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CE

Article #1 (1.5 contact hours)

Refereed Peer Review

Collecting, Processing, and Preparing Cerebrospinal Fluid in Dogs and Cats

KEY FACTS

- Ketamine and acepromazine are not recommended in the anesthetic protocol of patients with suspected intracranial disease.
- Herniation of the cerebellum through the foramen magnum may lead to apnea and death due to compression of the medullary respiratory centers and pathways.
- The most common difficulty encountered in the collection of CSF from the cerebellomedullary cistern is placement of the spinal needle too close to the occipital crest.

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ABSTRACT: Analysis of cerebrospinal fluid (CSF) is critical in the diagnostic evaluation of patients with neurologic disease. A multitude of diagnostic tests can be performed on CSF in an effort to identify the cause of neurologic abnormalities. In general, CSF analysis is a sensitive indicator of neurologic disease but rarely provides a definitive diagnosis. Although collection is easily performed with the appropriate technique, proper processing and preparation of CSF are necessary to ensure adequate sensitivity and improve the likelihood of identifying a specific disease process. Without proper collection, processing, and preparation of CSF samples, differentiation between pathologic and nonpathologic conditions may be difficult if not impossible.

Cerebrospinal fluid analysis is critical to the overall evaluation of patients with neurologic disease. Although sensitive for identifying many spinal and intracranial disease processes, CSF testing rarely provides a definitive diagnosis.¹ Therefore, analysis of CSF is typically only a part of the overall diagnostic evaluation of patients with suspected neurologic disease. Nonetheless, proper collection, processing, and preparation of CSF samples are necessary to ensure accurate interpretation of results. Common tests performed on CSF samples include the following²:

- Total nucleated cell and protein concentrations
- Differential cell counts
- Titers for antibodies against infectious organisms
- Culturing

COLLECTION

Collection of CSF involves much more than an understanding of neuroanatomy and the application of proper technique. Factors such as anesthetic risk and the prevailing neurologic condition often take precedence when deciding to collect CSF. Despite the relative ease with which fluid may be collected in most cases, complications associated with anesthesia of a compro-

mised patient and risk of herniation with increased intracranial pressure (ICP) necessitate a discussion of these concepts.

Anesthesia

Cerebrospinal fluid is collected with the patient under an adequate plane of anesthesia to prevent movement while performing the procedure.² Because a significant number of patients requiring CSF collection are at increased anesthetic risk, special precautions should be taken regarding drugs, dosages, and monitoring. Injectable anesthesia inhibits the ability to readily adjust the depth of anesthesia during collection. Therefore, patients should be intubated and gas anesthesia with supplemental oxygen administered following induction.³ This helps to ensure an adequate plane of anesthesia and easy maintenance or adjustment of this level should the procedure become prolonged or other complications arise. As with anesthesia for any procedure, the risk:reward ratio needs to be critically evaluated.

Herniation

Herniation is the shift of intracranial contents from an area of high pressure to an area of low pressure. In patients with elevated ICP, inserting a spinal needle into the subarachnoid space may result in a pressure gradient sufficient to cause a caudal shift of intracranial structures. This results in increased patient morbidity and mortality. Conditions often associated with an increased ICP include the following²:

- Neoplasia
- Hemorrhage
- Trauma
- Edema
- Generalized inflammation
- Granulomas
- Abscesses

Although not always readily available, imaging techniques should be performed to identify lesions that would preclude collection of CSF.

If CSF collection is deemed necessary in patients with suspected increased ICP, precautions should be taken before and during collection. Ventilation sufficient to maintain the partial pressure of carbon dioxide at approximately 30 mm Hg will likely prevent dilation of cerebral blood vessels.⁴ This will help minimize any additional increases in ICP secondary to increased cerebral blood flow. Aggressive hyperventilation is currently only recommended in patients with a deteriorating neurologic status and/or in which herniation is strongly suspected.⁵ The use of mannitol, an osmotic diuretic,

may be beneficial both prior to and during collection to reduce ICP.⁶

Collection Sites

Cerebrospinal fluid is most commonly collected from the cerebellomedullary cistern because it is a relatively convenient location from which to safely obtain an adequate sample for analysis. Fluid can also be collected from the lumbar cistern, although samples from this location are generally more difficult to obtain and thus may result in an inadequate sample volume with an increased likelihood of blood contamination. One milliliter of CSF per 5 kg body weight may be safely removed from either site.⁷ Although individual laboratory references vary, CSF from healthy dogs and cats generally contains less than 5 leukocytes/ μ l and less than 25 mg/dl of protein.² As discussed in the Technique section, lumbar samples may have an increased protein concentration that may exceed 25 mg/dl in healthy dogs and cats.^{2,7,8} Fluid is typically collected into a sterile glass tube, and anticoagulant is generally not needed. The presence of EDTA may result in false elevations of the protein concentration and, in small samples, may dilute the cell concentration, making interpretation of results unreliable.³

