

# Virology and Molecular Diagnostics

## Veterinary Diagnostic Services



The Virology and Molecular Diagnostics Section of the Veterinary Diagnostics Services (VDS) uses polymerase chain reaction (PCR) assays to detect pathogens (viruses as well as certain bacteria and protozoa) and serological tests to detect antibodies to pathogens.

## Contents

Contact Us .....	1
PCR Testing.....	1
PCR Test Turnaround Times .....	1
Specimen Selection and Collection for PCR .....	2
Swabs .....	3
Blood swabs for PRRS virus PCR .....	4
Environmental swabs (Swiffer™ cloth collection method) .....	4
Applicable to all swabs .....	4
Oral Fluids .....	4
Virus Isolation .....	5
DNA Sequencing .....	5
Avian PCR Tests.....	5
Additional Notes about Avian PCR Tests .....	7
Feline PCR Tests.....	7
Additional Notes about Feline PCR Tests.....	8
Canine PCR Tests .....	8
Additional Notes about Canine PCR tests .....	9
Ruminant PCR Tests .....	10
Additional Notes about Ruminant PCR Tests .....	13
Porcine PCR Tests.....	13
Additional Notes about Porcine PCR tests .....	15
Pooling Samples .....	16
Bacterial Typing .....	17
Serology.....	17
Serology Guidelines.....	18
Shipping Guidelines .....	19

## Contact Us

- Phone: 204-945-8220 in Winnipeg
- Email: [virology@gov.mb.ca](mailto:virology@gov.mb.ca)
- Website: [manitoba.ca/agriculture/vds](http://manitoba.ca/agriculture/vds)

## PCR Testing

- PCR is a method for amplifying a portion of deoxyribonucleic acid (DNA), using a set of two short synthetic oligonucleotides (primers). Detection of the amplified product (amplicon) indicates the presence of target pathogen in the sample.
- In conventional PCR assays, gel electrophoresis is used to compare amplicons with positive controls. Real-time PCR assays use oligonucleotide probes with fluorescent signals to detect the amplicon during the PCR. Real-time PCR is semiquantitative, in that a higher amount of target DNA in the sample will correlate with detection after fewer PCR cycles. To detect ribonucleic acid (RNA) viruses, a reverse transcriptase enzyme is used to make a DNA copy of the RNA target. PCR is then applied to the copy DNA.
- A positive PCR result only indicates the presence of target nucleic acid and does not necessarily indicate the presence of an infectious pathogen.

## PCR Test Turnaround Times

- Turnaround time (TAT) is based on when samples and complete paperwork are received by VDS. The TAT may increase by up to a day for serum samples that require centrifugation. Incomplete or erroneous submission forms will delay sample processing. For sets of more than five samples with unique identification (e.g., animal I.D., pen number), clients must email the multiple ID downloadable sheet in addition to the submission form.
- The standard turnaround time for all PCR tests is one to three business days.
  - Rush requests: Same day testing may be provided during a disease outbreak or with prior arrangement to accommodate a specific business need. Samples and complete paperwork must be received by 9 a.m.

- When sample volumes are high, turnaround times may be longer than usual. VDS staff will assess if a shorter than usual turnaround time is feasible. Clients will be informed when a rush request cannot be accommodated.

## Specimen Selection and Collection for PCR

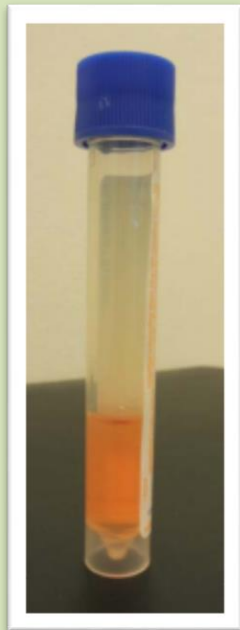
- Refer to the [PCR Assay List](#) for available tests and appropriate specimens.
- Sample as soon as possible after the onset of clinical signs.
- Specimens should be stored at 4°C and transported immediately to the laboratory to obtain best results.
- All samples must be in leak proof containers. All paperwork and container exteriors must be clean. This will help VDS to maintain quality of diagnostic tests and to contain pathogens.

