the exchange of nutrients and waste. The CSF also acts as a buffer between peripheral blood and the central nervous system (CNS), regulating the passage of substances in plasma into the brain. This regulation is accomplished via two mechanisms: 1) the blood brain barrier (BBB), and 2) the blood CSF barrier (BCB).

The brain and spinal cord are protected by three meninges, or meningeal membranes: the dura mater, arachnoid mater (or arachnoid membrane), and pia mater. CSF is located within the subarachnoid space between the arachnoid membrane and pia mater, and within the cerebral ventricles. The subarachnoid space forms sleeve-like extensions around the cranial and spinal nerves and terminates where the pia mater and the arachnoid mater fuse with the perineurium of these nerves.

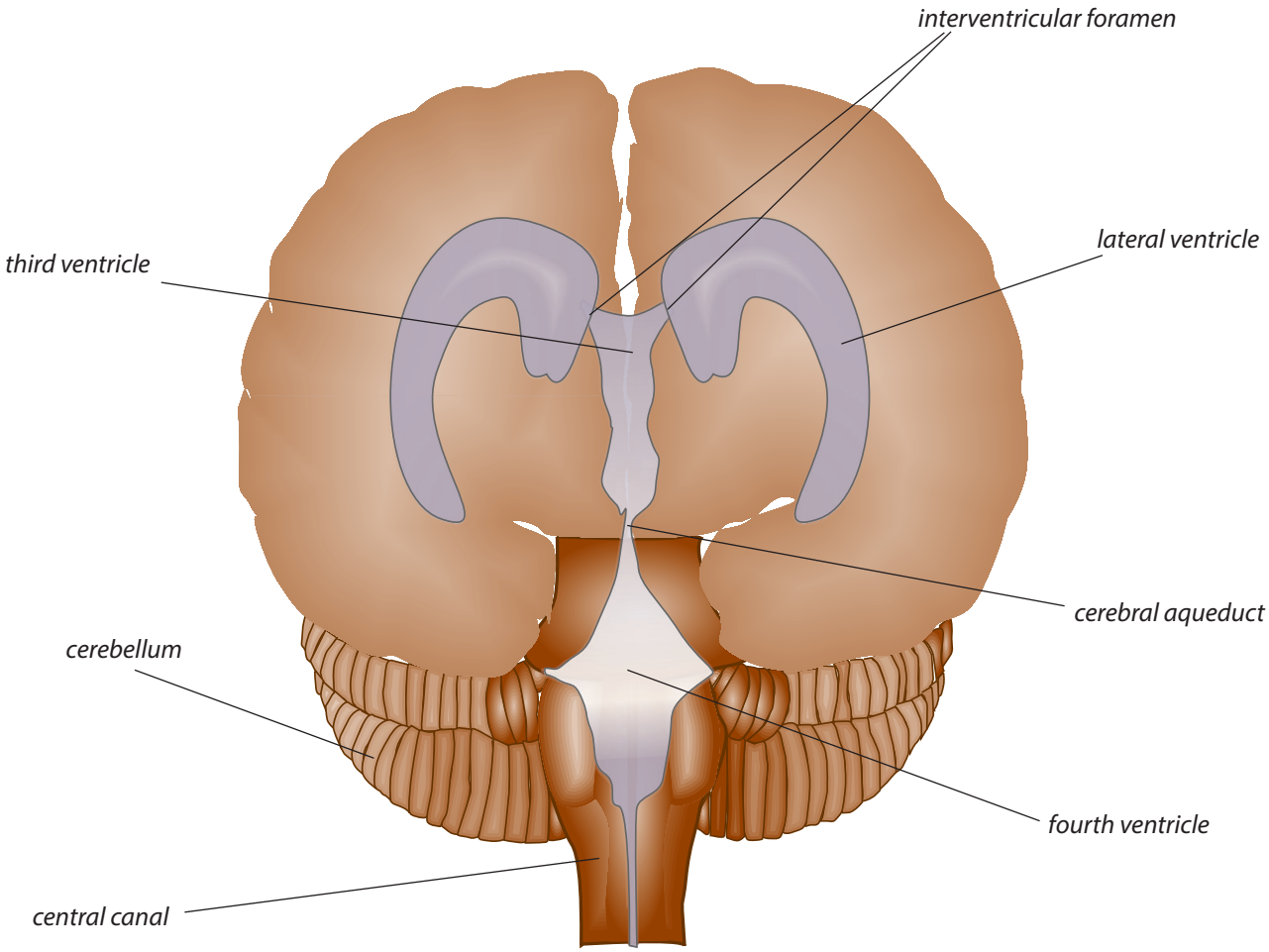
The choroid plexus is a network of capillaries in the pia mater that are lined by specialized epithelial cells (ependymal cells, or ependyma) that primarily function to produce and secrete CSF. Approximately 500 mL of CSF is produced daily in healthy adults and the total volume of CSF is replaced every 5-7 hours. CSF is secreted into the lateral and fourth ventricles of the brain where it circulates around the cerebral hemispheres and spinal cord (see illustration, page 66) and is eventually reabsorbed into the dural venous sinuses via the arachnoid membrane. The arachnoid membrane forms variable finger-like projections—villi (small) and granulations (large)—that penetrate dural sinuses and other venous structures in the brain.

