

# Association of Primate Veterinarians Guidelines for Cerebrospinal Fluid Aspiration for Nonhuman Primates in Biomedical Research

## Purpose

The Association of Primate Veterinarians (APV) recognizes that cerebrospinal fluid (CSF) collection may be required for both clinical and research purposes in nonhuman primates (NHP). Because there are inherent risks associated with the technique, the laboratory animal veterinarian should determine the need and utility of CSF collection for clinical evaluation. CSF collections for research purposes must be scientifically justified and approved by the institutional animal care and use committee (IACUC) or equivalent regulatory body hereafter referred to as IACUC. The following recommendations provide basic information for IACUCs, researchers using NHP models, and veterinary personnel to consider when developing policies and standard operating procedures for CSF collections.

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## Background

CSF analysis is a powerful tool that can augment detection of various processes occurring within the central nervous system (CNS). Examples include hemorrhage, inflammation, infection, neoplasia, and other clinical conditions. In addition, serial evaluation of compounds within the CSF, such as hormones, medications, neurotransmitters, biomarkers, metabolites, and viruses, may be extremely useful in both basic and translational research. CSF volume in the CNS is constantly being maintained by CSF formation and absorption.

## Technique and Procedural Considerations

CSF is typically collected via puncture of the cisterna magna or lumbar vertebral space. Collection of CSF by lumbar puncture may involve less risk of complications because the lumbar site is well below the termination of the spinal cord in most NHPs. The animal should be appropriately anesthetized, and the procedure should be performed using an aseptic technique, including hair clipping, surgical preparation of the collection site, and use of sterile materials. At a minimum, gloves, syringes, and needles should be sterile. Sterile surgical drapes may also be considered. CSF collection is typically performed in lateral or sternal recumbency, or in a sitting position, with flexion of the neck or spine to improve access to the cisterna or widen the intervertebral space, respectively.<sup>6,13,18</sup> Needle size and length selected should be appropriate for the species and size of the NHP.<sup>2,6,13,18</sup> The techniques, supplies, and equipment used, as well as pre-, intra-, and postprocedural care, may vary depending on the NHP species, research aims, and preference of the clinician.

Some research protocols may require consecutive CSF collections over time, and/or require CSF samples from unanesthetized subjects. For these protocols, serial collection of CSF

from conscious rhesus macaques may be accomplished through a chronically implanted cisternal catheter that is connected to a subcutaneous access port.<sup>7,8,12</sup>

In the creation of this document, every effort was made to find current references and literature regarding objective values for total CSF volume, limitations, or recommendations for the maximum number of dural punctures, frequency of collections, safe volume for withdrawal, and CSF replenishment rates. There is a paucity of literature in this area for most primate species. Furthermore, the techniques for collection vary as survey results collected from the APV membership indicate that facilities are successfully performing CSF collections using several different methods. We are therefore providing considerations and recommendations for CSF collection based on the best evidence currently available.

For reference, prior studies have determined the apparent CSF volume in adult male rhesus macaques (8.0 to 10.6 kg) was  $10 \pm 0.06$  mL and the CSF flow rate was  $0.018 \pm 0.003$  mL/min.<sup>15</sup> The CSF formation rates in rhesus macaques range from 28.6 to 44.2  $\mu$ L/min.<sup>4,11,14</sup> Further research specific to NHPs is needed in this area to facilitate the development of more prescriptive recommendations.

## Potential Adverse Effects

While few complications have been reported with CSF collections in NHPs, anatomic variation as well as inappropriate or poor procedural technique can result in a compromised sample and/or complications such as hemorrhage, herniation, infection, brain stem, spinal cord or nerve root damage, and animal discomfort. All these complications, while rare, can compromise animal welfare.

Due to the risks involved, CSF is usually collected by veterinarians or trained veterinary staff. Practices may vary with regard to unsuccessful CSF collection attempts. A common practice is the allowance of up to 3 unsuccessful attempts, or “sticks” by one individual. After the third failed attempt, another qualified individual can be allowed to attempt the CSF tap. If the second individual is unable to collect CSF after 3 attempts, the collection attempts are discontinued, and the animal should be provided appropriate analgesia while recovering from anesthesia. Changing collection sites (from cisterna to lumbar, for example) after failed attempts often results in a successful collection. Fluoroscopy may be used to assist in needle placement if attempts without imaging are unsuccessful. In addition to improving success rates, image guidance may reduce the incidence of traumatic puncture.<sup>5</sup>

Postprocedural monitoring should be provided continuously until the patient is returned to its home enclosure and able to remain consistently in an upright position without support. Patient monitoring should be done regularly for a minimum of 24 h or as indicated in the IACUC-approved protocol. Monitoring should include evaluation of basic NHP physiologic, behavioral, and locomotor parameters. In particular, animals

should be assessed for signs of neurologic impairment including but not limited to lethargy, depression, obtundation, and head-pressing. In human medicine, spinal headache is not uncommon following CSF aspiration and resulting intracranial hypotension. In consideration of these potential sequelae in NHPs, analgesia should be provided at the time of the procedure and extended 2 to 3 d, or as needed based on postprocedural observations for behavior associated with headache or spinal pain. The most effective analgesia is likely of the multimodal variety administered preprocedurally.

### IACUC Considerations and Recordkeeping

IACUCs should be aware of the CSF collection logistics and all possible complications and review them at least annually. All relevant information such as drug dosages, preprocedural preparation, CSF volume collected, frequency of collections, and postprocedural short- and long-term recovery events should be described in the IACUC protocol and entered in the medical record or laboratory logs where appropriate.

CSF can be a critical resource in a research protocol, but its collection can have consequences that impact animal welfare if the technique is not performed appropriately. Scientific justification should be provided for the number and frequency of collections performed. Specifically, the IACUC should also carefully evaluate each proposal involving CSF collection and consider the following issues:

- 1) Are CSF collections essential to address the scientific objectives stated in the protocol?
- 2) Are alternatives and less invasive methods of evaluating the CNS (such as imaging modalities) available and, if so, have they been considered?
- 3) Are the personnel performing the procedures appropriately trained and do they have adequate skills?
- 4) Will appropriate preprocedural anesthesia and analgesia be provided? The laboratory animal veterinarian should be consulted regarding the anesthesia and analgesia plan.
- 5) Is the volume of CSF to be collected appropriate for the age and size of the animal?
- 6) Are the number and frequency of meningeal punctures appropriate for the animal and have the risks of complications been minimized? If multiple CSF collections are performed as part of research investigations, the maximum number of collections per animal should be specified.
- 7) Is the plan for postprocedural monitoring adequate?
- 8) Have all possible postprocedural complications been considered and all reasonable interventional methods, inclusive of euthanasia, listed?

### Disclaimer

The position statements and/or guidelines produced by the APV are intended to be recommendations and guidance and are not a regulatory requirement. The Scientific Advisory Committee (SAC) within APV is tasked with the generation and revision of guidance documents for use by the membership and primate specialists worldwide. A subcommittee of current APV members and subject matter experts who have expertise in the area of interest are recruited to draft a document that is then sent out for comment and input from the SAC committee, the APV Board of Directors, and the APV membership. The final version was approved by the Board of Directors before being published on the APV website. We would like to extend special thanks to the committee members who worked on and

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