NEUROLOGIC

CHAPTER 117

Cerebrospinal Fluid Collection, Myelography, Epidurography and Discography

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CEREBROSPINAL FLUID COLLECTION

Cerebrospinal fluid (CSF) analysis is an important component of the diagnostic evaluation of patients with neurologic diseases, and abnormalities in the color, cellularity, and protein level of the CSF may contribute to or confirm the diagnosis. Cerebrospinal fluid abnormalities have to be viewed in the context of the neurologic examination, historical complaints, clinical findings, advanced imaging, and any laboratory abnormalities.

Spinal Needles

Spinal needles with a 22-gauge stylet are commonly used; 20-gauge needles may be acceptable in larger dogs. Spinal needles 1½ inches long are appropriate in most of dogs for cisternal tap and in cats for both lumbar and cisternal tap. Spinal needles 2½ inches long may be used in large dogs for both cisternal and lumbar tap. The 3½-inch-long needles are usually reserved for the lumbar tap in large and obese dogs.

Sample Stability

The CSF should be collected in a sterile plain glass tube without anticoagulant. Usually, 1 to 2 mL are collected; 1 mL per 5 kg body weight of CSF can be safely removed for analysis. Since cell morphology and cell recognition in the CSF are time dependent, the sample should be analyzed immediately, or no later than 4 to 8 hours, after collection. If a longer delay is expected, adding 50% hydroxyethyl starch (hetastarch) improves the stability of the sample for up to 48 hours.

COLLECTION TECHNIQUES

Collection Sites

Two sites are available for CSF tap: the cerebellomedullary cistern (cisternal tap) and the lumbar region (lumbar tap). Cisternal tap is easier, and a sample taken from this area is less likely to be contaminated with blood. CSF flows in a cranial to caudal direction, and cisternal tap is appropriate to investigate intracranial diseases. For diseases affecting the spinal cord, a lumbar tap is more likely to be diagnostic. Collection of CSF in dogs and cats is performed under general anesthesia. Patients should be intubated, and ventilator support must be available. The site of collection must be clipped and prepared aseptically, and sterile surgical gloves should be worn.

Cisternal Tap

Because the neck will be severely flexed when cisternal tap is performed, the patient should be intubated with a kink-proof endotracheal tube to avoid occluding airflow. The patient is positioned in right lateral recumbency for the right-handed operator. The head and entire spine is positioned close to the edge of the table facing the operator. The head is held by an assistant in 90° flexion with the nose parallel to the table. Placing some form of support under the neck (foam wedge or rolled up towel) to support the nose will help maintain the alignment between the spine of the axis and the external occipital protuberance. The assistant holding the head of the patient, being careful not to block access to the landmarks for CSF collection, is instructed to "tuck in the animal's chin" and push the occipital protuberance toward the operator (Figure 117-1).

The proper location for needle insertion can be estimated in several ways. It is the author's preference to locate the caudal aspect of the occipital protuberance with the index finger of the left hand while simultaneously locating the wings of the atlas with the thumb and the middle fingers. Pressing firmly with the index finger tip as the finger is simultaneously moved caudally, just behind the occipital protuberance, allows palpation of a slight depression in the muscle, and the caudal edge of this depression is the point of needle insertion. The

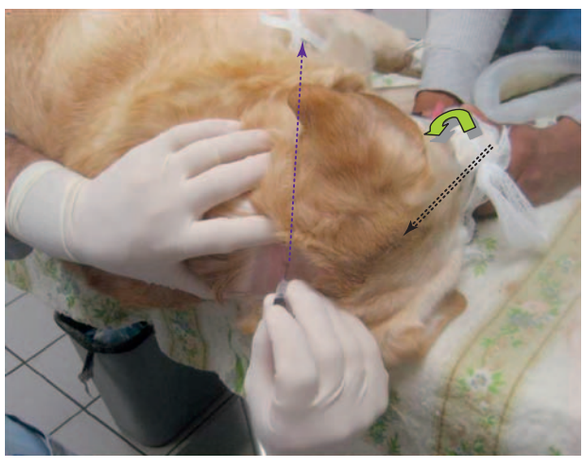


Figure 117-1 Cisternal tap. The patient is positioned in lateral recumbency with the entire spine and the head close to the edge of the table. The assistant, maintaining the head parallel to the table, is flexing the neck *(green arrow)* and pushing the occipital protuberance toward the operator *(double-dotted arrow)*. The needle is inserted in the muscle depression caudal to the occipital protuberance, at about a 30° angle along the midline, and is directed toward the tip of the shoulder joint *(single-dotted arrow)*. To facilitate recognition of the shoulder joint during collection, this landmark may be marked with bandage tape.