The choroid plexus epithelium and the arachnoid mater form the anatomic separation between the blood and the CSF, or BCB (see illustration, page 53). The BCB facilitates exchange and removal of small constituents of plasma, such as glucose and urea, and prevents the passage of larger molecules, such as proteins and some medications. The BBB functions similarly to the BCB by regulating sub-

Ventricular System



Ventricular System

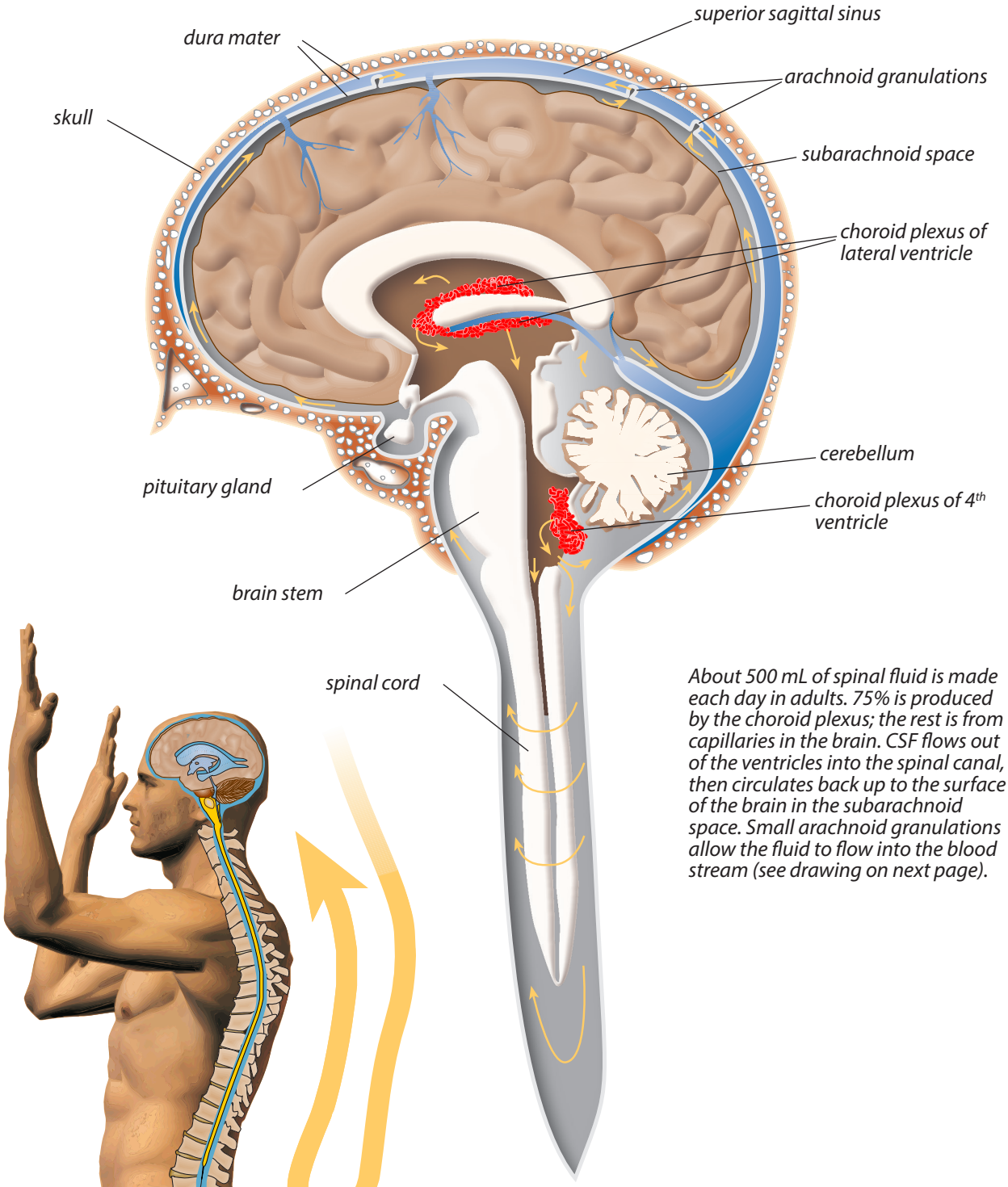


stances that enter and exit the brain; however, it is formed by capillary endothelial cells within the brain tissue. Breakdown of these physical barriers occurs in disease states (e.g., meningitis, CNS tumors, and infarcts) in which constituents of the peripheral blood can leak into the CSF and be detected visually and/or measured chemically (see *Table 3,* page 58 and *Table 4,* page 59).

CSF is typically procured via lumbar puncture, or LP (see *CSF Collection and Preparation*, page 54). An LP is clinically indicated in assessment for CNS infection, hemorrhage, malignancy (primary CNS or metastatic) and demyelinating processes. Therapeutic LP may be performed to relieve symptoms of benign intracranial hypertension or acute communicating hydrocephalus, or to deliver intrathecal antibiotics or antineoplastic drugs.

Normal CSF maintains only basal immune surveillance with few cellular and humoral immune system components (low cellularity, low protein and glucose concentrations, no red cells, etc). Biochemical testing and microscopic evaluation is performed to detect alteration in these parameters and aid in the diagnosis of CNS infection, intracranial hemorrhage, malignancy (primary or metastatic) or demyelinating diseases.

