Veterinary Diagnostic Services PCR Assays

Avian PCR Tests: Choose one specimen from the list for each requested test. Please contact VDS before requesting PCR testing on a specimen that is not listed below.

Pathogen	Recommended Specimens	Other Acceptable Specimens
Avian Influenza A virus (AIV) **see Note 1**	oropharyngeal swab lung cloacal swab (preferred for waterfowl)	trachea spleen brain
Avian orthoreovirus	joint swab synovium joint fluid	
Avian leukosis virus J strain	tumor tissue (spleen, liver, bursa)	
Chicken anemia virus (CAV)	spleen bursa	thymus bone marrow
Chlamydophila psittaci	nasopharyngeal swab conjunctival swab lung cloacal swab feces	liver spleen
Infectious bronchitis virus (IBV)	trachea or bronchus tracheal swab lung oviduct	kidney
Infectious bursal disease virus (IBDV)	bursa	
Infectious laryngotracheitis virus (ILTV)	trachea tracheal swab conjunctival swab oropharyngeal swab	
Marek's disease virus (MDV)	spleen tumor tissue	
Mycoplasma gallisepticum	sinus fluid or swab oropharyngeal swab nasopharyngeal swab trachea or tracheal swab	air sac lung
Newcastle disease virus (APMV-1) **see Note 1**	oropharyngeal swab lung cloacal swab	trachea brain spleen kidney

Ornithobacterium rhinotracheale	sinus fluid or swab oropharyngeal swab nasopharyngeal swab	trachea or tracheal swab lung
West Nile virus	brain cloacal swab	feces blood in heparin heart spleen lung liver kidney

Additional Notes about Avian PCR Tests

1. A real-time PCR assay that targets the matrix gene is used for initial detection of Influenza A virus. Positive samples will be tested for the highly pathogenic subtypes (H5 & H7). Non-negative results will be reported to the Canadian Food Inspection Agency, and confirmatory testing will be done at the National Centre for Foreign Animal Disease. The submitting veterinarian will be contacted by the CFIA. The same reporting procedure applies to Newcaste disease virus.

Pooling Samples

Pooling samples can reduce the sensitivity of PCR assays but may allow testing a larger subset of animals. **Animals exhibiting clinical signs and organ samples with gross lesions must always be sampled individually**. Pooled samples (of the same type) can be acceptable for the purpose of pathogen surveillance programs.

VDS will accept the following pools:

AIV – oropharyngeal or cloacal swabs – up to 5; do not mix swab types.
IBDV – bursa – up to 3.
CAV – spleen or bursa – up to 3.

Cat PCR Tests: Choose one specimen from the list for each requested test. Please contact VDS before requesting PCR testing on a specimen that is not listed below.

Pathogen	Recommended Specimens	Other Acceptable Specimens
Feline Upper Respiratory Tract Panel (FHV-1, FCV, *C. felis*, *M. felis*)	combined conjunctival & oropharyngeal swabs (extracted together for one PCR panel) conjunctival swab	oropharyngeal swab nasal swab
Felid herpesvirus 1 (FHV-1)	conjunctival swab oropharyngeal swab	nasal swab lung
Feline calicivirus (FCV)	oral swab (ulcerated mucosa) conjunctival swab oropharyngeal swab	nasal swab lung
Chlamydophila felis	conjunctival swab nasal swab	
Mycoplasma felis	conjunctival swab	
Mycoplasma haemofelis	blood in EDTA	
Feline panleukopenia virus	rectal swab feces jejunum	

Additional Notes about Feline PCR Tests

1. In most cases that present with conjunctivitis and rhinitis, the URT Panel will be the most appropriate test for a complete and cost-effective diagnostic approach.

2. Nasal swabs are appropriate for respiratory tract pathogens if rhinitis is the predominant clinical sign. Lung or fluids from transtracheal aspirate or bronchoalveolar lavage can be tested for FHV-1 and FCV if clinical or pathology findings indicate the rare pneumonic manifestations of infection.

3. If bacterial culture is also warranted, a bacterial transport medium swab (nasal, conjunctival, rectal) should be collected in addition to the virology swab.

4. If not already done, a concurrent CBC is strongly recommended when testing blood samples for *Mycoplasma haemofelis*.

Dog PCR Tests: Choose one specimen from the list for each requested test. Please contact VDS before requesting PCR testing on a specimen that is not listed below.

Pathogen	Recommended Specimens	Other Acceptable Specimens
Anaplasma phagocytophilum	blood in EDTA	
Borrelia burgdorferi	synovial fluid, synovium lymph node skin (tick attachment site)	blood in EDTA
Mycoplasma haemocanis	blood in EDTA	
Canine distemper virus	conjunctival swab oropharyngeal swab blood in EDTA lymph node cerebrospinal fluid brain	urinary bladder lung stomach intestine nasal swab
Canine parvovirus	rectal swab feces jejunum	
Leptospira spp.	urine	kidney, liver

Additional Notes about Canine PCR tests

1. It is possible to detect *Borrelia burgorferi* DNA in blood during the acute stage of Lyme disease, but the PCR test will generally have low sensitivity on blood samples. The PCR test for *B*. *burgdorferi* should be done in conjunction with serologic testing for antibody (IDEXX SNAP 4Dx^{TM}).

