

SAMPLE SUBMISSION PROTOCOL FOR DNA PCR AND SEQUENCING

PCR

Interpretation of PCR results needs to be done in appropriate clinical context. To reduce misinterpretation and misuse of results, we only accept samples submitted by licensed veterinarians. We offer several consensus primer polymerase chain reaction (PCR) tests to detect the presence of nucleic acid from an infectious agent. This requires that the agent be present in the sample. The PCR amplifies a short segment of DNA specific to a gene from the target pathogen. A positive result indicates a band consistent in size as the positive control was amplified and validated by sequencing, confirming that the amplified product is not a non-specific amplification. Insufficient pathogen present in the sample may result in a negative test.

These consensus protocols are broad spectrum, and sensitivity is likely to vary with different agents in a group. These protocols have been used to characterize a number of novel agents. As many of the agents that these assays may be expected to identify are not yet known, sensitivity of these assays is obviously also unknown, and these assays should be considered experimental.

Viruses that we will not test for include poxviruses of mammals. We will not accept macaques herpesvirus samples. We will not accept bacterial agents in unfixed samples unless known not to be of concern. Ethanol is an acceptable fixation solution that will not degrade DNA.

COLLECTING SAMPLES

Ante-mortem:

Tissue biopsies, nasal washes, cloacal swabs, conjunctive swabs, choanal swabs or oral swabs are the most commonly submitted samples. It is important to select a sample likely to contain maximal amounts of the agent of concern. Collect tissue into a small sterile tube and keep on ice or refrigerate immediately after collecting. If tissues are not to be mailed within 1-2 days, then store the sample frozen. If collecting a swab, use a sterile cotton tip swab, swab the site vigorously enough to exfoliate cells, and place in a red top tube or other sterile tube with no additives. Do not add saline. Label tubes with species, ID number or name, type of sample, investigator's name, and sampling date.

Post-mortem:

Tissues likely to contain maximal amounts of the agent of concern should be submitted. Histopathology may be useful for tissue selection. Only a very small sample (approximately 0.1g) is needed. Place samples into clear, small, sterile plastic tubes or red top blood collection tubes with no additives. Label tubes with species, ID number or name, type of sample, investigator's name, and sampling date. Refrigerate samples until shipment. Freeze if samples will be stored longer than 2 days. Fresh or frozen samples are preferred. Although we have occasionally had success with tissues that have been

fixed in formalin for less than 14 days, formalin is very damaging to nucleic acids and may cause false negative results.

CURRENT DNA CONSENSUS PROTOCOLS OFFERED

Virus protocols:

- Adenoviridae
- Herpesviridae
- Papillomaviridae
- Non-mammalian Poxviruses
- Boca/Parvoviridae
- Polyomavirus

Bacterial protocols:

- Chlamydiales
- Mycobacteria
- Bacterial ID from isolated culture submission
- Mycoplasma
- Lawsonia

Protozoal protocols:

- Coccidia
- Cryptosporidium
- Leishmania
- Hepaticystis

Metazoan parasite protocols:

- Pentastomids
- Nematode
- Trematode
- Cestode
- Entamoeba
- Blastocystis
- Acanthocephala

Fungi protocols:

- Fungal ID
- Microsporidia

Real-time protocols (qPCR):

- Agamid Adenovirus (AgA)
- Intranuclear Coccidia (INC)
- Tortoise Herpesvirus 2 (THV2)

Additional tests, like barcoding, are under development and may be available on request

FEES

Standard PCR is \$100.00/test/sample. Turnaround time is 2-3 weeks.

Real-time PCR is \$90.00 for the first sample and \$30.00 for each additional sample. Discounts may be given for additional samples. Turnaround time is 2 weeks.

DATA USE

Data from samples may be used for medical education and research, including presentations at scientific meetings and publication in journals or textbooks. Names of clients will not be used to identify data resultant from samples, and all reasonable measures will be taken to maintain client confidentiality. We feel strongly that the advancement of medical science in diseases of nondomestic animals is important, and strongly encourage publication of rigorous studies. We are happy to work with submitting veterinarians toward appropriate publications.

SHIPPING SAMPLES

Notify lab via e-mail (ChildressA@ufl.edu) before shipping. Include a submission form with billing address, telephone and email contact. Please do not ship samples after Wednesday due to the fact that the University of Florida is closed during the weekend. Ship all samples on ice packs or dry ice via FedEx, UPS, or DHL to:

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