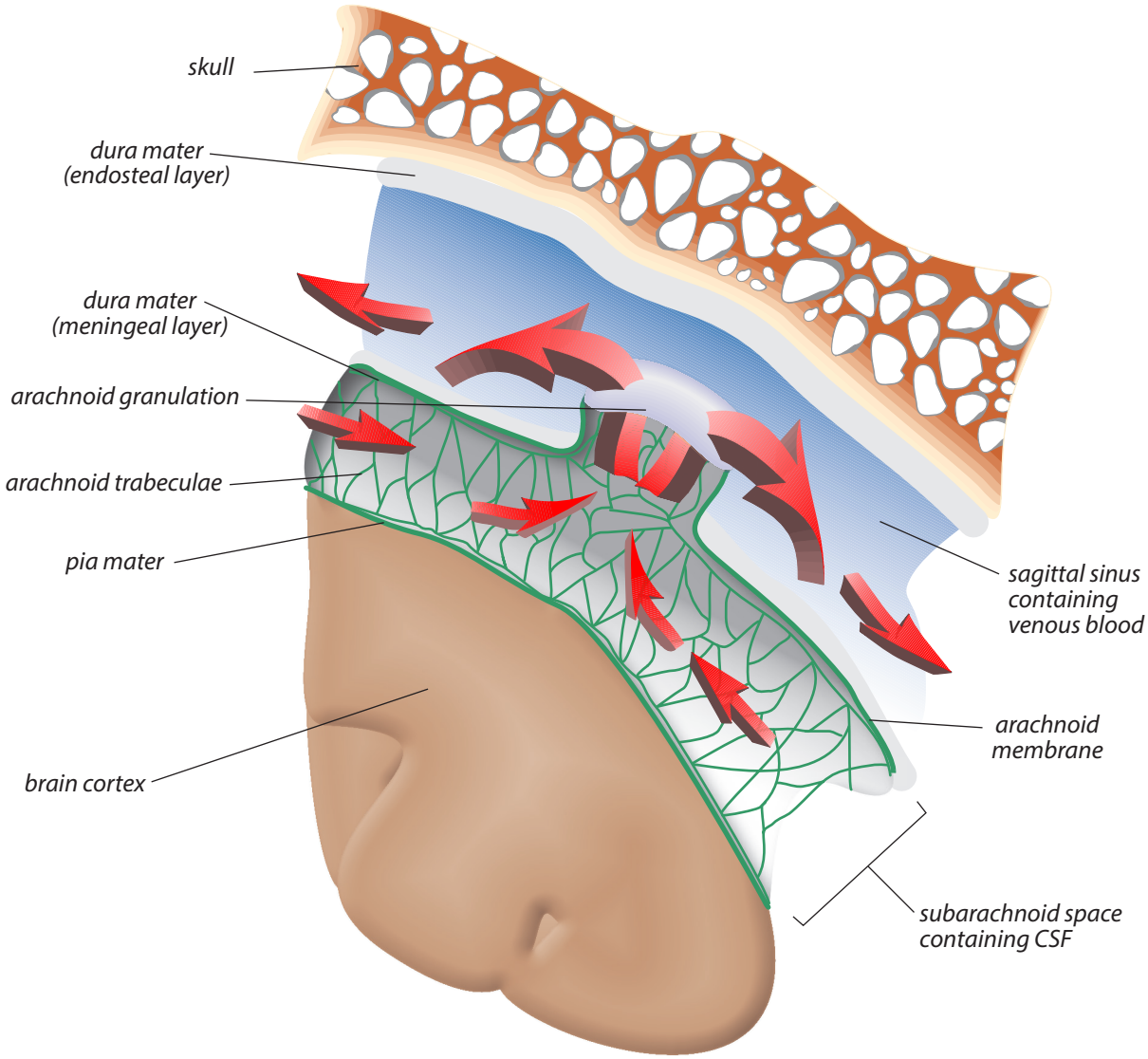
CSF Flow



About 500 mL of spinal fluid is made each day in adults. 75% is produced by the choroid plexus; the rest is from capillaries in the brain. CSF flows out of the ventricles into the spinal canal, then circulates back up to the surface of the brain in the subarachnoid space. Small arachnoid granulations allow the fluid to flow into the blood stream (see drawing on next page).

CSF, after leaving the ventricles, circulates over the brain and down and up the spinal canal, eventually exiting into the venous blood stream.

Arachnoid Granulations



The arachnoid villi and granulations (Pacchionian bodies) are finger-like projections of the arachnoid membrane that penetrate the dura mater and protrude into the lumen of the subdural sinuses and other veins. The villi are small microscopic projections and the granulations are large (up to 1.0 cm in diameter). They act as one-way valves, allowing the CSF to be reabsorbed into the venous blood stream.