2. If not already done, a concurrent CBC is strongly recommended when testing blood samples for *Anaplasma phagocytophilum* and *Mycoplasma haemocanis*.

3. The most appropriate specimen for the Canine distemper virus PCR will be determined by the clinical manifestation or histopathology findings.

Horse PCR Tests: Choose one specimen from the list for each requested test. Please contact VDS before requesting PCR testing on a specimen that is not listed below.

Pathogen	Recommended Specimens	Other Acceptable Specimens
Anaplasma phagocytophilum	blood in EDTA	
Bacillus anthracis	blood in a red-top tube	
Equid herpesvirus (EHV 1 & EHV 4)	nasal swab nasopharyngeal swab nasopharyngeal wash spinal cord brain cerebrospinal fluid fetal lung fetal liver	fetal spleen
Equine arteritis virus (EAV)	blood in EDTA nasal swab lung fetal lung placenta	fetal spleen fetal liver fetal lymph node
Influenza A virus	nasal swab (deep) nasopharyngeal swab nasopharyngeal wash endotracheal wash transtracheal wash bronchoalveolar lavage lung	
Leptospira spp.	urine kidney fetal kidney	liver spleen brain blood in EDTA eye (uvea) placenta
Neorickettsia risticii	blood in EDTA feces	colon spleen
West Nile virus	brain spinal cord cerebrospinal fluid	

Ruminant PCR Tests: Choose one specimen from the list for each requested test. Please contact VDS before requesting PCR testing on a specimen that is not listed below.

Pathogen	Recommended Specimens	Other Acceptable Specimens
Anaplasma marginale	blood in EDTA	
Bacillus anthracis	blood in a red-top tube	
Bovine Coronavirus	rectal swab feces small intestine large intestine	nasal swab lung
Bovine herpesvirus 1 (IBRV)	nasal swab conjunctival swab fetal liver fetal lung	trachea lung semen
Bovine respiratory syncytial virus (BRSV)	nasal swab lung	
Bovine Rotavirus A	rectal swab feces small intestine	
Bovine viral diarrhea virus (BVDV)	serum blood in EDTA lymph node nasal swab oral mucosa (ulcerated) fetal thymus fetal spleen semen	rectal swab feces intestine lung spleen fetal lung fetal liver
Chlamydophila abortus	placenta uterine fluids	fetal liver fetal lung
Coxiella burnetii	placenta uterine fluids	fetal liver fetal lung
Cryptosporidium parvum	rectal swab feces small intestine	
Leptospira spp.	urine kidney fetal kidney	liver spleen lung brain placenta
Malignant catarrhal fever virus (OHV-2)	blood in EDTA lymph node or tonsil	ulcerated mucosa or any organ with typical lesions
Mycobacterium avium paratuberculosis	ileum mesenteric lymph node	

Mycoplasma bovis	lung synovium joint exudate or swab	nasal swab milk
Neospora caninum	fetal brain (cattle)	fetal lung
Toxoplasma gondii	placenta (sheep & goats) fetal brain	fetal lung fetal liver fetal kidney
Ureaplasma diversum	placenta (cattle)	fetal lung fetal stomach content

Additional Notes about Ruminant PCR Tests

1. Single PCR tests for BVDV, BHV-1, *Neospora caninum* and *Ureaplasma diversum* are included in the fee for bovine fetus necropsies.

2. Single PCR tests for *Chlamydophila abortus*, *Coxiella burnetii* and *Toxoplasma gondii* are included in the fee for ovine and caprine fetus necropsies.

3. When *Anaplasma marginale* infection is suspected, a CBC should be ordered along with the PCR test. Both tests can be done on the same EDTA blood sample.

Swine PCR Tests: Choose one specimen from the list for each requested test. Please contact VDS before requesting PCR testing on a specimen that is not listed below.

Pathogen	Recommended Specimens	Other Acceptable Specimens
Brachyspira spp. **see Note 1**	colon feces	cecum
Influenza A virus (SIV) Subtyping – **see note 2**	nasal swab lung oral fluids	trachea (ulcerated mucosa)
Lawsonia intracellularis	ileum feces	jejunum colon
Leptospira spp.	urine kidney fetal kidney	liver spleen
Mycoplasma hyopneumoniae	lung nasal swab tonsil oral fluids	
Mycoplasma hyorhinis **see Note 3**	joint swab synovium joint fluid	organs affected by polyserositis (lung, heart, liver, spleen) nasal swab tonsil oral fluids
Mycoplasma hyosynoviae **see Note 3**	joint swab synovium joint fluid	tonsil nasal swab oral fluids
Porcine circovirus-2 (PCV-2)	serum tonsil lymph node oral fluids fetal lung	lung fetal heart
Porcine epidemic diarrhea virus (PEDV)	rectal swab feces oral fluids environmental swab **– see Note 4** jejunum	
Porcine parvovirus	fetal lung	
Porcine reproductive and respiratory syndrome virus (PRRSV) - North American & European strains	serum blood swab lung, fetal lung oral fluids nasal swab semen	tonsil lymph node environmental swab brain

