

SAMPLE SUBMISSION PROTOCOL FOR REVERSE TRANSCRIPTASE RNA-BASED PCR AND SEQUENCING

RT-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 reverse transcriptase - polymerase chain reactions (RT-PCR) that detects the presence of viral RNA from various viral groups. This requires the presence of virus in the sample submitted. The RT-PCR amplifies a short segment of cDNA specific to the virus. A positive result indicates a band consistent in size with the positive control was amplified and additional sequencing was performed to confirm that the amplified product is virus and not a non-specific amplification.

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 flaviviruses, arenaviruses of mammals, filoviruses, poxviruses of mammals, paramyxo and reoviruses of mammals, rhabdoviruses of mammals, bunyaviruses of mammals, and aphthoviruses.

COLLECTING SAMPLES

Ante-mortem:

Tissue biopsies, lung/tracheal washes, whole blood, cloacal 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 freeze immediately after collecting. 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. Samples should be kept frozen before and during shipment.

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. Samples should be kept frozen before and during shipment. Shipment should be on dry ice. 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 RNA CONSENSUS PROTOCOLS OFFERED

Astroviridae
Caliciviridae
Orthoreovirus
Aquareovirus
Paramyxovirinae:
 Ferlavirus/Morbillivirus/Respirovirus/Avulavirus/Rubulavirus/Sunshine
Pneumovirinae:
 Pneumovirus/Metapneumovirus
Non-mammalian Rhabdoviridae
Arenavirus
Enterovirus
Coronavirus
Bornavirus
Circovirus

Additional tests are under development and may be available on request

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\$130.00/test/sample for PCR and sequencing. Turnaround time is 2-3 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.

SHIP SAMPLES TO:

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 on the weekends. Ship all samples on dry ice via priority FedEx, UPS, or DHL to:

April Childress
University of Florida
College of Veterinary Medicine
2015 SW 16th Ave
Building 1017, Room V2-186
Gainesville, FL 32608
352-294-4420
ChildressA@ufl.edu