

CAHFS Electron Microscopy Submission Form Diagnostic Ultrastructural Pathology

(Lab use only) Animal species:	Specimen(s):			Specimen Label/ Accession #
Date:	H.E. slide: <input type="checkbox"/> YES <input type="checkbox"/> NO			
Please submit samples with full information: CAHFS standard submission form, pathology report, H&E slide Disease diagnosis/target cell-structure/EM suspected :				
Approximate time (hours) before tissue fixation:	_____ in formalin	_____ in glutaraldehyde	_____ in Karnovsky	
Is the pathogen or lesion in the sample: (Check One)	<input type="checkbox"/> very rarely found	<input type="checkbox"/> regularly found	<input type="checkbox"/> frequently found	

Instructions for handling and submission: <https://cahfs.vetmed.ucdavis.edu/tests-and-fees/diagnostic-services/electron-microscopy>

Test code	# of samples	Tissue ultrastructure
8504		Sample retrieval from wet tissue (formalin, glutaraldehyde, paraformaldehyde)
8506		Sample retrieval from formalin-fixed paraffin embedded (FFPE) block
		Cell pelletization
		Other: _____
		Other: _____
* For research project, please contact electron microscopy staff		

TRIMMING (EM Lab use only)

Initials & Date

HANDLING AND SUBMISSION INSTRUCTIONS FOR EM TESTING

Diagnostic Pathology

- *Techniques:* ultrastructure on fixed tissue embedded in plastic
- *Electron microscopy fixative*
 - For optimal results tissues must be fixed as soon as possible in one of the following EM fixatives (optimal method):
 - 2.5% Glutaraldehyde, 0.1M Sodium Cacodylate buffer
 - Karnovsky's fixative
 - Alternative method of fixation
 - 10% Neutral Buffered Formalin (NBF)
- *Sample harvest and fixation in 2.5% Glutaraldehyde, 0.1M Sodium Cacodylate buffer or equivalent EM fixative*
 - **Biopsy** - Any tissue collected from an animal must be trimmed into pieces of approximately 1.0 mm³ and placed in EM fixative immediately
 - **Postmortem** - Any tissue collected from an animal carcass should be placed in **EM fixative** ideally within a period of 6 hours after death. However, the diagnosis can be accomplished on tissue that have been refrigerated at 4 Celsius for 24 to 48 hours. In general, tissue must be trimmed into pieces of about 1.0 mm³ (1 x 1 x 1 mm). **If the organ has a complex architecture a different container must be used for each different tissue zone, area or structure.**
 - Kidneys, adrenal gland (cortical and medullary)
 - Lungs, liver, pancreas etc. (parenchyma, airways, ducts)
 - Nervous system (gray and white matter, nuclei or pathway)
 - Pathological tissue architecture (Tumor: different patterns or antigen expression)
- **10% Neutral Buffered Formalin (NBF):** Biopsy or any tissue collected from a fresh animal carcass should be placed in 10% NBF ideally within a period of 3 hours after death. However, the diagnosis can be accomplished on tissue that have been refrigerated at 4 Celsius for 24 to 48 hours. **If the organ has a complex architecture, ensure that all the different tissue zone, area or structure must be included; trimming will be performed by EM lab staff.**
- **Paraffin-embedded tissue:** This alternative option offers poor ultrastructural quality often precluding detailed diagnosis but is recommended when no other material is available. The best results are obtained from tissues fixed in NBF from biopsy or a fresh animal carcass ideally collected within a period of 1 hour after death. Additionally, material that is retrieved from FFPE block present two disadvantages: 1) Fixation with 10% formaldehyde buffer is suboptimal for electron microscopy and, 2) embedding in paraffin reduces the macromolecular integrity due to the strong mechanical/chemical/thermic processing stress. Normally, the inferior quality of the tissue ultrastructure precludes a precise organelles identification. If histological examination is unavoidable for localization of the microorganism or lesion due to its infrequency in the sample, we recommended postfixing the tissue in 3% Glutaraldehyde 0.1 M cacodylate buffer before this is embedded in paraffin. **Sample retrieval and trimming will be performed by EM lab staff**