Technique

Regardless of the location used to collect the fluid, the area should be clipped and prepared as for a surgical procedure and sterile gloves worn. Proper positioning of the patient is very important and greatly facilitates the ability to successfully collect CSF.

Cerebrospinal fluid cell counts, cytopreparatory techniques, and protein quantitation can typically be performed on 1 ml of fluid. However, it is preferable to obtain another 2 to 3 ml in order to perform CSF cultures, infectious titers, or chemistry evaluation if desired.⁷

Cerebellomedullary Cistern

To collect CSF from the cerebellomedullary cistern, the patient is positioned in right lateral recumbency if the practitioner is right-handed and left lateral recumbency if left-handed.³ The axis of the patient's body is positioned parallel to the edge of the table, and the head is flexed so that it is perpendicular to the body (Figure 1). The holder must ensure that the nose is kept in line with the spine so the skull is not rotated. Care must also be taken to ensure that the endotracheal tube remains patent while the head is flexed. The landmarks used include the occipital protuberance and the wings of the atlas.^{3,9} For a right-handed individual, the left hand is used to palpate these landmarks. The thumb and middle finger are placed on the wings of the atlas,



Figure 1—Proper patient positioning for collection of CSF from the cerebellomedullary cistern. The patient's body is parallel to the table, and the head is flexed so that it is perpendicular to the long axis of the body.

and the index finger is placed on the occipital protuberance. An imaginary line is formed connecting the wings of the atlas, and the occipital protuberance is used as a reference for midline. Insertion of the spinal needle should occur along the cranial border of the wings of the atlas, being careful to stay on midline (Figure 2).²

A 20- or 22-gauge, 1.5-inch disposable spinal needle is adequate for most situations, although a 2.5-inch needle may be required for large dogs. A stylet is important to avoid introducing skin cells into the subarachnoid space with subsequent cyst formation. The needle is inserted slowly and the stylet removed frequently to assess for flow of CSF. A loss of resistance may be felt as the needle penetrates the dorsal atlantooccipital ligament and enters the subarachnoid space.² However, this should not be used as the sole means for assessing the depth of the needle. Cautious advancement and frequent removal of the stylet to evaluate for CSF flow are mandatory. The fluid is allowed to drip into a sterile tube, and the needle is withdrawn.

Lumbar Cistern

To collect CSF from the lumbar cistern, the patient may be positioned in either right or left lateral recumbency. Fluid is collected from the L5-6 or L6-7 interspace in dogs and the lumbosacral space in cats.² Placing a towel between the legs often assists in opening the interspace and facilitates collection. For right-handed individuals, the left hand is used to palpate the dorsal spinous processes and isolate the appropriate location. Typically, the L-6 dorsal spine is the most caudal that can be palpated. However, evaluating survey radiographs assists in visualization and determining the likelihood of palpating the various spinous processes.

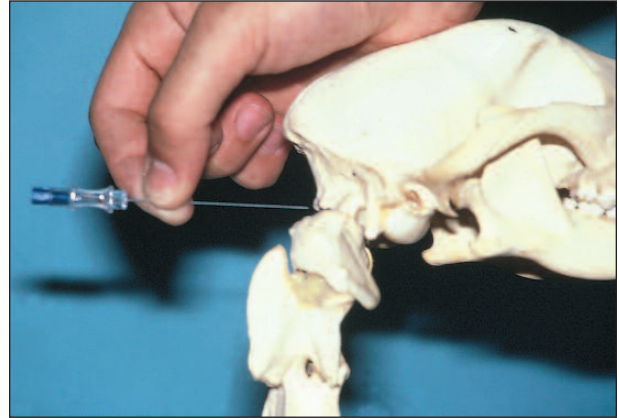


Figure 2—The needle is inserted along the midline just cranial to the wings of the atlas. The occipital protuberance is used to facilitate identification of midline.