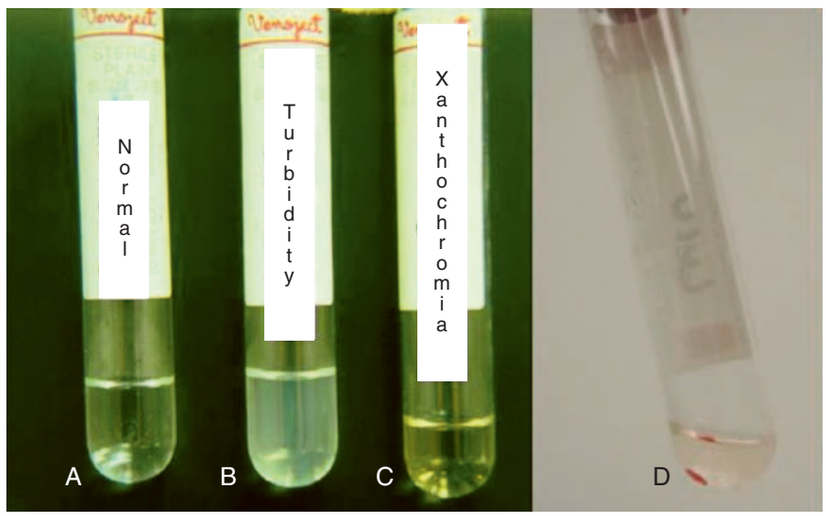
needle is inserted at approximately a 30° angle along the midline, and it is directed toward the tip of the shoulder joint. The skin is punctured first, and the needle is slowly advanced into the underlying muscle and fascia. In many instances, a slight sudden loss of resistance (slight "pop") may be felt as the atlanto-occipital membrane and dura mater are penetrated simultaneously. However, this sensation is not consistent, and in small dogs and cats this may be difficult to feel. Often the clinician must rely on the length of the needle used and on his/her tridimensional reconstruction of the anatomic area. The needle is usually advanced with the stylet in place and in small increments; the stylet can be removed between each movement to check whether CSF is present in the needle. Advancing the needle without stylet increases the chance that the needle will become obstructed with tissue or blood clot Additionally, there will be less damage if the nervous tissue is penetrated with the stylet in place. If the tip of the needle hits bone, the needle is slightly withdrawn and redirected slightly cranially or caudally to the original trajectory. When the desired level has been reached, the palm of the left hand is placed on the skull for support and the hub of the needle is grasped with the thumb and index finger. The stylet is removed with the right hand and is observed for fluid flow. If fluid is not seen, the stylet is replaced, and the needle is further advanced 1 to 2 mm at a time. After each advancement the stylet is removed to observe for fluid flow. When the desired level has been reached but no fluid has emerged, a slight rotation of the needle may be enough to dislodge any obstruction and allow the CSF to flow. To increase CSF flow, pressure can be applied to the jugular veins. If whole blood flows from the needle, the needle should be withdrawn and the procedure repeated using a fresh needle. This indicates that a venous sinus has been penetrated. Because these structures are in the extradural space, the CSF is not contaminated with blood and the procedure can be repeated. If CSF is tinted with blood, either a dural vessel has been penetrated or the blood may be part of the disease process. In the first case, the CSF may clear as the CSF drips from the needle, and the CSF may then be collected. Rotating the needle may also help to clear this type of bleeding. Centrifugation of the fluid may be used to differentiate hemorrhage from contamination or from a disease process. If hemorrhage is part of the disease process, centrifugation of the CSF will result in a yellow color (xanthochromia) from the hemoglobin breakdown products from the erythrocytes present; in the case of contamination, the supernatant will be clean and colorless (Figure 117-2).

Small amounts of iatrogenic hemorrhage do not interfere significantly with evaluation of the CSF.