Whole Blood	Feces	Semen	Serum
<ul style="list-style-type: none"> <li>• Use tubes containing an anti-coagulant: EDTA (purple top), heparin (green top) or citrate (blue top). Submit a minimum of 2-3 ml.</li> </ul>	<ul style="list-style-type: none"> <li>• Submit approximately 2 ml in a 10 ml screwcap tube or up to 50 ml in a 100 ml urine container (or equivalent container with a screw cap). Sample containers must be securely closed. Outer surfaces must be clean and dry. <b>Do not submit feces in plastic bags or gloves.</b></li> </ul>	<ul style="list-style-type: none"> <li>• Submit 5 ml individual samples in plain red top serum tubes for PRRSV PCR. Do not submit semen in extender.</li> </ul>	<ul style="list-style-type: none"> <li>• Refer to the <a href="#">Serology</a> section below.</li> </ul>

## Swabs

- **Nasal, oropharyngeal, laryngeal or cloacal swabs**

- Use screw cap tubes containing viral transport medium (e.g., Starplex Scientific Inc., Multitrans System, S160-100) and polyester or dacron swabs with plastic handles. Check expiry dates. Viral transport fluid must be pink. Discard the tube if fluid is yellow (sign of bacterial overgrowth).
- If viral transport media tubes are not available, place a few millilitres of saline in a red-top tube or other sterile container with a secure lid.
- After swabbing the animal, **vigorously swirl** the swab in the viral transport media to dispel the contents of the swab into the fluid. Both nostrils from the same animal can be pooled in one tube. Do not pool swabs from different animals into one tube.
- Remove the swab from the fluid, while pressing and rolling the swab firmly along the inside wall of tube to squeeze residual contents from swab.
- Discard the swab.



**Normal viral transport medium with pink color**



**Spoiled viral transport medium. Do not use if fluid is yellow.**

- **Blood swabs for PRRS virus PCR**
  - Use a 2 ml screw cap tube containing 0.5 ml of sterile saline and polyester or dacron swabs with plastic handles.
  - If you are using alcohol to disinfect, wipe alcohol off and allow the area to dry completely.
  - Prick the ear vein or other appropriate vein with the needle and totally soak the swab in the blood. Use only one blood swab per screw cap tube.
  - Immerse the swab in the tube of saline. Cut the plastic handle end to fit the swab end into the tube. Leave the swab in the tube.
  
- **Environmental swabs (Swiffer™ cloth collection method)**
  - Submit the fluid sample only and not the cloth.
  - Note that chemical disinfectants and petroleum compounds may inhibit PCR.
  
- **Applicable to all swabs**
  - Securely close the screw cap (do not over-tighten or they will leak).
  - Label each tube clearly with **black** permanent marker.
  - Package tubes in a clean plastic zipper lock bag or a box. **Avian influenza submissions must be double-bagged.**
  - Seal the paperwork in a plastic zipper lock bag.
  - Immediately refrigerate at 4°C (do not freeze).
  - Submit a VDS multiple ID downloadable sheet.
  - Refer to [Shipping Guidelines](#) below.

## Oral Fluids

- VDS offers PCR testing on oral fluids for PRRSV, PEDV and other swine pathogens. Oral fluids are a mixture of saliva and oral mucosal transudate. Saliva is produced by the salivary glands. Oral mucosal transudate enters the mouth by crossing the buccal mucosa from the capillaries. Oral fluid samples inevitably also contain respiratory secretions and fecal material. Oral fluids can contain both pathogens and antibodies.
  
- Submit a minimum of 5 ml of oral fluid sample in a clean tube for PCR testing. Keep oral fluid samples refrigerated at all times and submit on the day of collection. Do not submit rope.
  
- Find a video presentation on oral fluid collection at:  
[cfsph.iastate.edu/video.php?link=oral-fluid-collection-in-pigs](https://cfsph.iastate.edu/video.php?link=oral-fluid-collection-in-pigs)  
 Contact VDS for information about oral fluids collection methods.

## Virus Isolation

- Many of the samples used for PCR can also be used for virus isolation, if collected and stored properly. VDS will forward samples to referral laboratories for virus isolation, upon request.
- If virus isolation is anticipated, the clinic must instruct VDS to store the samples at -70 °C immediately after the samples have been processed.

## DNA Sequencing

- DNA sequencing is a molecular tool used to characterize the genome of a microorganism. DNA sequencing identifies which strain of virus is present in the clinical sample and in some cases, helps to differentiate field and vaccine strains of viruses. Only samples testing strongly positive by conventional or real-time PCR can be used for DNA sequencing.

