

# 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.

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## Contact Us

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## 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

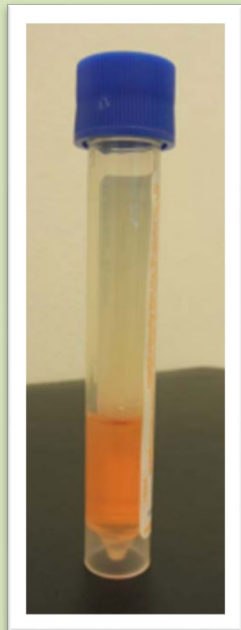
- Refer to the [PCR Assay List](#) for available tests and appropriate specimens.
- Sample as soon as possible after the onset of clinical signs.
- Specimens should be stored at 4°C and transported immediately to the laboratory to obtain best results.
- All samples must be in leak proof containers. All 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>

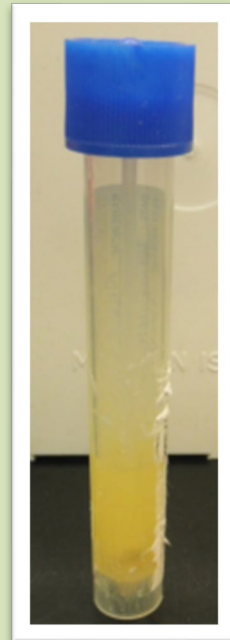
## Swabs

- **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). **This list includes both conventional and real-time PCR assays.**

## 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>	cloacal swab lung oropharyngeal swab (preferred for waterfowl) brain

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

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

## 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
<i>Chlamydomphila felis</i> real-time PCR -also see Feline Upper Respiratory Tract Panel	conjunctival swab nasal swab
Felid herpes virus 1 (FHV-1) real-time PCR -also see Feline Upper Respiratory Tract Panel	conjunctival swab oropharyngeal swab lung nasal swab
Feline calicivirus (FCV) real-time PCR -also see Feline Upper Respiratory Tract Panel	conjunctival swab lung nasal swab oral swab (ulcerated mucosa) oropharyngeal swab

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

## 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	blood in EDTA lymph node skin (tick attachment site) synovial fluid, synovium
Canine distemper virus real-time PCR	blood in EDTA brain cerebrospinal fluid conjunctival swab intestine lung lymph node nasal swab oropharyngeal swab stomach urinary bladder
Canine parvovirus real-time PCR	feces jejunum rectal swab
<i>Leptospira</i> spp. real-time PCR	Kidney liver urine
<i>Mycoplasma haemocanis</i> real-time PCR	blood in EDTA

## 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 lymph node skin (tick attachment site) synovial fluid, synovium
Equid herpesvirus (EHV 1 & EHV 4) PCR	blood in EDTA brain cerebrospinal fluid fetal liver fetal lung fetal spleen nasal swab nasopharyngeal swab nasopharyngeal wash spinal cord
Equine arteritis virus (EAV) PCR	blood in EDTA fetal liver fetal lung fetal lymph node fetal spleen lung nasal swab placenta
Equine Influenza A virus (EIV) PCR	bronchoalveolar lavage endotracheal wash lung nasal swab (deep) nasopharyngeal swab nasopharyngeal wash transtracheal wash
<i>Leptospira</i> spp real-time PCR	blood in EDTA brain colon eye (uvea) fetal kidney kidney liver placenta spleen urine
<i>Neorickettsia risticii</i> PCR	blood in EDTA feces
West Nile virus PCR	brain

	cerebrospinal fluid spinal cord
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## 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	feces large intestine lung nasal swab rectal swab small intestine
Bovine herpesvirus 1 (IBRV) real-time PCR -also see Bovine Respiratory Panel	conjunctival swab fetal liver fetal lung lung nasal swab semen trachea
Bovine Respiratory real-time PCR Panel (BVDV, PI3, IBRV, BRSV)	lung nasal swab
Bovine respiratory syncytial virus (BRSV) real-time PCR -also see Bovine Respiratory Panel	lung nasal swab
Bovine Rotavirus A real-time PCR -also see Calf Enteric Panel	feces rectal swab small intestine
Bovine viral diarrhea virus (BVDV) real-time PCR -also see Bovine Respiratory Panel	blood in EDTA feces fetal liver fetal lung fetal spleen fetal thymus intestine lung lymph node nasal swab

	oral mucosa (ulcerated) rectal swab semen serum skin biopsy (e.g., ear notch) spleen
Calf Enteric real-time PCR Panel (Bovine Coronavirus, Bovine Rotavirus, <i>C. parvum</i> )	feces rectal swab small intestine
<i>Chlamydophila abortus</i> real-time PCR	fetal liver fetal lung placenta uterine fluids
<i>Coxiella burnetii</i> PCR	fetal liver fetal lung placenta uterine fluids
<i>Cryptosporidium parvum</i> real-time PCR -also see Calf Enteric Panel	feces rectal swab small intestine
<i>Leptospira</i> spp. real-time PCR	brain fetal kidney kidney liver lung placenta spleen urine
Malignant catarrhal fever virus (OHV-2) real-time PCR	blood in EDTA lesions lymph node or tonsil ulcerated mucosa or any organ with typical
<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> (MAP) real-timePCR	feces ileum mesenteric lymph node
<i>Mycoplasma bovis</i> real-time PCR	joint exudate or swab lung milk nasal swab ocular swab synovium
<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	lung nasal swabs

