LSU | LADDL
Louisiana Animal Disease Diagnostic Laboratory

Molecular Diagnostics General Guidelines - PCR Testing

1) Submit samples as soon as possible for testing as degradation can affect testing quality.
2) Keep samples refrigerated before shipment.
3) Keep samples cold during transport (i.e., ice pack) (**non-incubated** *Tritrichomonas* exception).
4) Write name on submitted sample.

* **See Fee Schedule for current prices**. A discount is offered for some tests when submitting several samples, see Fee Schedule or call LADDL for pricing and details.

Results are reported within 2 business days.

For additional testing in other departments please submit additional samples according to the guidelines for that department. If you have any questions, please call LADDL at (225) 578-9777.

IMPORTANT NOTE

Recently vaccinated animals may give a false positive result by PCR, please interpret results accordingly.

Sample Type	Submission Requirements
CSF (cerebral spinal fluid)	At least 0.5ml in a sterile tube such as red top (EDTA acceptable but not recommended)
FFPE (formalin-fixed paraffin-embedded)	Send freshly cut FFPE sections with thickness up to 10 µm and area of 250 mm^2. Up to 8 sections can be combined in 1 tube. Send a duplicate tube for archiving.
Swabs	- Use non-culture swabs - **Preferred**: flocked swabs - **Acceptable**: foam, polyester, rayon swabs on plastic handles - **Not Recommended**: cotton swabs and wooden handled swabs - **NO** growth/culture medium, transport medium, or agar on swab or in tube - **NO** calcium alginate swabs - Send a double swab or 2 single swabs if possible - Send in red top or EDTA tube or broken off in a microcentrifuge or other capped tube - Avian swabs can be pooled according to the Avian Influenza guidelines below
Tissue	25-100 mg of fresh, fixed or frozen tissue
Tracheal wash	At least 1 ml in a sterile tube such as red top (EDTA acceptable but not recommended)
Urine	At least 1 ml in a sterile tube such as red top (EDTA acceptable but not recommended)
Whole blood	1-4 ml in Vacutainer with EDTA (lavender top) For blood under 1 ml use a Microtainer with EDTA (lavender top) **DO NOT** send serum

Bacteria	Preferred Sample Submission
Anaplasma marginale	Whole blood, tissue (spleen, lung, liver, kidney).
Anaplasma platys	Whole blood.
Bartonella henselae	Whole blood, tissue (spleen).
Chlamydophila psittaci	Ocular, cloacal or choanal swab, tissue (i.e., lung, liver, kidney).
Chlamydiaceae (including C. muridarum, suis, psittaci, abortus, felis, pecorum, pneumonia, trachomatis, caviae) - positive result only, species not specified	Ocular, cloacal or choanal swab, tissue (i.e., lung, liver, kidney).
Ehrlichia canis	Whole blood (buffy coat from EDTA blood), tissue (spleen, kidney, bone marrow).
Francisella noatunensis	Fish tissue (spleen, kidney).
Johne's Disease (Mycobacterium avium subspecies paratuberculosis)	Bovine feces (tested individually or pools of up to 5 samples)
Leptospira interrogans serovars (pathogenic) - positive result only, doesn't specify which serovar.	Blood (1st week of illness), urine or tissue (kidney).
Mycoplasma genus sequencing PCR	Nasal swab, tissue (i.e., lung, liver, kidney), fluid (i.e., whole blood, nasopharyngeal wash).
Mycoplasma bovis	Nasal swab, tissue, fluid.
Mycoplasma gallisepticum	Tracheal or palatine cleft swab.
Mycoplasma synoviae	Tracheal or palatine cleft swab.
Rhodococcus equi	Nasal swab, tracheal wash, tissue.
Streptococcus equi equi	Nasal swab, tracheal wash, tissue.

Parasites	Preferred Sample Submission
Tritrichomonas foetus	Use InPouch TF (BioMed Diagnostics) or smegma sample in 1.5 ml PBS in red top tube. See 'Tritrichomonas foetus Testing Guidelines' on our website for more information.
Trypanosoma cruzi	Whole blood (buffy coat from EDTA blood), tissue.

Other	Preferred Sample Submission
Canine c-kit PCR mutation exon 11	Canine MCT FFPE samples.

