UCDAVIS VETERINARY MEDICINE
California Animal Health & Food Safety Laboratory System

## CAHFS Electron Microscopy Submission Form

# Identification and Screening of Virus Propagated by Cell Culture

| Date: | | Specimen Label/ Accession # |
|---|---|---|
| ***Please submit samples with full information: CAHFS standard submission form, pathology report***<br>Instructions for handling and submission: https://cahfs.vetmed.ucdavis.edu/tests-and-fees/diagnostic-services/electron-microscopy | | |
| Disease/virus(es) suspected: | | |

| Test Code | # of samples | Test Requested |
|---|---|---|
| 11157 | | TEM - Virus identification and screening on propagated by cell culture |
| | | TEM - Virus morphometry |
| | | TEM - Virus quantification |
| | | TEM - |
| | | |

* For research project, please contact electron microscopy staff

**Negative staining**

| | Case # | Animal species | Cell line | Isolate | PCR Ct value at inoculation | PCR Ct value at submission |
|---|---|---|---|---|---|---|
| 1 | | | | | | |
| 2 | | | | | | |
| 3 | | | | | | |
| 4 | | | | | | |
| 5 | | | | | | |
| 6 | | | | | | |
| 7 | | | | | | |
| 8 | | | | | | |
| 9 | | | | | | |
| 10 | | | | | | |
| 11 | | | | | | |
| 12 | | | | | | |
| 13 | | | | | | |
| 14 | | | | | | |
| 15 | | | | | | |

**Plastic embedding by pelletization**

| | Case # | Animal species | Cell line | Isolate | PCR Ct value at submission | Cytopathic effect (%) |
|---|---|---|---|---|---|---|
| 1 | | | | | | |
| 2 | | | | | | |
| 3 | | | | | | |
| 4 | | | | | | |
| 5 | | | | | | |
| | | | | | | |

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**Virus propagated by cell culture**

Please contact Electron Microscopy staff first. Electron Microscopy staff will advise you what is the optimal sample and proper harvest method, fixation and processing protocol for individual diagnosis request (https://cahfs.vetmed.ucdavis.edu/tests-and-fees/diagnostic-services/electron-microscopy).

- For confirmation of known agents:
  - Samples may be submitted for negative stain only.

- For identification of unknown agents:
  - Samples with control cell line should be submitted for negative stain and isolate embedding.

- For cell line with unfamiliar diagnostic cytopathic effect:
  - Samples may be submitted for negative stain and embedding.
  - A control culture should be submitted along with the original sample. This helps us rule out any possible common contamination and enables us to provide accurate diagnoses.

- Quantity, fixation, and shipping:
  - Negative stain –
    - A minimum 1.0mL of Liquid suspension should be sent in ice pack overnight to CAHFS.
    - Virus concentration should be > $10^8$/ml or RT-PCR Ct , <25,
    - Cell culture presenting 80% of cytopathic effect,
    - For icosahedral, naked or enveloped viruses, if cells are grown on substrates (flasks), freeze-thaw or scrape the cells and submit the suspension.

  - Cell embedding (Fixative can be provided as needed) -
    - For bacteria, submit samples between 0.5-0.75 $OD_{595}$.
    - Cells are grown in liquid suspension: spin the cells at 2000 rpm for 10 minutes in a 1.5mL Eppendorf tube and discard the supernatant. Add 1mL of fixative and re-constitute it (2.5% glutaraldehyde in 0.1M Sodium cacodylate buffer pH 7.2-7.4 or Karnovsky) and send it to VDL at room temperature.
    - If the cells are grown in flasks remove the supernatant and add the fixative to the flask for 2-4 hours at 4 degree Celsius to fix the cells. Then scrape the cells and place them in fixative. Ship the samples to CAHFS at room temperature.
    - If the sample is an unknown agent, grows the sample in flask, scrape half the cells from the flask and send it for negative staining, the other half should be fixed in the flask (as mentioned above) for tissue embedding. It's critical for us to have the same cell culture for both negative staining and embedding to provide accurate diagnoses.