Midline is located by palpating the dorsal spinous processes and/or the wings of the ilium as may be necessary in obese animals (Figure 3). The needle is inserted just caudal to the interspace of interest, along the cranial border of the caudal dorsal spinous process (Figure 4). The needle is slowly advanced cranially once bone is contacted. Typically, a slight leg kick is observed once the needle penetrates the interarcuate ligament as it irritates the cauda equina. The needle should be advanced until it lies on the ventral aspect of the spinal canal. The stylet is removed and, if necessary, the needle is slowly withdrawn until the bevel is in the ventral subarachnoid space and CSF flow is evident. Fluid is collected or the needle is readjusted as needed.

Protein and Cell Concentrations at Collection Sites

Samples collected from the lumbar and cerebellomedullary cisterns in healthy animals vary with regard to the protein and cell concentrations.^{7,8} Lumbar samples typically have a higher protein and lower cell concentration than do samples from the cerebellomedullary cistern. Analysis of CSF collected from the cerebellomedullary and lumbar cisterns of 31 healthy dogs revealed that the mean protein concentration of the lumbar samples was 29 mg/dl and the mean total nucleated cell concentration was 0.55 cells/ μ l. In contrast, CSF from the cerebellomedullary cistern had a mean protein concentration of 14 mg/dl and a mean total nucleated cell concentration of 1.45 cells/ μ l.⁸ Although reasons for these differences are not definitive, the disproportionately low cell concentration in lumbar samples may result from increased cell lysis or outward migration of cells as CSF flows in a caudal direction.¹⁰ The difference in protein concentration is thought to arise from the slower circulation of CSF in the lumbar

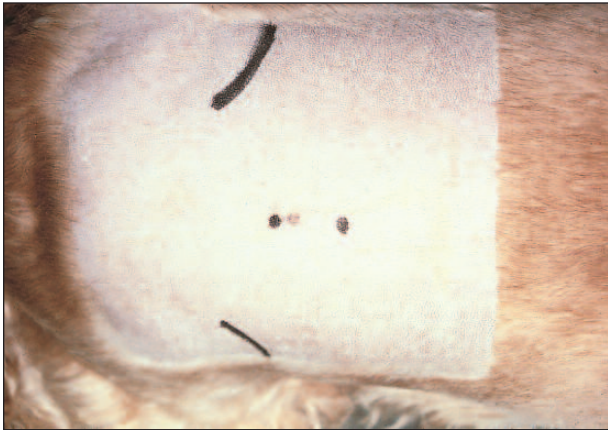


Figure 3—The landmarks for collecting CSF from the lumbar region include the wings of the ilium (*black lines*) and the L-5 and L-6 dorsal spinous processes (*black dots*).

region with subsequent local protein accumulation.⁷ Despite a statistically significant difference, the clinical significance of the differences in cell counts between collection sites is questionable due to the low number of cells in normal CSF and the fact that they are counted in whole numbers.

The collection site itself is important in enhancing the clinician's capacity to identify neurologic disease. In a study of 145 dogs with focal neurologic abnormalities, CSF collected caudal to the lesion revealed abnormalities in both protein and cell concentrations much more frequently than fluid collected cranial to the lesion.¹¹ These differences likely result from the caudal flow of CSF along the neuraxis. In general, the lesion should be localized and fluid collected from a location caudal to the lesion.

PROCESSING

Following collection, the practitioner has several options regarding processing and analysis of a CSF sample. The sample may be processed and evaluated on site using one of the various cytopreparatory techniques described later or sent to a clinical pathologist for interpretation following preparation. Alternatively, the sample may be collected and mixed with autologous serum that will facilitate preservation of the sample up to 48 hours while it is shipped to a laboratory.¹²

Delayed processing of samples may result in the preparation of slides that are inadequate for interpretation due to disrupted cell structure.^{1,3,7,12} Common cellular changes observed with delayed evaluation include pyknosis, lysis, and disintegration of the cytoplasmic and nuclear membranes of cells.¹² In general, if no methods are undertaken to increase the stability of cells in a CSF sample, samples should be processed within 30 minutes