<i>Toxoplasma gondii</i> real-time PCR	fetal brain fetal kidney fetal liver fetal lung placenta (sheep & goats)
<i>Ureaplasma diversum</i> real-time PCR	fetal lung fetal stomach content placenta (cattle)

## 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 *Chlamydia 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>	feces cecum colon
Influenza A virus (SIV) real-time PCR SIV Subtyping real-time PCR - <b>Refer to Note 2</b>	lung nasal swab oral fluids trachea (ulcerated mucosa)
<i>Lawsonia intracellularis</i> real-time PCR	colon feces ileum jejunum
<i>Leptospira</i> spp. real-time PCR	fetal kidney kidney liver spleen urine
<i>Mycoplasma hyopneumoniae</i> real-time PCR	environmental swabs – <b>Refer to Note 4</b> laryngeal swabs lung nasal swab

	oral fluids – <b>Refer to Note 5</b> piglet processing fluid tonsil
<i>Mycoplasma hyorhinis</i> real-time PCR <b>Refer to Note 3</b>	joint fluid joint swab nasal swab oral fluids organs affected by polyserositis (lung, heart, liver, spleen) pleura or peritoneum synovium tonsil
<i>Mycoplasma hyosynoviae</i> real-time PCR <b>Refer to Note 3</b>	joint fluid joint swab nasal swab oral fluids synovium
<i>Mycoplasma suis</i>	blood in EDTA
<i>Mycoplasma suis</i> real-time PCR	blood in EDTA spleen
Porcine circovirus-2 (PCV-2) real-time PCR	fetal heart fetal lung lung lymph node oral fluids piglet processing fluid serum tonsil
Porcine circovirus-3 (PCV-3) real-time PCR	fetal heart fetal lung lung lymph node oral fluids piglet processing fluid serum tonsil
Porcine Coronavirus (PEDV, TGEV, DeltaCoV) real-time PCR Panel	environmental swab – <b>Refer to Note 4</b> feces jejunum oral fluids – <b>Refer to Note 5</b> rectal swab
Porcine Deltacoronavirus real-time PCR -also see Porcine Coronavirus Panel	environmental swab feces jejunum oral fluids rectal swab

Porcine epidemic diarrhea virus (PEDV) real-time PCR -also see Porcine Coronavirus Panel	environmental swab – <b>Refer to Note 4</b> feces jejunum oral fluids – <b>Refer to Note 5</b> rectal swab
Porcine parvovirus real-time PCR	fetal lung
Porcine reproductive and respiratory syndrome virus (PRRSV) real-time PCR - North American & European strains	blood swab brain environmental swab fetal lung laryngeal swabs lung lymph node nasal swab oral fluids piglet processing fluid semen serum tonsil
Porcine sapovirus (SAPO)	feces fecal swab oral fluids
Rotavirus (A, B, C) real-time PCR	feces jejunum rectal swab
Senecavirus A real-time PCR -vesicular lesions must be reported to CFIA; do not submit to VDS if vesicular lesions are present	environmental swab feces nasal swab oral fluids rectal swab
Suid herpes virus 2 (CMV) real-time PCR	lung nasal swab turbinate mucosa
Transmissible gastroenteritis virus (TGEV) real-time PCR -also see Porcine Coronavirus Panel	environmental swab feces jejunum oral fluids – <b>Refer to Note 5</b> rectal swab

## 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.
  - **Lawsonia intracellularis** – ileum – up to 2.

- **Mycoplasma hyosynoviae** – tonsil – 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.
- **PCV-2** – serum – up to 5.
- **PRRSV** – serum or blood swabs – up to 5.
- **SIV** – nasal swabs – up to 2.
- 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>○ Mycoplasma gallisepticum / Mycoplasma synoviae combination (MG/MS)</li> <li>○ Mycoplasma meleagridis (MM)</li> <li>○ Swine Influenza A virus (SIV)</li> </ul>
<b>Tuesday</b>	<ul style="list-style-type: none"> <li>○ Bovine leukemia virus (BLV) *</li> <li>○ Mycobacterium avium subsp. paratuberculosis (MAP) *</li> <li>○ <i>M. hyopneumoniae</i> (IDEXX parent test) (follow-up confirmatory BioChek on Wednesday, if necessary)</li> <li>○ Neospora caninum*</li> <li>○ PRRSV</li> </ul>
<b>Wednesday</b>	<ul style="list-style-type: none"> <li>○ Avian encephalomyelitis virus (AEV)</li> <li>○ Avian reovirus (REO)</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> </ul>
<b>Thursday</b>	<ul style="list-style-type: none"> <li>○ Mycoplasma hyopneumoniae (IDEXX parent test) (follow-up confirmatory BioChek on Friday, if necessary)</li> <li>○ PRRSV</li> </ul>
<b>Friday</b>	<ul style="list-style-type: none"> <li>○ TGEV/PRCV</li> </ul>

\*May be rescheduled to later day in week, depending on workload.

## 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.