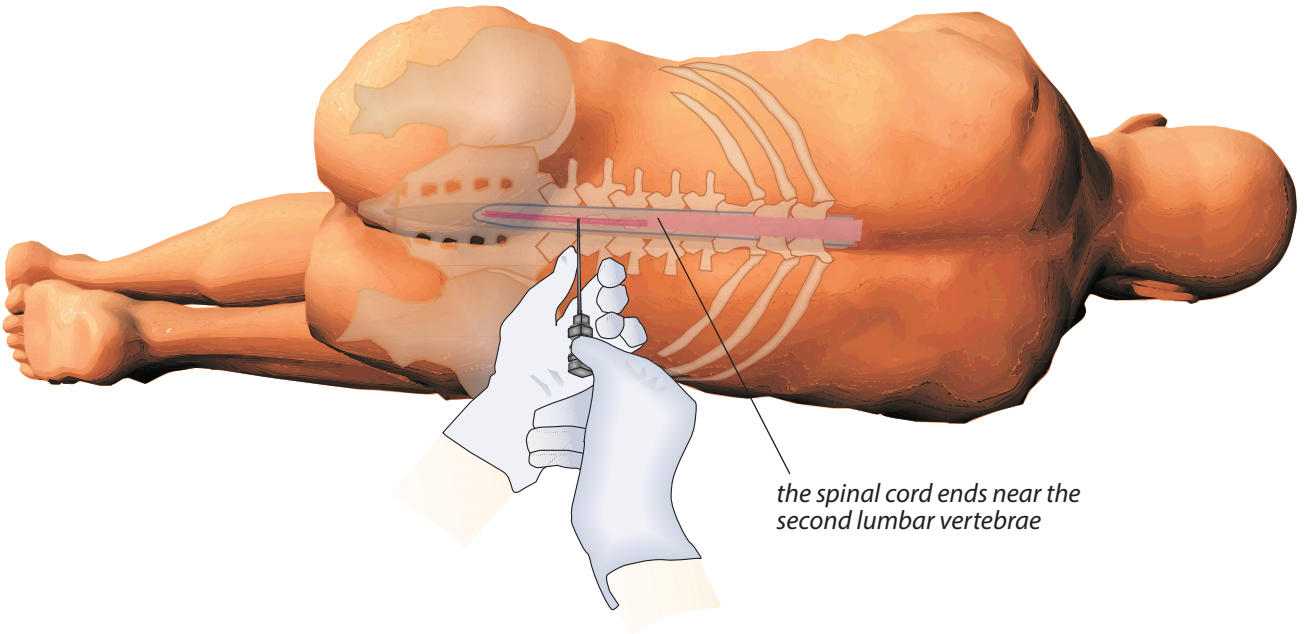
Collection and Preparation

CSF is obtained by inserting a needle into the subarachnoid space between lumbar vertebrae, a procedure referred to as a lumbar puncture (LP) or more colloquially as a "spinal tap". Patients are placed in the fetal, or lateral recumbent, position (see illustration below) with neck and knees flexed to maximize the opening between adjacent vertebral bodies. A sitting position with the neck flexed may also be employed in some circumstances. The lateral recumbent position is preferred, however, as this allows for more accurate evaluation of the opening pressure and decreases the risk of post-LP headache.

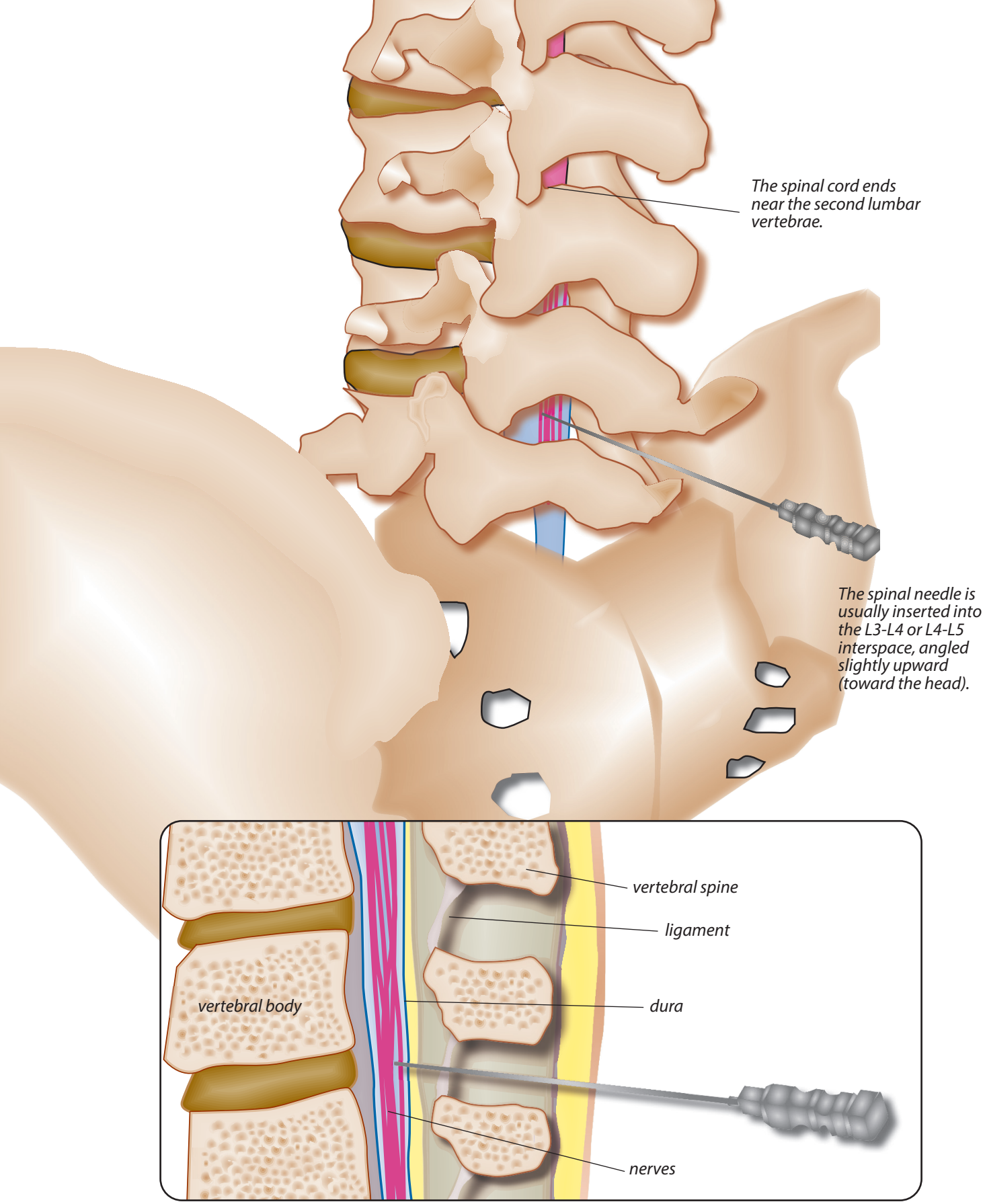
The L3-L4 or L4-L5 interspaces are used to gain access to the spinal subarachnoid space in order to avoid the conus medullaris (termination of the spinal cord) which typically occurs in the L2 region or higher in almost all individuals. Nerve fibers below the conus medullaris, termed the cauda equina, move freely within the subarachnoid space and are not damaged during an LP. The midpoint of a line visualized between the right and left superior iliac crests represents the L3-L4 intervertebral space and is typically used as the anatomical landmark for the LP. This interspace (or the one below between L4 and L5 vertebral bodies) is palpated and, using sterile technique and local anesthetic, a 20- to 22-gauge spinal needle with stylet is slowly inserted into the subarachnoid space between the two vertebral bodies (see illustration, facing page). Entry into the subarachnoid space is usually confirmed by a tactile "popping" sensation as the ligamentum flavum (the ligament connecting laminae of adjacent vertebrae) is penetrated. CSF should drain freely and should never be actively aspirated. If CSF does not flow passively, the needle can be gently rotated in case it is impinging upon nerves or other structures. Care should be taken not to advance the needle completely across the spinal canal as this could result in injury to the vascular structures located along the posterior surface of the vertebral bodies and result in a traumatic