Lumbar Tap

Lumbar tap is usually more difficult, and blood contamination tends to occur more often. Collection is usually from L5-L6 or L4-L5 in dogs. In cats, L6-L7 can also be used. Lumbar collection can be obtained with the animal in lateral or ventral recumbency. It is the author's preference to collect the fluid with the animal in ventral recumbency. In this position is easy to align the spine and to maintain the hind limbs cranially extended. This widens the interarcuate space, which facilitates the needle penetration in the vertebral canal (Figure 117-3).

For a collection at the L5-L6 site, the tip of the L6 spinous process is palpated. The needle is inserted just lateral to the midline alongside the caudal edge of the L6 spinous process. The needle is inserted at approximately a 45° angle (caudally) from an imaginary line perpendicular to the long axis of the spine; it is then directed cranially and ventrally through the ligamentum flavum into the vertebral canal. After the interarcuate space is passed, the needle penetrates the dura and enters the subarachnoid space. Due to the mechanical stimu-



74

Figure 117-2 Cerebrospinal fluid (CSF) appearance. **A,** Normal CSF has a waterlike appearance. **B,** Turbidity is usually due to an increased number of cells (more than 200 WBCs/ L, more than 400 red blood cells/ L) and occasionally is due to an increased protein level. Elevated protein levels will also cause increased viscosity. A marked increase in the protein level often causes the CSF to clot. **C,** Xanthochromia (yellowish color) is indicative of recent hemorrhage as part of the disease process; xanthochromia is the result of the hemoglobin breakdown products from the erythrocytes. Xanthochromia after centrifugation of the CSF sample differentiates between iatrogenic and ongoing hemorrhage in the subarachnoid space as part of the disease process. **D,** In case of iatrogenic blood contamination after centrifugation, the supernatant will be clear and colorless.

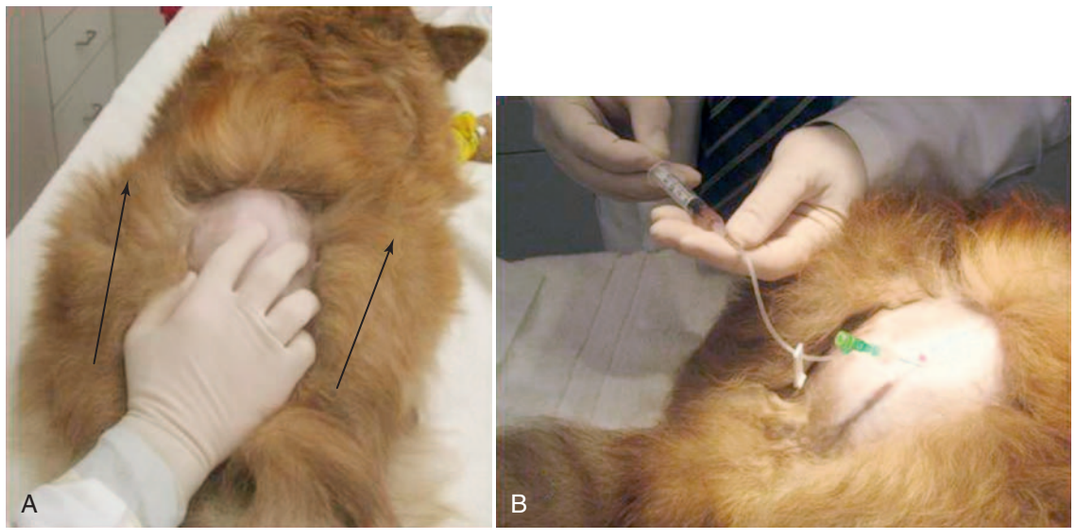


Figure 117-3 Lumbar tap. **A,** With the animal in sternal recumbency it may be easier to maintain the spine in alignment while opening the interarcuate space by extending the hindlimbs cranially *(arrows)*. This may facilitate the needle placement in the vertebral canal. **B,** After the needle is in the proper position, to facilitate collection a 2.5-mL syringe with a short injection set is connected to the needle and gentle suction is applied to complete the cerebrospinal fluid collection.

lation of the spinal cord and/or nerve roots at this level, a twitch of the pelvic limbs and/or tail it is usually noted. The needle is advanced to the floor of the vertebral canal and then slightly retracted 1 to 2 mm. At this point the stylet is withdrawn. CSF tends to flow more slowly in lumbar collection than in cisternal collection. To facilitate collection it is the author's preference to connect a 2.5-mL syringe with a short injection set to the needle and to apply gentle suction to complete the CSF collection. If the collection fails, the operation may be repeated on the contralateral side first, and then at the more cranial L4-L5 site.