Virus	Preferred Sample Submission
Avian Influenza Virus (AIV)	Diagnostic testing for properly submitted samples. * * See end of document for submission requirements.
Bluetongue Virus (BTV)	Whole blood or vascularized tissue (spleen, lung).
Bovine Leukemia Virus (BLV)	Whole blood (buffy coat) or tissue (spleen, liver, lymph node).
Bovine Viral Diarrhea Virus (BVDV)	Whole blood (buffy coat) or lymphatic tissue (i.e., spleen, thymus).
Bovine Respiratory Syncytial Virus (BRSV)	Nasal swabs, tissues (lung), bronchoalveaolar lavage.
Canine Distemper Virus (CDV)	CSF, tissue (brain, lung, spleen), nasopharyngeal swab, nasopharyngeal wash.
Canine Influenza Virus (CIV)	Nasal swab, tracheal wash, tissue.
Eastern Equine Encephalitis Virus (EEE)	Mammalian tissue (brain stem), avian oropharyngeal swab, avian tissue (brain, kidney, spleen), avian whole blood.
Epizootic Hemorrhagic Disease (EHD)	Whole blood or vascularized tissue (spleen, lung).
Equine Herpesvirus Type 1 (EHV-1)	Nasal swab, tracheal wash, tissue, whole blood or buffy coat from EDTA or citrated blood. For increased likelihood of detection, a nasal swab AND a buffy coat is recommended.
Equine Herpesvirus Type 4 (EHV-4)	Nasal swab, tracheal wash, tissue.
Equine Influenza Virus (EIV)	Nasal swab, tracheal wash, tissue.
Equine Rhinitis A Virus (ERAV)	Urine, nasal wash, or pharyngeal swab.
Exotic Newcastle Disease (END) Avian Paramyxovirus Type 1 (APMV-1)	Diagnostic testing for properly submitted samples. See end of document for submission requirements.
Feline Herpesvirus (FHV)	Ocular swab, nasopharyngeal swab, nasopharyngeal wash, tissue (lung, trachea, turbinate, spleen, tonsil).
Infectious Bovine Rhinotracheitis (IBR / BHV-1)	Bovine semen, nasal swabs, tissue.
Infectious Laryngotracheitis Virus (ILTV)	Trachea swab, tracheal wash, tissue.
Koi Herpesvirus (KHV)	Fish tissue.
West Nile Virus (WNV)	Avian whole blood, avian oropharyngeal swab, avian tissue (brain stem, kidney, spleen), mammalian tissue (brain).
White Spot Syndrome Virus (WSSSV)	Whole crawfish, refrigerated or frozen the day of capture. 60 animals for a cultivated pond survey, 120 animals for a wild-caught survey. Please call lab for more instructions.

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Submission requirements for AIV and END/APMV-1 PCR testing*

- Specimens should be held on ice pack immediately following collection until transferred to the testing laboratory or other refrigerated storage.
- Tubes should be stored and transferred in an upright position to reduce chance of leakage.
- IAV and APMV-1 have been shown to be stable in BHI when stored at refrigeration (4°C) for up to 96 hours, with consideration given to the length of time needed at the laboratory for sample processing.
- If samples have been frozen (-70°C), they should remain frozen until delivered to the testing laboratory.
- Specimens should never be stored in the freezer portion (-20°C) of a standard refrigerator/freezer unit with an automatic defrost cycle (specimens will go through freeze/thaw, which is detrimental to the survival of virus and viral nucleic acid).

Sampling source	Preferred Specimen	Sample Collection
Gallinaceous poultry (e.g., chickens, turkeys, pheasants, quail)	**Tracheal or oropharyngeal (TR/OP) preferred**	• FOR FADs – typically 5 swabs/ pool in at least 3 mls of BHI. • Up to 11 swabs/pool in at least 5.5 mls of BHI pooled by sample route and species for TR/OP swabs from gallinaceous species only.
	Cloacal swab (CL) may be used	• Up to 5 swabs/pool at least 3 mls of BHI pooled by sample route and species.
Domestic waterfowl (production)	**CL preferred**, TR/OP swab may be used	• Up to 5 swabs/pool from a single flock and species in at least 3 mls of BHI.
Wild/captive waterfowl species	**TR/OP and CL** swabs may be used	• Collect USDA Wildlife Services Surveillance samples by pooling 1 CL and 1 OP swab from a **single bird** in one 3 ml BHI tube; this approach may also be used for captive waterfowl that are openly housed. • Captive flocks in closed, common housing may be pooled 5 swabs/pool in at least 3 mls BHI by sample route and **species.**
Other wild/free living/captive /pet species	Typically **CL swabs**; fresh fecal samples may be used – call the NVSL for guidance	• Captive flocks in closed, common housing may be pooled 5 swabs/pool in at least 3 mls BHI by sample route and **species group** (e.g., passerines).
Any avian species	Tissue samples	• Pool by system from a **single** bird (e.g., respiratory, enteric, reproductive) - mince tissue and place in 3 mls BHI.

***For more information, see the official document:**
https://www.aphis.usda.gov/animal_health/lab_info_services/downloads/WIAV0020.pdf

Check for the newest version of the above document on the government website:
https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/emergency-management/hpai/fadprep-hpai