Rotavirus (A, B, C)	rectal swab feces jejunum	
Suid herpesvirus 2 (CMV)	nasal swab turbinate mucosa	lung
Swine Deltacoronavirus (SDCV)	rectal swab feces oral fluids environmental swab jejunum	
Transmissible gastroenteritis virus (TGEV)	rectal swab feces oral fluids environmental swab jejunum	

Additional Notes about Porcine PCR tests

1. A real-time PCR assay is used to detect DNA from the genus *Brachyspira*. When a positive is obtained, sequence analysis is done to determine the species.

2. A real-time PCR assay that targets the matrix gene is used for initial detection of Influenza A virus. Subtyping will automatically be done unless declined by the client at the time of submission. Subtyping involves additonal real-time PCR assays for the H1, H3, N1 and N2 genes. When there are multiple positives among a set of samples, one of the strongest positives (lowest Ct value) will be selected for subtyping.

3. *Mycoplasma hyorhinis* causes polyserositis and polyarthritis in 3 to 12 week old pigs (generally under 10 weeks of age; possibly up to 15 weeks). Unlike *Mycoplasma hyopneumoniae*, it is not a significant cause of pneumonia. In the absence of pleuritis, positive PCR results on lung are probably detecting the DNA of commensal organisms from the upper respiratory tract. Virulent strains of *Haemophilus parasuis* cause identical lesions in the same age group (Glasser's disease), so separate samples should be submitted for bacterial culture. *Mycoplasma hyosynoviae* causes polyarthritis at 10 to 30 weeks of age. Testing joint samples for both *M*. *hyorhinis* and *M*. *hyosynoviae* is warranted when polyarthritis occurs during the overlapping age range (approximately 10 to 15 weeks). To detect pigs that are carrying *M*. *hyorhinis* or *M*. *hyosynoviae* in the upper respiratory tract, PCR testing can be done on oral fluids, nasal swabs or tonsil – positive results will not be indicative of active infection/disease causation.

4. Swabbing environmental surfaces (trucks, wash bays, pens, loading docks, etc.) to test for porcine coronaviruses should only be done according to the OSHAB protocol. VDS will only accept fluid samples for testing, not dry or moist pads. Clients can find the protocol here: http://www.opic.on.ca/images/pdfs/Trailer_WashBaySamplingProtocol.pdf or contact VDS for a copy.

Pooling Samples

Pooling samples can reduce the sensitivity of PCR assays but may allow testing a larger subset of animals. **Animals exhibiting clinical signs and organ samples with gross lesions must always be sampled individually**. Pooled samples (of the same type) can be acceptable for the purpose of pathogen surveillance programs.

VDS will accept the following pools:

PRRSV – serum or blood swabs – up to 5.
PCV-2 – serum – up to 5.
SIV – nasal swabs – up to 2.
***Mycoplasma* spp.** – tonsil – up to 2.
Lawsonia intracellularis – ileum – up to 2.
PEDV, **TGEV**, **SDCV** & **porcine rotaviruses** – feces – up to 5 samples sealed in a plastic specimen container with a screw cap (medical urine container). A small portion from each pig is sufficient; total sample volume should not exceed 50 mL (the container should be no more than half full). Nitrile or latex gloves should never be used as specimen containers.

Rectal swabs – up to 3.

Oral fluids and environmental swabs are by definition pooled samples. VDS will not pool these samples further.

Bacterial Typing

Certain bacteria cultured and identified in the Microbiology Section may be further characterized by PCR testing. Bacterial typing is not done directly on samples.

Clostridium perfringens **Typing**: Isolates are tested for the exotoxin genes alpha, beta, epsilon and iota, and for the enterotoxin (cpe) and beta2- toxin genes. Genotypes (A - E) are based on the combination of exotoxin genes that are present in the isolate: A (alpha), B (alpha, beta and epsilon), C (alpha and beta), D (alpha and epsilon), E (alpha and iota).

E.coli **Typing**: Isolates are tested by PCR for the following virulence factor genes: F4 (K88), F5 (K99), F18ab/F18ac (FedA1), heat-labile toxin (LT), heat stable enterotoxins (STa and STb), Shigatoxins Stx1 (vt1) and Stx2 (vt2), attaching and effacing (Intimin or eae). Testing for virulence factors relevant to extraintestinal infection will be referred to the *Escherichia coli* Laboratory at the University of Montreal.

Pasteurella multocida **Typing**: Groups A and D are important in pigs. Type A is mostly associated with pneumonia and type D with progressive atrophic rhinitis. Isolates are tested by PCR for the capsular serotype (A, B, C, D, E, F), and type D isolates are tested for the toxin gene that is needed to cause progressive atrophic rhinitis.