The clinic must provide the following information:

- VDS Case number
- sequencing test to be performed
- correct identity of sample(s) to be tested by the reference lab
- reference lab to which samples will be sent

## List of PCR Assays

The Virology and Molecular Diagnostics section of the Veterinary Diagnostics Services (VDS) uses polymerase chain reaction (PCR) assays to detect pathogens (viruses as well as certain bacteria and protozoa).

## Avian PCR Tests

Select one specimen from the list for each requested test. Please contact VDS before requesting PCR testing on a specimen that is not on the following list.

Pathogen	Specimens
Avian Influenza A virus (AIV) real-time PCR <b>Refer to Note 1</b>	oropharyngeal swab lung cloacal swab (preferred for waterfowl) trachea

	spleen brain
Avian orthoreovirus real-time PCR	joint swab synovium joint fluid
Avian leukosis virus J strain PCR	tumor tissue (spleen, liver, bursa)
Chicken anemia virus (CAV) PCR	spleen bursa thymus bone marrow
<i>Chlamydophila psittaci</i> real-time PCR	nasopharyngeal swab conjunctival swab lung cloacal swab feces liver spleen
Infectious bronchitis virus (IBV) real-time PCR	trachea or bronchus tracheal swab lung oviduct kidney
Infectious bursal disease virus (IBDV) real-time PCR	bursa
Infectious laryngotracheitis virus (ILT) real-time PCR	trachea tracheal swab conjunctival swab oropharyngeal swab
Marek's disease virus (MDV) real-time PCR	spleen tumor tissue
<i>Mycoplasma gallisepticum</i> PCR	sinus fluid or swab oropharyngeal swab nasopharyngeal swab trachea or tracheal swab air sac lung
Newcastle disease virus (APMV-1) real-time PCR <b>Refer to Note 1</b>	oropharyngeal swab lung cloacal swab trachea brain spleen kidney
<i>Ornithobacterium rhinotracheale</i> PCR	sinus fluid or swab oropharyngeal swab nasopharyngeal swab trachea or tracheal swab

	lung
West Nile virus PCR	brain cloacal swab feces blood in heparin heart spleen lung liver kidney

### Additional Notes about Avian PCR Tests

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 (CFIA), 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 Newcastle disease virus.

## Feline PCR Tests

Select 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	Specimens
Feline Upper Respiratory Tract real-time PCR Panel (FHV-1, FCV, <i>C. felis</i> , <i>M. felis</i> )	combined conjunctival & oropharyngeal swabs (extracted together for one PCR panel) conjunctival swab oropharyngeal swab nasal swab
Felid herpes virus 1 (FHV-1) real-time PCR -also see Feline Upper Respiratory Tract Panel	conjunctival swab oropharyngeal swab nasal swab lung
Feline calicivirus (FCV) real-time PCR -also see Feline Upper Respiratory Tract Panel	oral swab (ulcerated mucosa) conjunctival swab oropharyngeal swab nasal swab lung
<i>Chlamydomphila felis</i> real-time PCR -also see Feline Upper Respiratory Tract Panel	conjunctival swab nasal swab
<i>Mycoplasma felis</i> real-time PCR -also see Feline Upper Respiratory Tract Panel	conjunctival swab



Feline leukemia virus (detects viral RNA & proviral DNA).	blood in EDTA conjunctival swab oropharyngeal swab lymph node bone marrow
<i>Mycoplasma haemofelis</i> real-time PCR	blood in EDTA
Feline panleukopenia virus real-time PCR	rectal swab feces jejunum

## Additional Notes about Feline PCR Tests

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.

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 forms of these infections.

If bacterial culture is also warranted, collect a bacterial transport medium swab (nasal, conjunctival or rectal) in addition to the virology swab.

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

## Canine PCR Tests

Select one specimen from the list for each requested test. Please contact VDS before requesting PCR testing on a specimen that is not listed.