(continued page 56)

Lumbar Puncture Technique



Lumbar puncture is performed by inserting a 20 gauge (or 22 gauge for children) spinal needle with a stylet between the third and fourth or fourth and fifth lumbar vertebrae (L3-L4 or L4-L5). This location is used because the spinal cord stops near L2 (or above), and a needle introduced below this level will miss the cord.

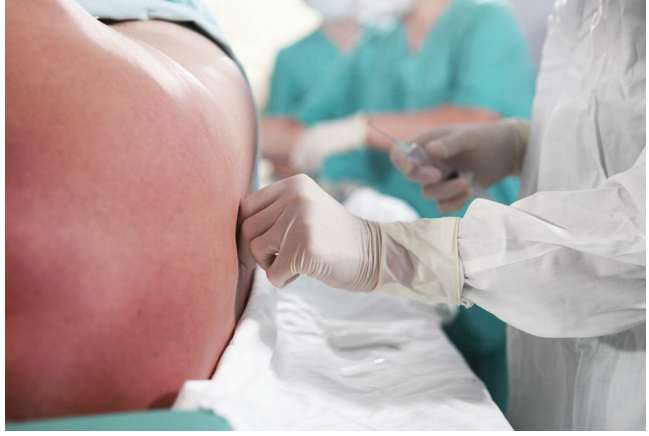


The needle is advanced between the third and fourth or fourth and fifth lumbar vertebrae. Only nerves/nerve roots are present here since the cord (conus medullaris) terminates at or above the second lumbar vertebra. The individual nerve roots below the cord (cauda equina) float away from the advancing needle and are not damaged during the LP. Piercing the dorsal or epidural veins on the posterior surface of the vertebral bodies may lead to a traumatic tap.

tap (see *A Closer Look At ... Traumatic Tap vs. Subarachnoid Hemorrhage*, page 66).

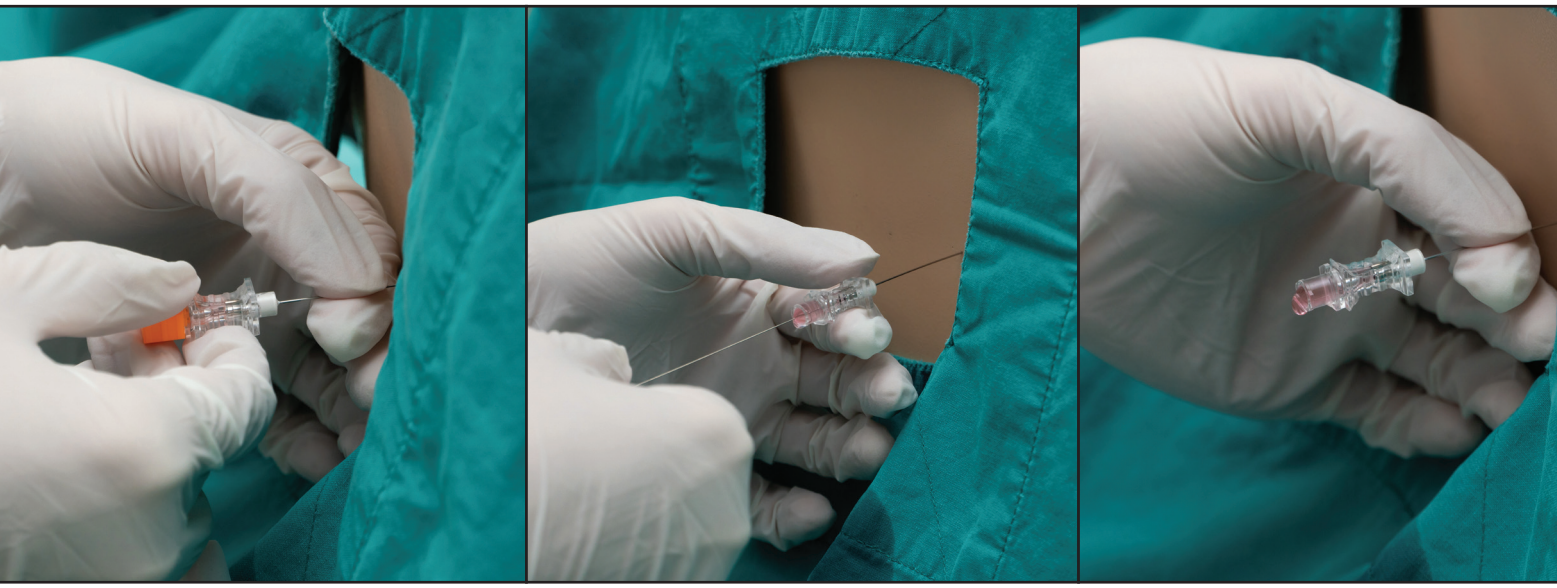
Once fluid appears, a three-way stopcock and manometer are attached to the needle hub and the opening pressure is measured. Pressures vary depending on the age and body-mass index, BMI (see *Table 1,* page 57). Opening pressures greater than 250 mm H_2O are consistent with intracranial hypertension that may be seen in various disease states (e.g., meningitis, CNS hemorrhage, tumors), obesity, patients who are tense or straining during the LP, congestive heart failure, superior vena cava syndrome, or other conditions that potentially prevent adequate CSF absorption (e.g., certain congenital malformations).