Risks and Complications

Inadvertant needle penetration of the parenchyma at the cerebellomedullary angle may cause brainstem dysfunction ranging from temporary vestibular abnormalities to cessation of voluntary respiration and death. Foramen magnum herniation, with the cerebellar vermis herniating caudally and compressing the brainstem, may also be a lethal complication. This risk has been decreased by the use of MRI, which should be performed before CSF collection. MRI may identify patients where the cerebellum is already herniated or prone to herniation (Figure 117-4).

In these patients mannitol and hyperventilation may be used to decrease intracranial pressure (ICP) before CSF collection. To avoid puncturing an already herniated cerebellum, a lumbar tap is the preferred method for CSF collection.

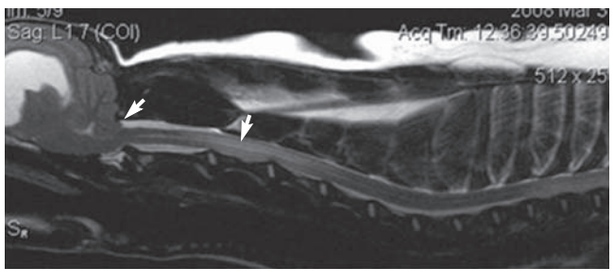


Figure 117-4 Contraindications for cisternal tap. MRI T2 sagittal plane of a dog with obstructive hydrocephalus causing cerebellar herniation *(left arrow)* and syringomyelia *(right arrow)*. In this patient, the needle placement in the cerebellomedullary cistern would likely puncture the herniated cerebellar vermis; therefore, cisternal tap is contraindicated in this case.

MYELOGRAPHY

Myelography may be performed by either cervical or lumbar injection. With the patient in lateral recumbency the needle is placed as described above for CSF collection. Ideally, lumbar myelography injection should be made at L5-L6 as the incidence of complications increases with injections at sites cranial to this. To study lesions in the lumbosacral area, cisternal myelography is advised, which may decrease the risk of contaminating the area with an epidurography. In the cervical injection the bevel of the needle should be directed caudally and in the lumbar injection cranially. Injection should be performed by connecting the syringe to the spinal needle with a short injection set prefilled with contrast medium. Cervical injection is easier but lumbar injection is usually preferred. Cervical injection is usually associated with the accumulation of contrast medium into the cerebral ventricular system, which may increase the risk of postmyelographic seizures. This may be alleviated by maintaining (just after the injection) the patient tilted at about 30° with the head elevated. However, a lesion causing severe cord swelling or severe spinal cord compression may not allow the contrast medium to flow caudally to the lesion, resulting in equivocal interpretation of the study that will require a subsequent lumbar myelogram. Lumbar injection can be performed under pressure, forcing the contrast medium to pass the lesion outlining the cranial and the caudal edge of the spinal cord lesion. Serial radiographs may help to locate the needle in the desired spot. Fluoroscopic guidance can also facilitate proper needle placement.

CSF should be collected before the injection of the contrast since the most common contrast mediums used (iohexol or iopamidol, 240 to 300 mg/mL) tend to induce an inflammatory reaction. A test injection (with 0.5 to 1.0 mL, depending on patient size) of contrast medium should be performed, and after radiologic confirmation (by radiographs or fluoroscopy) that the contrast is in the subarachnoid space, the remainder of the calculated dose can be injected. The volume of contrast to inject varies from 0.3 mL/kg to 0.45 mL/kg body weight; this depends on the site of the injection and the expected location of the lesion. For cervical myelogram with lumbar injection, the higher dose is recommended. After the contrast medium is injected, a series of radiographs in lateral and ventrodorsal position are taken. Additional oblique views are often needed in dogs with herniated disc to further identify the site of the lesion. Stress view radiographs in linear traction, dorsiflexion, and ventriflexion are usually performed in dogs with caudal cervical spondylomyelopathy, and stress view radiographs with maximum flexion and extension (both in lateral and ventrodorsal position) of the lumbosacral junction are usually performed in dogs with lumbosacral diseases. Transient exacerbation of neurologic signs may be seen after myelography; this is usually caused by transient chemical myelitis secondary to contrast injection. This risk may be higher in patients affected by an inflammatory myelopathy or chronic spinal cord compression. This usually resolves within a few days. Accidental injection of the contrast medium within the spinal cord parenchyma or within the central spinal canal may cause worsening of the neurologic status. In most cases, patients recover when iatrogenic trauma occurs in the lumbar region, but contrast medium injection in the cervical region may be fatal (Figure 117-5).