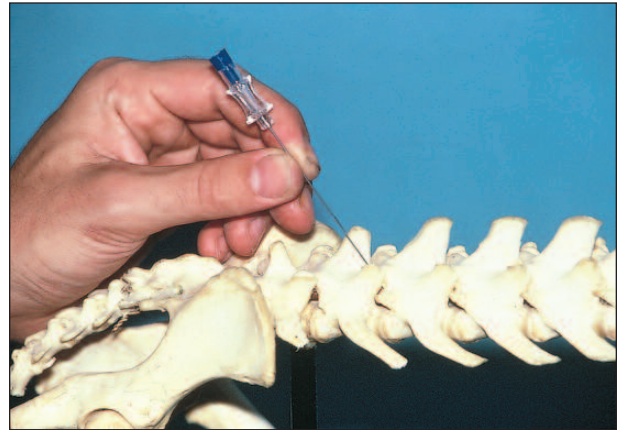


Figure 4—The spinal needle should be inserted into the L5-6 or L6-7 interspace in dogs. The needle in this image is being inserted into the L5-6 interspace. Note the angle at which the needle is inserted.

from the time of collection.^{1,2,3,7} The low protein content of CSF is thought to contribute to the rapid deterioration of cells, necessitating rapid processing.¹²

If the sample is going to be sent to an outside laboratory for processing and interpretation, two aliquots of CSF (approximately 0.25 ml each) should be collected and placed into sterile tubes. Blood is withdrawn and the serum harvested. Into one of the tubes, 0.03 ml of serum (approximately one drop from a 25-gauge needle) should be added and mixed with the aliquot of CSF. The tubes should be labeled and subsequently stored in a refrigerator and/or shipped with ice packs to a diagnostic laboratory for evaluation within 48 hours following collection. It is important that the serum be clear and that it only be added to one aliquot of CSF. The sample without serum added will be used for protein quantitation and nucleated cell concentrations as the disrupted cytomorphology does not tend to inhibit evaluation of the total nucleated cell concentration. The sample with serum added will be used for cytology, and a differential cell count will be generated.

CYTOPREPARATORY TECHNIQUES

Although values in healthy dogs and cats vary, CSF typically contains less than 5 nucleated cells/ μ l.² In general, CSF nucleated cell concentrations of less than 500 cells/ μ l require a concentration technique in order to obtain sufficient cell numbers for cytologic evaluation.^{1,3} It is often assumed that a normal total nucleated cell concentration obtained by a hemocytometer represents CSF from a healthy individual. However, cytologic evaluation following a concentration technique may identify abnormalities crucial to the overall interpretation of the CSF results.¹³ For instance, cytologic evalua-

tion may identify abnormal distributions of cells typically found in CSF, abnormal cell populations, infectious organisms, or cells with abnormal structure. For this reason, it is recommended that CSF be cytologically evaluated following a concentrating technique regardless of the total nucleated cell concentration.

There are advantages and disadvantages to each concentration technique. In general, characteristics that need to be considered include the following:

- The ability to preserve the morphology of the cells
- The ability to concentrate cells in order to allow for a representative differential count
- The time and costs involved

Direct Smear

The low concentration of cells in normal CSF precludes the ability to produce a useful direct smear. In addition, the normally low protein concentration does not provide adequate cellular support and thus cellular distortion is common during the smearing of fluid on the slide. This technique may be acceptable with nucleated cell concentrations greater than 500 cells/ μ l and the addition of a few drops of autologous serum.¹

Simple Centrifugation

One milliliter of CSF may be centrifuged at low speeds for 5 minutes. The supernatant may be withdrawn with a pipette and subsequently used for noncytologic tests. A drop of serum is added to the cells, and this mixture is then gently agitated. One drop may be gently smeared on a glass slide. This technique is generally considered inadequate for cell preservation and recovery and is thus not recommended.¹⁴

Membrane Filtration

This technique offers practitioners a relatively simple, inexpensive procedure that allows for excellent cell recovery of CSF samples.^{1,14,15} Membrane filtration was initially used in humans to allow for retention of tumor cells as blood was passed through membranes with pores large enough to allow typical blood components to pass through but small enough that the large tumor cells would be retained.¹⁵ When membrane filtration is applied to CSF samples, the membranes are small enough that the cellular components are retained and microscopic evaluation of the membrane itself may be performed.¹⁶ Cellular recovery is in the range of 90% to 100% of cells.^{16,17}

Advantages of this technique include the following¹⁴:

- Excellent cellular recovery
- Immediate preservation of cells

- Enhanced likelihood of identifying abnormal cell types (i.e., fungal, neoplastic)

Disadvantages—Despite retention of adequate cellular structure, interpretation may still be difficult for the following reasons:

- With membrane filtration, the cells are rounded, partially enmeshed in the three-dimensional filter matrix, making evaluation of some cells difficult.¹
- Giemsa stains, which allow for better contrast between cellular components than does hematoxylin, cannot be used on these filters.^{1,14}
- Highly cellular or proteinaceous samples may obstruct the filter, making interpretation difficult due to overcrowding.¹⁵

Despite the excellent cellular recovery and ease with which membrane filtration may be performed, the potential for interpretation difficulties precludes the recommendation of this procedure for general practitioners. Detailed descriptions of this procedure may be found elsewhere.^{1,15}

Sedimentation

Sedimentation is the recommended technique for general practitioners who lack the availability of a cytocentrifuge.¹⁴

Advantages of this technique include the following:

- It is a moderately rapid, simple technique that provides adequate cell yield (approximately 60%).
- The distribution of cell counts on the sediment generally reflects the true distribution of the cells in the CSF sample.¹⁸
- The results obtained allow for good cellular quality and adequate concentration of cells even in CSF with normal nucleated cell concentrations.¹ When compared with the membrane filtration technique, the cells are more spread out and flattened, allowing for better evaluation of nuclear and cytoplasmic detail.¹

Disadvantages—Transformation (activation) of some CSF mononuclear cells can occur,¹⁹ which may result in the following:

- An increase in cell size
- Development of vacuoles
- Augmentation of phagocytic activity

Preparation

Sedimentation slides should be prepared in advance of CSF collection. A heated scalpel is used to cut the proximal 2 cm off a 15-mm-diameter centrifuge tube

(Figure 5). Alternatively, a clean syringe barrel may be used. The smooth side of the cylinder is placed in heated petroleum jelly and then on a clean slide. Once a watertight seal has developed (approximately 30 minutes), the sample is ready for use.

Procedure

Up to 1 ml of freshly collected CSF is placed in the sedimentation chamber for approximately 30 minutes. The supernatant is aspirated carefully by placing a pipette just under the CSF surface and following the sample down as the aspiration process begins. The supernatant may be retained for further analysis. The cylinder is subsequently removed, and the remaining CSF is collected by gently touching the center of the CSF sample with a small piece of absorbent paper. The slide is immediately air-dried by vigorous waving. Drying with excessive heat or delayed or incomplete drying may lead to poor cytologic quality. The remaining petroleum jelly can be scraped off with a scalpel, and the slide can be stained and evaluated or sent to a veterinary clinical pathologist. In addition, Wright's or Wright's–Giemsa stain may be used to enhance cellular detail. However, cellular distortion increases rapidly if the slide is not promptly air-dried.¹⁴

Cyto centrifugation

Cyto centrifugation is the technique used most frequently in medical cytologic laboratories.¹⁴ The equipment is expensive but produces a rapid result and is easy to use, providing good cytologic detail.^{20,21} Cell recovery is comparable to that of sedimentation but is considerably less than that of membrane filtration.¹⁶ A distinct advantage of cyto centrifugation is the small quantity of CSF necessary. Good results can be obtained with as little as 0.2 ml of fluid.¹⁴ The technique involves placement of CSF into a centrifuge chamber and centrifug-

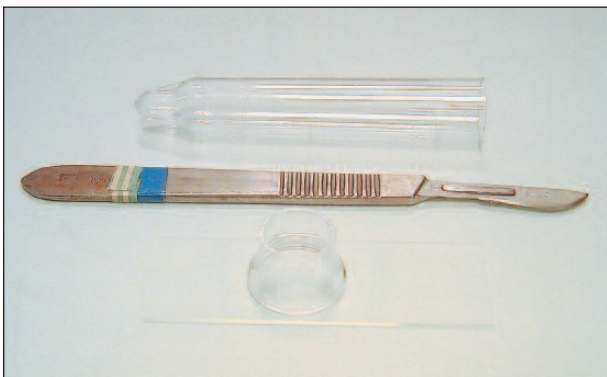


Figure 5—The proximal 2 cm of the centrifuge barrel is cut with a heated scalpel, and petroleum jelly is used to adhere the smooth surface to a glass slide.

ing at 500 to 1000 revolutions/min for 5 minutes. The addition of a small amount of 30% albumin (0.05 ml) may facilitate cell capture.²⁰ In general, it is recommended that two slides be made for examination.