Complications of LP are relatively uncommon and often minor (e.g., headache, back pain) when precautions are followed and proper technique employed. Epidural or subdural hematomas are extremely rare, but potentially serious complications that may occur in patients with coagulopathy, if receiving anticoagulant medications, or with significant thrombocytopenia (platelet count <20,000). Although correction of the coagulation abnormalities is recommended prior to performing a LP, the benefit of CSF evaluation may outweigh the potential bleeding risk in some clinical scenarios. A space-occupying lesion or abnormal intracranial pressure risks potentially fatal uncal, trans-tentorial, or cerebellar herniation during an LP, which is contraindicated in these situations. An increase in intracranial pressure by itself is not a contraindication for LP as these are expected in some scenarios such as in obese patients and patients with CNS infection. LP should also be avoided in patients with localized skin or soft tissue infection (e.g., paraspinal abscess) overlying the LP site given the risk of intrathecal dissemination of microorganisms.



Patient positioned and prepped for lumbar puncture.

CSF may be alternatively collected from a ventricular access device (e.g. drain, shunt, reservoir). Ventriculoperitoneal (VP) shunts (the most common ventricular access device) are small plastic tubes or catheters used to treat hydrocephalus and divert excess CSF from the ventricles of the brain to another site (e.g., chest or abdominal cavity). A VP shunt (or other ventricular access device) is accessed by inserting an LP needle at the apex of the shunt reservoir which sits under the skin near the occipital region. Direct ventricular puncture or tapping can also be performed in patients with rapid-onset hydrocephalus who do not have a ventricular access device or in patients with possible or imminent brain herniation. In infants and very young children where the fontanelle is still open, a needle is passed through this into the subdural space. If the fontanelle is closed, this becomes a neurosurgical procedure whereby a hole is drilled in the skull. The fluid obtained in these procedures is subdural (not cerebrospinal) fluid and is usually withdrawn for therapeutic purposes to relieve symptoms of a subdural hematoma or post-infectious subdural effusion, as opposed to diagnostic purposes; thus laboratory testing may not be indicated (see *Laboratory Evaluation and Testing* on the following pages).



Lumbar puncture yields slightly blood-tinged fluid.

Laboratory Evaluation and Testing

Laboratory evaluation of CSF is critical for the prompt diagnosis and management of many CNS diseases. Together with opening pressure and physical examination (see sections on *Interpretation: Macroscopic Findings*, page 64 and *Interpretation: Microscopic Findings*, page 72), assessment of chemical and cytologic composition, presence of certain biomarkers, and microbiologic study results can provide valuable information about the state of the CNS and potential disease process.

CSF is typically collected in 3-4 sterile tubes without additives by the clinical provider performing the procedure and are labeled sequentially according to the order of collection. The proceduralist is responsible for recording the opening pressure and total volume of CSF procured. Similar volumes should be attempted to be obtained for each tube and may range from 1 mL up to around 5 mL depending on the patient's age. Up to 20 mL of CSF can be safely removed from most adults, and infants can usually tolerate removal of up to 8 mL.

The sample should be processed within 1 hour of collection since delays can lead to a myriad of spurious results (e.g., cellular degeneration, increased glucose, etc.). Prompt delivery to and evaluation by the laboratory of the CSF sample is paramount. Upon receipt, the tubes are immediately sent to the appropriate section of the laboratory for specific testing. Centrifugation is performed initially as it helps to remove cells (e.g., RBCs) and debris that have the potential to obscure accurate specimen analysis. Refrigeration or freezing of samples may be necessary if immediate evaluation cannot be performed. Samples intended for microbial studies, however, should never be refrigerated as this can select for the growth of certain organisms.

The first tube is often preferred for chemical (e.g., protein, glucose, etc.) and serologic studies. Subsequent tubes are typically reserved for microbial studies (tube 2) and cell counts (tubes 3 or 4) in order to minimize the chance of bacterial contamination and obtain accurate cell counts,

Table 1: Reference Values, Normal CSF

Parameter	Adult	Neonate	Child
Total Volume (mL)	90-150	10-60	60-100
Appearance	Clear, colorless	Clear, colorless	Clear, colorless
Opening pressure ($mm\ H_2O$)	90-180 (BMI <30) <250 (BMI >30)	10-100	10-150

Adapted from Henry's, chapter 30.

Table 2: Recommended CSF Division and Use

Collection Tubes	Tests Performed	Volume collected (adult)	Volume collected (child)
Tube #1	Chemistry (glucose, protein) Serology Cell count and differential (if traumatic tap)	3-5 mL	1 mL
Tube #2	Microbiology (culture, Gram stain) Serology, Chemistry	3-5 mL	1 mL
Tube #3	Cell count and differential Chemistry (if traumatic tap)	3-5 mL	1 mL
Tube #4	Morphology Flow cytometry Cell count & differential	3-5 mL	1 mL

Adapted from CLSI; Tietz, chapter 45.

respectively (see *Table 2,* page 57). Alterations in these recommendations may be necessary depending on the clinical indication and how the sample is obtained (e.g., if there is evidence of a traumatic tap). Sample division may also differ depending on the performing laboratory.