Intracranial subarachnoid hemorrhage is also a rare but fatal complication associated with lumbar myelography.

EPIDUROGRAPHY

Epidurography is usually combined with myelography and discography to help visualize diseases of the cauda equine. However, MRI has largely replaced the use of these two studies. Epidurography is particularly indicated in dogs where the dural sac does not extend to the sacrum and is usually performed after myelography. With the area aseptically prepared and the patient in lateral recumbency, the spinal needle is inserted between the spinal process of L7 and S1, between the sacrum and the first coccygeal vertebra, or between one of the caudal intervertebral spaces. The volume of contrast to inject is 0.1 to 0.2 mL/kg body weight.

DISCOGRAPHY

Discography is usually used in conjunction with epidurography for evaluation of L7-S1 disk diseases. The contrast is injected directly into the nucleus pulposus of the L7-S1 disk and radiographs in lateral and dorsoventral projection are taken. The volume of contrast is 0.1 to 0.3 mL/kg of body weight. In a normal disc, it is very difficult to inject contrast and usually no more then 0.1 mL may be injected. In a degenerated and/or herniated disc 2 to 3 mL may be easily injected and the contrast may also flow out in the spinal canal through the degenerated disc. The combination of discography/

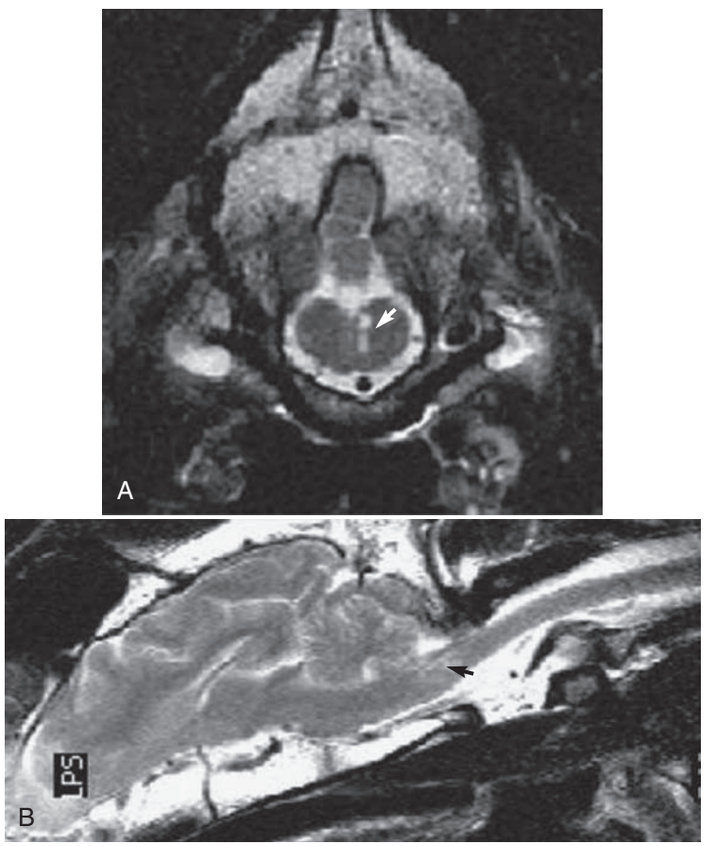


Figure 117-5 Fatal complication of improper needle placement during myelography. MRI T2 weighted of a German Shepherd Dog presented with depressed mental status, poor gag reflex, reduced tongue movement, and reduced sensation of the mandibular and maxillary branches of the trigeminal nerve. Myelography to rule out compressive spinal disease was attempted at a different facility 3 days prior to this MRI. The dog never recovered properly from the myelography and the owner elected euthanasia 3 weeks later. **A,** Transverse plane: the hyperintense line traversing almost the entire caudal brainstem in a dorsoventral direction is the damage created by the needle tract *(arrow)*. **B,** The diffuse hyperintensity in the caudal brainstem *(arrow)* is most likely edema secondary to the intraparenchymal needle placement. (Courtesy Dr. Rodolfo Cappello and Dr. Annette Wessmann.)

epidurography may be performed using a single needle puncture. Discography is performed and radiographs are taken first; the needle is withdrawn to the epidural space and additional contrast is injected, followed by radiographs.