Pathogen	Specimens
<i>Anaplasma phagocytophilum</i> real-time PCR	blood in EDTA
<i>Borrelia burgdorferi</i> real-time PCR	synovial fluid, synovium lymph node skin (tick attachment site) blood in EDTA
<i>Mycoplasma haemocanis</i> real-time PCR	blood in EDTA
Canine distemper virus real-time PCR	conjunctival swab oropharyngeal swab blood in EDTA lymph node

	cerebrospinal fluid brain urinary bladder lung stomach intestine nasal swab
Canine parvovirus real-time PCR	rectal swab feces jejunum
<i>Leptospira</i> spp. real-time PCR	urine kidney, liver

### Additional Notes about Canine PCR tests

It is possible to detect *Borrelia burgdorferi* 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™).

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

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

### Equine PCR Tests

Select 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	Specimens
<i>Anaplasma phagocytophilum</i> real-time PCR	blood in EDTA
<i>Bacillus anthracis</i> PCR	blood in a red-top tube
<i>Borrelia burgdorferi</i> real-time PCR	blood in EDTA synovial fluid, synovium lymph node skin (tick attachment site)
Equid herpesvirus (EHV 1 & EHV 4) PCR	nasal swab nasopharyngeal swab nasopharyngeal wash spinal cord

	brain blood in EDTA cerebrospinal fluid fetal lung fetal liver fetal spleen
Equine arteritis virus (EAV) PCR	blood in EDTA nasal swab lung fetal lung placenta fetal spleen fetal liver fetal lymph node
Equine Influenza A virus (EIV) PCR	nasal swab (deep) nasopharyngeal swab nasopharyngeal wash endotracheal wash transtracheal wash bronchoalveolar lavage lung
<i>Leptospira</i> spp real-time PCR	urine kidney fetal kidney liver spleen brain blood in EDTA eye (uvea) placenta colon spleen
<i>Neorickettsia risticii</i> PCR	blood in EDTA feces
West Nile virus PCR	brain spinal cord cerebrospinal fluid

## Ruminant PCR Tests

Select 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	Specimens
<i>Anaplasma marginale</i> real-time PCR	blood in EDTA spleen
<i>Bacillus anthracis</i> PCR	blood in a red-top tube
Bovine Coronavirus real-time PCR -also see Calf Enteric Panel	rectal swab feces small intestine large intestine nasal swab lung
Bovine Respiratory real-time PCR Panel (BVDV, PI3, IBRV, BRSV)	nasal swab lung
Bovine herpesvirus 1 (IBRV) real-time PCR -also see Bovine Respiratory Panel	nasal swab conjunctival swab fetal liver fetal lung trachea lung semen
Bovine respiratory syncytial virus (BRSV) real-time PCR -also see Bovine Respiratory Panel	nasal swab lung
Bovine Rotavirus A real-time PCR -also see Calf Enteric Panel	rectal swab feces small intestine
Bovine viral diarrhea virus (BVDV) real-time PCR -also see Bovine Respiratory Panel	serum blood in EDTA lymph node skin biopsy (e.g., ear notch) nasal swab oral mucosa (ulcerated) fetal thymus fetal spleen semen rectal swab feces intestine lung spleen fetal lung fetal liver
Calf Enteric real-time PCR Panel (Bovine Coronavirus, Bovine Rotavirus, <i>C. parvum</i> )	rectal swab feces small intestine

<i>Chlamydophila abortus</i> real-time PCR	placenta uterine fluids fetal liver fetal lung
<i>Coxiella burnetii</i> PCR	placenta uterine fluids fetal liver fetal lung
<i>Cryptosporidium parvum</i> real-time PCR -also see Calf Enteric Panel	rectal swab feces small intestine
<i>Leptospira</i> spp. real-time PCR	urine kidney fetal kidney liver spleen lung brain placenta
Malignant catarrhal fever virus (OHV-2) real-time PCR	blood in EDTA lymph node or tonsil ulcerated mucosa or any organ with typical lesions
<i>Mycobacterium avium paratuberculosis</i> real-timePCR	ileum mesenteric lymph node feces
<i>Mycoplasma bovis</i> real-time PCR	lung synovium joint exudate or swab nasal swab milk ocular swab
<i>Neospora caninum</i> real-time PCR	fetal brain fetal lung
Parainfluenza virus 3 (PI3) real-time PCR -included only in the Bovine Respiratory Panel	nasal swabs lung
<i>Toxoplasma gondii</i> real-time PCR	placenta (sheep & goats) fetal brain fetal lung fetal liver fetal kidney
<i>Ureaplasma diversum</i> real-time PCR	placenta (cattle) fetal lung fetal stomach content