Common analytes that are routinely evaluated in CSF include glucose, total protein, albumin, IgG, lactate, lactate dehydrogenase (LDH), and glutamine. These analytes are measured as a concentration (an amount of the substance per volume of CSF); however, because many of these analytes are derived from the plasma and not produced or secreted by the CSF under normal conditions, a blood sample is often collected and evaluated contemporaneously with the CSF specimen to provide a CSF to serum ratio.

A normal CSF glucose level is around 60-80% of serum glucose. Microbial organisms, particularly bacteria, require glucose for growth and propagation. Thus, in CNS infection, the glucose levels are often decreased (bacterial meningitis >> viral meningitis). A CSF:serum of less than 0.4 (or CSF glucose concentration of <18 mg/dL) has a high sensitivity and specificity for distinguishing bacterial meningitis from aseptic or viral meningitis.

Proteins are relatively large molecules that typically do not readily cross the BCB and should be present only at very low levels in normal CSF. CSF total protein concentrations are usually higher in neonates and decrease gradually during the first year of life. Older adults also demonstrate increased CSF total protein concentrations. Albumin and IgG are the most prevalent proteins in CSF, and those that are normally present in CSF are derived from the serum. The combined measurement of albumin and IgG is used to evaluate the integrity and permeability of the BCB. Increased permeability of the BCB or disruption to the usual CSF circulation (e.g., CNS hemorrhage, inflammation, hypoxia) can cause an increase in total or individual CSF protein concentration.

Lactate (or lactic acid) is abnormally produced in the CNS with tissue destruction and/or anaerobic metabolism and is independent of blood lactate. Elevated CSF lactic acid levels occur in conditions that decrease the flow of oxygen to brain tissues secondary to hypoxia, ischemia,

Table 3: Biochemical Studies—Normal Values

Tests	Adult	Neonate[†]
Glucose (mg/dL)	40-70 (fasting)	60-80
Total Protein (mg/dL)[*]	15-45 (<60 years of age) 15-60 (≥60 years of age)	15-100 (term) 115-170 (preterm)
IgG (mg/dL)	<4.0	
Albumin (mg/dL)	10-35	
Lactate (mg/dL)[*]	10-24	10-60
LDH (U/L)	<30 (10% of serum)	<70
Glutamine (mg/dL)	5-20	

Adapted from Henry's, chapter 30. Tietz, chapter 45.

[*] Normal ranges may differ depending on instrument calibration

[†] Beyond the neonatal stage, children variably reach adult levels at around 6-10 years of age

seizures, hemorrhage or infection. CSF lactate levels are also frequently used to assess injury severity and progression and/or recovery in traumatic brain injuries. Lactate dehydrogenase (LDH) is another product of anaerobic metabolism and produced in the CSF as a result of cell death. Elevated LDH levels are seen in similar CNS disorders as elevated lactate levels (see *Table 4*, below).

Evaluation of other analytes may be requested in certain clinical situations. Spectrophotometric identification of bilirubin, oxyhemoglobin and methemoglobin is used in the setting of possible subarachnoid hemorrhage. Evaluation of neurodegenerative diseases and dementia often requires β-amyloid and tau proteins (Alzheimer's disease) and neuroglial autoantibodies (e.g., MBP, NMDAR, LGI-1, CASPR, MOG) in autoimmune encephalitis or potential paraneoplastic syndromes (see *A Closer Look At…Multiple Sclerosis,* page 61). Testing for specific tumor markers, usually in the setting of known prior malignancy, may suggest metastatic disease to the CNS (see *Table 5,* page 60).

If CNS infection is suspected, select tests may be performed to detect and identify microbes. Viral meningitis is the most common type of CNS infection. However, it is important to distinguish between viral and bacterial etiologies as the treatment, course, and outcomes differ significantly.

One of the quickest and easiest tests for detecting bacteria in the CSF is performing a Gram stain. A glass slide is prepared from the CSF sample and examined under the microscope for bacterial forms. Fungal elements may also be highlighted with Gram stain. CSF culture is a slower process used to detect causative organisms and provide antibiotic sensitivities in bacterial or fungal infections. A

Table 4: Disorders with Abnormal Biochemical Results in CSF

Tests	Elevated	Decreased
Glucose	Coma Systemic (diabetic) hyperglycemia Epidemic encephalitis	CNS tumor* Meningitis (bacterial, fungal, TB) Systemic hypoglycemia CNS hemorrhage
Total protein	CVA CNS hemorrhage CNS mass (abscess, tumor) Meningitis (bacterial, fungal, viral, TB) Coma (traumatic)	CSF leak (trauma) Large volume removal of CSF Intracranial hypertension Hyperthyroidism Children 6 months to 2 years
IgG/Albumin ratio	Multiple sclerosis Chronic inflammatory diseases	
Lactate (lactic acid)	Meningitis (bacterial) CNS tumor Cerebral ischemia, CVA Cerebral trauma Seizures Increased intracranial pressure	
LDH	Meningitis (bacterial) CNS tumor Cerebral ischemia, CVA Cerebral trauma Seizures Increased intracranial pressure	Hypoglycemia, hyponatremia, dehydration Hepatic or uremic encephalopathy

CNS tumor: may be primary or metastatic.
CVA = cerebral vascular accident (stroke); TB = tuberculosis

negative Gram stain or culture does not rule out an infection because the microbes may be present in small numbers or unable to grow in culture due to prior antibiotic therapy. Molecular polymerase chain reaction (PCR) testing can detect bacterial, viral, fungal or parasitic genetic material (DNA, RNA) and is particularly helpful if the microbe does not grow in routine culture or if the patient has been on antibiotics. Unlike bacterial and fungal infection, PCR is the only method that can reliably detect viruses. Less common infectious agents may require specific testing for antibodies (e.g., West Nile virus, Lyme disease, etc.) or proteins or antigens released by the microbes (e.g., *Cryptococcus*, *Histoplasma*, VDRL [see *A Closer Look At Neurosyphilis,* page 426]). These specialized tests are usually ordered only if there is a high index of clinical suspicion.