REFERENCES

The reference list can be found on the companion Expert Consult Web site at www.expertconsult.com.

CHAPTER 118

Muscle and Nerve Biopsy

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Biopsy of muscle and/or nerve can be an essential aid in the diagnosis and therapy of suspected neuromuscular disease of small animals. Examination of the specific components of the motor unit, as well as of sensory and autonomic nerves, permits definition and classification of the underlying pathology.

MUSCLE BIOPSY

Conventional biopsy and formalin fixation techniques, as used with most organ systems, severely limit the quality of information that may be obtained from muscle specimens. Development of specialized enzyme histochemical techniques, using frozen specimens, has greatly increased the understanding both of normal muscle and of the underlying pathologic processes of many neuromuscular diseases.

Selection of Muscle

The selection of a muscle for biopsy is guided by a number of criteria:

1. The muscle should be affected by the disease process but should not be end-stage. This choice may be based on electrophysiologic data, including abnormal electromyographic (EMG) results, and on clinical abnormalities suggesting muscle involvement (atrophy, hypertrophy, apparent pain, weakness).
2. The muscle should be easily identified surgically, with low associated morbidity, and with fibers oriented in a single direction.
3. Specimens should be harvested from muscles for which there is previous interpretive experience. Standard muscles include the lateral head of the triceps brachii (distal third), vastus lateralis (distal third), cranial tibial (proximal third), and temporalis muscles. Biopsy specimens from both a thoracic and a pelvic limb muscle, or other distant locations, and from a proximal and a distal muscle, are necessary for optimal diagnosis of generalized neuromuscular disease.
4. Muscle biopsy specimens should be harvested from a site remote to tendinous insertions and aponeuroses.
5. Specimens should be free of artifact induced by previous disease, intramuscular injections, and EMG needle insertion.
6. Some specialized procedures may require biopsy specimens from specific muscles or regions within muscles. For example, diagnosis of congenital myasthenia gravis is based on the demonstration of decreased numbers of acetylcholine receptors in biopsies of external intercostal muscle.

Open Muscle Biopsy Procedure

Open biopsies are done most often under general anesthesia following an electrodiagnostic study. After routine surgical preparation, the skin and fascia overlying the muscle are incised, allowing visualization of myofiber orientation. A specimen for fixation is harvested first. Two incisions are made with a No. 11 scalpel blade, parallel to the direction of the myofibers and approximately 2 cm long, 0.25 cm apart, and 0.5 cm deep. Specialized muscle clamps are placed at either end of the incised strip of muscle (to minimize myofiber contraction), and the isolated muscle is freed from the surrounding muscle with a scalpel blade or scissors. The specimen should be immediately immersed in fixative, usually glutaraldehyde (either sodium phosphate–buffered glutaraldehyde or Karnovsky's fixative). Clamps may be removed 24 hours following fixation. If a specialized muscle clamp is not available, the specimen may be sutured to the wooden stem of a cotton-tipped applicator. Specimens for freezing and routine histochemical staining (approximately 0.5 to 1 cm in cross section and 1 to 1.5 cm long) may be harvested from adjacent muscle. It is not necessary to maintain these specimens in a stretched position. To reduce artifact, handling of the specimen should be kept to a minimum. Wound closure is routine. External dressings are not necessary. Complications (infection, hematoma) are uncommon and usually are the result of animals interfering with the biopsy site. Collection of a muscle biopsy specimen may be contraindicated in dogs or cats with coagulopathy.

Percutaneous Muscle Biopsy Procedure

Percutaneous needle or punch muscle biopsy is not recommended routinely due to the limited size and poor orientation of the biopsy specimens obtained.

Specimen Processing and Transport

Ideally, muscle biopsy specimens should be frozen immediately following harvesting or transported to specialized laboratories for processing and interpretation. Specimen blocks are mounted on thin cork squares using tissue-embedding medium, with the muscle fibers oriented vertical to the cork, and frozen for approximately 20 seconds in isopentane (2-methylbutane) cooled to approximately –150° C in liquid nitrogen. Rapid freezing of the specimen is critical for preservation of morphologic detail and prevention of artifacts. Frozen blocks may be stored in airtight containers at –80° C

Chapter 117

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