



AAEP General Recommendations for Diagnostic Sample Handling and Storage for Delayed Submission to a Diagnostic Laboratory

Note: These guidelines are intended to be a reference for veterinarians conducting necropsies and collecting samples in their respective practices. They are neither regulations nor directives and should not be interpreted as such. It is the responsibility of attending veterinarians, along with the clinical histories, and in consultation with their laboratories, to utilize relevant information to determine optimal sample collection and handling protocols.

Overview:

Delayed submission of diagnostic samples from necropsies or other critical diagnostic workups requires correct handling and storage of tissues to ensure samples maintain their diagnostic viability from the time of collection until processing and analysis. When samples cannot be delivered to the laboratory in a timely fashion (<48 hours post procurement), the following recommendations may assist in preserving sample integrity. Some samples, unfortunately, cannot be held without sample degradation, and test results from such samples must be interpreted with caution. Practitioners are encouraged to contact their laboratory for further information and guidance whenever possible.

Ideally, samples should be received in the laboratory within 48 hours of collection. If this is not possible or a laboratory cannot accept samples, collecting 2 full sets of tissues, one for freezing and one for formalin fixation, is a reliable way to preserve samples for the most common diagnostic tests. Photographs should be taken at the time of necropsy and collection to assist with animal identification and to document lesions and findings.

Temperature for sample refrigeration: 40°F (4°C)

Temperature for sample freezing: 5°F to 13°F (-15°C to -10°C)

Notes:

- Use sample containers that can withstand freezing temperatures such as plastic tubes and vials
- Do not store samples in a self- defrosting freezer due to the freeze-thaw cycle and possible damage to the sample

Recommendations for specific testing modalities are as follows:

Bacteriology

- Tissue samples for culture (aerobic or anaerobic), feces, and other fluids should be placed in sealable plastic bags or sterile containers and frozen
- Swabs in aerobic transport should be frozen. (Amies® transport medium with charcoal is recommended as it supports fastidious bacteria)
- Samples or swabs in anaerobic transport media (ATM) should be frozen.
Note: There are no specific references for preservation of samples in ATM by freezing, therefore, it is also recommended to place tissues (must be >2cm thickness if possible) for anaerobic culture in a sterile, sealable container and freeze
- Intestinal contents may be preserved for aerobic and anaerobic culture by ligating sections of intestine and freezing

Clinical Pathology

- Samples intended for complete blood count or cytology should be placed in EDTA tubes and refrigerated, but may not be diagnostic after 24-48 hours of procurement and refrigeration
- A differential smear from EDTA blood should be made and allowed to dry before shipping to lab
- Serum may be preserved for biochemical analysis by separating serum from the clot and transferring to a clean plastic tube or plain red-top tube and frozen

Histology

- Samples (0.5 cm maximum thickness) should be fixed in 10% formalin (1:10 of formaldehyde to distilled water or saline) and maintained at room temperature. Do not allow samples to freeze
- *Note: Containers should be filled with a 1:10 tissue to formalin ratio to prevent incomplete fixation and sample autolysis*

Molecular Diagnostics

- Tissue, body fluids, and swabs should be frozen. Contact the laboratory for the effects of freezing on specific molecular tests
- Do not send swabs in bacterial transport media unless approved by laboratory due to possible PCR inhibition
- EDTA blood should be refrigerated and not frozen unless directed by the laboratory

Parasitology

- Fecal samples may be refrigerated for up to 7 days. Feces for fecal ova and parasites cannot be frozen due to damage to the parasitic ova
- Parasites submitted for identification (worms, ticks, etc.) may be preserved in 70% isopropyl alcohol and stored at room temperature

Serology

- Serum samples should be separated from the clot and transferred to a clean plastic tube or plain red-top tube and frozen

Toxicology

- Specific toxicological samples may require special handling. Consultation with a toxicologist and/or laboratory is recommended prior to sample collection whenever possible.
- Tissue samples, stomach contents, urine samples, and body fluids (peritoneal fluids, etc.) should be placed in sealable containers and frozen
- Food or hay samples should be placed in plastic bags and frozen

Virology

- Tissue samples, feces, and other body fluids should be placed in sealable bags or sterile sealable tubes and frozen
- It is preferable to send swabs in viral transport media if available. If not, place swab in a sterile container (such as a plain red-top tube) containing a few drops of saline and freeze. Contact the laboratory for the effects of freezing on specific virology tests.

Additional information on sampling and diagnostic testing can be found at:

<https://www.vet.cornell.edu/animal-health-diagnostic-center>

Mittel, L.D (2017) Veterinary Diagnostic Testing. In Pusterla, N. and Higgins, J. (Editors). *Interpretation of Equine Laboratory Diagnostics*. (pp 1-11) John Wiley and Sons, Inc.