Table 5: CSF Biomarkers

Tests	Normal value	Suggested disease state if elevated	
Antibody index	<0.4	Chronic inflammatory process (>1.5)	
Alpha-fetoprotein (AFP)	<1.5 mg/mL	CNS dysgerminomas and meningeal carcinomas	
Human chorionic gonadotropin (hCG)	<0.21 U/L	CNS dysgerminomas and meningeal carcinomatosis	
β-Glucuronidase	<49 mU/L	47-70 mU/L >70 mU/L	Adenocarcinoma AML
Carcinoembryonic antigen (CEA)	<0.6 ng/mL	Metastatic adenocarcinoma	
Lysozyme (muramidase)	4-13 µg/mL	CNS tumors; AMML	

AML = acute myeloid leukemia; AMML = acute myelomonocytic leukemia
Normal values vary greatly depending on the reference laboratory.

Table 6: CSF Findings in Viral vs. Bacterial Meningitis

Tests	Viral	Bacterial
Opening pressure	normal	>300 mmH_2O
Color/clarity	normal	Cloudy / purulent
WBC count	<250 /µL Lymphocyte predominant	>2000 / µL Neutrophils >1,200 /µL
Protein	Normal-to-mild increase	>220 mg/dL
Glucose	Normal-to-mild decrease	<40 mg/dL
Lactate	20-40 mg/dL	>40 mg /dL

Adapted from: Shahan, et al.

A Closer Look At…

Multiple Sclerosis and Oligoclonal Bands

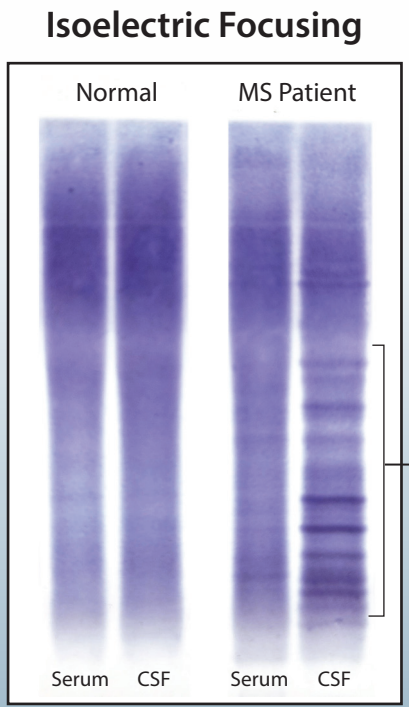
Multiple sclerosis (MS) is included in a group of demyelinating CNS disorders in which the injurious inflammatory component of the disease is believed to be autoimmune-driven. MS is typically characterized by a relapsing/remitting course with inevitable progression, albeit often protracted. The initial phase of MS can manifest with non-specific features of CNS dysfunction: numbness in the limbs or face (typically unilateral), vision loss, weakness, vertigo, balance problems, ataxia and pain, among others are common symptoms that can occur singly or with several at once. Diagnosis is usually made in young adults who experience episodes (or "attacks") with at least partial (sometimes full) resolution of symptoms. Imaging shows widespread confluent and plaque-like demyelination and oligodendrocyte destruction.

Patients with suspected MS usually undergo an LP to evaluate the biochemical composition of the CSF. Active tissue injury in MS is always associated with CNS inflammation manifested by lymphocyte pleocytosis, and elevated total protein and IgG. Inflammatory infiltrates are dominated by T lymphocytes and macrophages, which impair the integrity of the BCB. When this happens, B-cells can enter the CSF and secrete immunoglobulin (Ig) into the normally immune-poor CSF.

Mature B-cells (or B lymphocytes, to include the terminally differentiated plasma cells) are not normally found in the CSF or are present in extremely low numbers. In the periphery, B-cells produce and secrete Ig to aid with the body's cellular immune functions and protect against infections. Abnormal intrathecal production of Ig in MS is confirmed by protein electrophoresis where the abnormal Igs migrate as discrete bands, so-called oligoclonal bands. Oligoclonal bands are two or more bands in the CSF that fix as IgG, and are not present in the contemporaneous serum sample. Although highly sensitive (85-95%), oligoclonal bands are not specific for MS. Other immune system-based CNS disorders, including human immunodeficiency virus (HIV), neurosyphilis, subacute sclerosing panencephalitis, among other etiologies, will also show oligoclonal bands in the CSF and should be excluded by additional testing.

The inflammation in MS leads to the presence of oligoclonal bands (OCBs) in the CSF. OCBs are a result of the clonal expansion of B-cells within the CNS.

These bands represent immunoglobulins produced by these B-cells and can be detected through CSF protein electrophoresis. The presence of OCBs is a hallmark of intrathecal immunoglobulin synthesis, indicating an ongoing immune response within the CNS. Although characteristic of MS, OCBs are not specific for this diagnosis.



Bands indicate synthesis of immunoglobulin. In the MS patient, there are multiple additional bands (OCBs) due to intrathecal synthesis.