## Additional Notes about Ruminant PCR Tests

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

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

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

## Porcine PCR Tests

Pathogen	Specimens
<i>Brachyspira</i> spp. real-time PCR <b>Refer to Note 1</b>	colon feces cecum
Influenza A virus (SIV) real-time PCR SIV Subtyping real-time PCR - <b>Refer to Note 2</b>	nasal swab lung oral fluids trachea (ulcerated mucosa)
<i>Lawsonia intracellularis</i> real-time PCR	ileum feces jejunum colon
<i>Leptospira</i> spp. real-time PCR	urine kidney fetal kidney liver spleen
<i>Mycoplasma hyopneumoniae</i> real-time PCR	lung nasal swab tonsil oral fluids – <b>Refer to Note 5</b> environmental swabs – <b>Refer to Note 4</b> laryngeal swabs piglet processing fluid
<i>Mycoplasma hyorhinis</i> real-time PCR <b>Refer to Note 3</b>	joint swab synovium joint fluid pleura or peritoneum organs affected by polyserositis (lung, heart, liver, spleen) nasal swab

	tonsil oral fluids
<i>Mycoplasma suis</i> real-time PCR	blood in EDTA spleen
<i>Mycoplasma hyosynoviae</i> real-time PCR <b>Refer to Note 3</b>	joint swab synovium joint fluid nasal swab oral fluids
Porcine circovirus-2 (PCV-2) real-time PCR	serum tonsil lymph node oral fluids fetal lung lung fetal heart piglet processing fluid
Porcine epidemic diarrhea virus (PEDV) real-time PCR -also see Porcine Coronavirus Panel	rectal swab feces oral fluids – <b>Refer to Note 5</b> environmental swab – <b>Refer to Note 4</b> jejunum
Porcine Coronavirus (PEDV, TGEV, DeltaCoV) real-time PCR Panel	rectal swab feces oral fluids – <b>Refer to Note 5</b> environmental swab – <b>Refer to Note 4</b> jejunum
Porcine parvovirus real-time PCR	fetal lung
Porcine reproductive and respiratory syndrome virus (PRRSV) real-time PCR - North American & European strains	serum blood swab lung, fetal lung oral fluids nasal swab semen tonsil lymph node environmental swab brain piglet processing fluid laryngeal swabs
Rotavirus (A, B, C) real-time PCR	rectal swab feces jejunum
Suid herpes virus 2 (CMV) real-time PCR	nasal swab turbinate mucosa lung

<p>Senecavirus A real-time PCR -vesicular lesions must be reported to CFIA; do not submit to VDS if vesicular lesions are present</p>	<p>environmental swab oral fluids nasal swab rectal swab feces</p>
<p>Porcine Deltacoronavirus real-time PCR -also see Porcine Coronavirus Panel</p>	<p>rectal swab feces oral fluids environmental swab jejunum</p>
<p>Transmissible gastroenteritis virus (TGEV) real-time PCR -also see Porcine Coronavirus Panel</p>	<p>rectal swab feces oral fluids – <b>Refer to Note 5</b> environmental swab jejunum</p>

**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 be done automatically. Subtyping involves additional real-time PCR assays for the H1, H3, N1 and N2 genes.

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); submit separate samples 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 indicate active infection/disease causation.

4-Environmental surface swabs (trucks, wash bays, pens, loading docks, etc.) to test for porcine coronaviruses should only be done according to the Ontario Swine Health Advisory Board (OSHAB) protocol. Clients can contact VDS for a copy of this protocol. VDS will only accept fluid samples for testing, not dry or moist pads.



5-Some pathogens may be detected in porcine oral fluids but will generally be present in low quantities, limiting the sensitivity of PCR testing. Consider other specimen types when clinical signs are present.

## Pooling Samples

- Pooling samples will 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 & CAV** – bursa – up to 3.
  - **PRRSV** – serum or blood swabs – up to 5.
  - **PCV-2** – serum – up to 5.
  - **SIV** – nasal swabs – up to 2.
  - **Mycoplasma hyosynoviae** – tonsil – up to 2.
  - **Lawsonia intracellularis** – ileum – up to 2.
  - **PEDV, TGEV, SDCV & porcine rotaviruses** – feces – from up to 5 animals 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). Do not use nitrile or latex gloves as specimen containers. Rectal swabs – up to 5.
- 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:** Porcine isolates suspected to be enterotoxigenic are tested by PCR for the following virulence factor genes: F4 (K88), F18, heat-labile toxin (LT) and heat stable enterotoxins (STa and STb). The panel also includes the attaching and effacing factor (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.