Advantages of this technique include the following:

- The appearance of cells in appropriately cyto centrifuged samples is considered good to excellent.
- The cells are generally well spread, allowing distinct evaluation of nuclear and cytoplasmic morphology.

Disadvantages of this technique include the following³:

- The expense of the equipment
- The potential for increased vacuolation of cells secondary to the centrifugation process

STAINING

Preparations made in general practice may be stained and evaluated in-house or sent via mail to a veterinary clinical pathologist for interpretation. The standard Romanowsky stains, Wright's and Wright's–Giemsa, provide good cellular detail on air-dried CSF samples. The various quick staining methods such as Diff-Quik® (Canlab, Baxter Travenol Labs, Toronto) are convenient and provide adequate cellular detail.¹⁴ If the slides are going to be mailed to a veterinary clinical pathologist for evaluation, they should be sent unstained in a suitable container.

CONCLUSION

Cerebrospinal fluid analysis is a sensitive indicator of neurologic disease, and concentration techniques can facilitate recognition of abnormalities. Samples with total nucleated cell concentrations less than 500 cells/ μ l require concentration, and it is recommended that all samples be evaluated cytologically following a concentration technique. There are advantages and disadvantages to each technique. In general, sedimentation offers the most practical means for CSF preparation in general practice.

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ARTICLE #1 CE TEST

The article you have read qualifies for 1.5 contact hours of Continuing Education Credit from the Auburn University College of Veterinary Medicine. Choose the best answer to each of the following questions; then mark your answers on the postage-paid envelope inserted in *Compendium*.

1. Regarding CSF collection, hyperventilation is most appropriate in patients in which
 - a. intracranial disease is suspected despite a normal computed tomogram or magnetic resonance image.
 - b. CSF collection takes longer than 5 minutes.

- c. CSF is collected from the cerebellomedullary cistern.
 - d. brain herniation is suspected subsequent to or during CSF collection.
2. Mannitol may be used to decrease ICP through
 - a. osmotic diuresis.
 - b. free-radical scavenging.
 - c. decreased CSF production.
 - d. increased permeability of the blood-brain barrier.
 3. In general, CSF samples should be processed within _____ minutes.
 - a. 10
 - b. 30
 - c. 60
 - d. 120
 4. A CSF sample that will be shipped to a laboratory for processing and evaluation should be
 - a. stored in a refrigerator without additive and shipped with ice packs.
 - b. mixed with autologous serum and kept at refrigerator temperature until shipped with ice packs.
 - c. collected into a sterile glass tube with an anticoagulant and shipped at room temperature.
 - d. mixed with glucose in order to prevent cellular disruption and shipped with ice packs.
 5. The most commonly used CSF concentrating technique in commercial laboratories is
 - a. cytocentrifugation.
 - b. direct smearing.
 - c. sedimentation.
 - d. membrane filtration.
 6. When compared with CSF collected from the cerebellomedullary cistern, lumbar collection typically
 - a. yields greater volumes.
 - b. is easier.
 - c. requires that the patient be awake during the procedure.
 - d. is associated with a higher protein concentration.
 7. In which of the following situations should CSF-concentrating procedures be instituted?
 - a. in all samples, regardless of the total nucleated cell concentration
 - b. only when the sample contains more than 500 cells/ μ l
 - c. only when the sample volume collected is very small
 - d. when the sample is contaminated with blood
 8. Adding _____ to the CSF sample may facilitate cell capture while performing the cytocentrifugation technique.
 - a. alcohol
 - b. albumin
 - c. water
 - d. ethanol
 9. While collecting CSF from the cerebellomedullary cistern, the patient should be in
 - a. sternal recumbency with the head parallel to the table.
 - b. lateral recumbency with the head parallel to the axis of the body.
 - c. a position that is most comfortable for the practitioner.
 - d. lateral recumbency with the head perpendicular to the axis of the body.
 10. The approximate cell yield with the membrane filtration technique is
 - a. less than that with the sedimentation technique.
 - b. 90% to 100%.
 - c. less than 50%.
 - d. less than that with the cytocentrifugation technique.