## Serology

- VDS offers Enzyme Linked Immunosorbant Assay (ELISA) for the detection of antibodies against various pathogens and Indirect Fluorescent Antibody Test (IFA) for *Porcine reproductive and respiratory syndrome virus* (PRRSV) antibody. These serological tests can be used to determine if:
  - an animal has been infected by a particular pathogen
  - a specific pathogen is linked to a clinical disease
  - an animal has elicited an antibody response following vaccination
- ELISA tests are performed according to the schedule below. It may not be feasible to accommodate requests for testing outside of this schedule.
- Samples must arrive before the testing day to allow for sample preparation. Samples arriving the day of testing may be delayed until the next scheduled day.

## ELISA Test Schedule

<b>Monday</b>	<ul style="list-style-type: none"> <li>○ Swine Influenza</li> <li>○ Mycoplasma gallisepticum / Mycoplasma synoviae combination (MG/MS)</li> <li>○ Mycoplasma meleagridis (MM)</li> </ul>
<b>Tuesday</b>	<ul style="list-style-type: none"> <li>○ PRRSV</li> <li>○ <i>M. hyopneumoniae</i> (IDEXX) (follow-up Oxoid on Wednesday)</li> </ul>
<b>Wednesday</b>	<ul style="list-style-type: none"> <li>○ Avian encephalomyelitis virus (AEV)</li> <li>○ Chicken anemia virus (CAV)</li> <li>○ Infectious bronchitis virus (IBV)</li> <li>○ Infectious bursal disease virus (IBDV)</li> <li>○ Newcastle disease virus (NDV)</li> <li>○ Avian orthoreovirus (REO)</li> </ul>
<b>Thursday</b>	<ul style="list-style-type: none"> <li>○ PRRSV</li> <li>○ Mycoplasma hyopneumoniae (IDEXX)</li> </ul>
<b>Friday</b>	<ul style="list-style-type: none"> <li>○ TGEV/PRCV</li> <li>○ Mycoplasma hyopneumoniae (Oxoid/Dako) (follow-up IDEXX on Tuesday)</li> </ul>
<b>Weekly</b>	<ul style="list-style-type: none"> <li>○ Bovine leukemia virus (BLV)</li> <li>○ Mycobacterium avium paratuberculosis</li> <li>○ Neospora caninum</li> </ul>

## Serology Guidelines

1. After collection, keep the blood samples at room temperature, until the serum has separated from the clot.
2. Submit serum only. Even if serum separation tubes are used for collection, serum must be poured off into separate clean tubes. Freeze and thaw cycles during shipping or storage can lead to hemolysis, if serum is not separated from the clot.
3. Do not submit serum samples that are hemolyzed (dark-red color) or grossly lipemic (milky appearance).
4. Do not refrigerate serum samples for more than three to five days at 2° - 7°C. If samples need to be stored for a longer period, the serum must be removed from the clot and frozen at -20°C.
5. Swine and Cattle: Submit a minimum of 1 ml serum for each test requested.
6. Poultry: 0.5 ml serum must be submitted from each bird.

7. Be sure to close tubes tightly to avoid leakage.
8. Tubes exteriors must be clean and dry. Label the tubes on the side, using black fine tip permanent marking pen.
9. Place the tubes with serum in consecutive numerical order in cardboard boxes designed to hold the tubes. Do not submit in bags.
10. Refer to the following Shipping Guidelines.

## Shipping Guidelines

Pack tightly specimens in a foam cooler as follows:

1. Cover the bottom of the cooler with cold freezer packs. Do not ship with wet ice.
2. Cover with one layer of packing material (e.g., crumpled packing paper or bubble wrap).
3. Place packaged sample tubes on top of the packing material.
4. Cover the tubes with more packing material.
5. Place one or more cold freezer packs on top and fill the rest of the cooler with packing material to prevent samples from moving during shipping.
6. Include the sample list (sealed in a zipper lock bag) in the cooler.
7. Make sure the top and bottom of the shipping box is sealed well with packing tape.
8. Ship refrigerated to Veterinary Diagnostic Services within 24 hours.