

BIVI Health Management Center

Sample Submission Guidelines

Revised 8/12/2008

Proper sample collection, organization, and preparation is crucial in order for our laboratory to provide the most accurate results. Once samples are collected, effort must be made to minimize further deterioration. Please follow the general guidelines below to assure a quality sample. Any questions regarding sample collection and handling are welcome; please contact us (contact information at the end of this document).

Sample Collection and Handling:

1. Collect samples using sterile technique drawing as much blood as possible (see #6 below). This means use a clean needle, syringe (if used) needs to be rinsed with sterile water several times before moving to next animal, using sterile collection tube every time. Using a Vacutainer, blood collection system, is aseptic and has little contamination risk
2. Do not force blood through needles as this may contribute to hemolysis.
3. Do not use Purple-Top (EDTA treated) tubes, and do not use outdated Corvac or Serum Separator Tubes (SST).
4. Freshly collected blood samples should be transported from farm to clinic in an insulated container with several ice packs and maintained on ice. This helps insure that the serum samples do not deteriorate, hemolyze and minimizes the growth of any possible contaminants that may be present (**See contamination section**).
5. Process the samples immediately allowing clotting and then separating by centrifuging and pouring off into plastic snap-top serum tubes. Never freeze whole blood before pouring off serum even if in serum separator tubes. ****Be sure to double snap all tubes!****

Benefits of using Snap Cap Tubes:

- Double snap caps resist leakage-but you must make sure it's snapped!
 - Less cracking due to freeze/thaw which equals added safety
 - Easily re-sealed--glass tubes build up pressure causing lids to pop off
 - Less mess when opening tubes as long as the lids are double snapped
 - Decreased handling time for technicians, ensuring the quickest turnaround time
 - Externally-shrouded cap minimizes environmental contamination
 - We recommend BD Falcon (#352059) brands of polystyrene, clear, sterile, plastic tube with snap caps or contact us for Diagnostic Submission Kits. Investing in quality tubes will increase the quality of diagnostic service.
6. Send serum only, no whole blood or clots. **Allow 0.5ml of serum per test requested (1.0ml minimum)** so that inconclusive or false-Neg results due to

insufficient quantity are not caused. *This is very important especially when requesting several tests. Some tests require more serum than others so please be sure to send more serum than is necessary.*

Sample Organization, Submission Forms and Shipping

NOTE: Accurate and complete submission form information ensures the highest level of service from our lab. Please carefully review all submission form information prior to submitting for testing.

Requirements for Submission Forms:

- Use a separate submission form per case
- Do not combine multiple cases in one tube box or on one submission form

Submission Form Cover Sheet

Complete all fields on the submission form coversheet, including:

1. Primary / Veterinarian – the veterinarian directly involved with the producer for this case. The “Primary” is our first point of contact with any questions or communication we have about the case.
2. Project Type, Producer / Project Name and Site / Bleed fields should all be specific to that case.
3. Case Result Distribution List – list names and contact information for all persons to receive case results. Indicate whether receiving Interim, Finals or both types of case results.
4. Diagnostic Test Requests – check what testing to receive and if testing for PCR, indicate individuals or number of samples/pool.
5. Comments – any special instructions needed for completing testing.

Sample Information Page(s)

1. Number the tubes consecutively, 1 through ‘X’, matching the actual tube number with the tube number pre-printed on the serology submission form. Completely fill out a submission form and include all information. All information included in form fields will be included in your diagnostic report. Use permanent marker to identify tubes. Grease pencil marks are easily rubbed off, and labels tend to fall off.
2. Place the tubes in blood boxes in numerical order. This will help prevent the accidental loss or mix up of tubes during unpacking, and greatly improve the log-in process.
3. Keep a copy of the paperwork submitted, and retain a portion of each serum sample in your freezer (if desired). This guards against unforeseen mishaps (lost or broken tubes) and may save you from having to re-bleed. Acute serum samples may also be retrieved from the freezer for pairing with convalescent samples. Paired sera should be tested together to maximize the diagnostic value of the test results.

Shipping Samples

1. Do not freeze samples. Ship samples in an insulated shipping box immediately with ice packs using UPS (preferred) selecting either “Next-day Air” or “Next-day Air saver” shipping options. ***This type of express shipping is especially imperative for PRRS antigen detection via Virus Isolation or PCR. Please do not ship over the weekend*** or by regular US-mail if possible. If you would like shipping materials, please contact us at the address or phone number in the ‘Contact’ section below. Ship samples according to Department of Transportation Hazardous Materials Regulations. **(See Department of Transportation Hazardous Materials Regulations section)**
2. During warm months, it is very important that specimens are packed with extra ice packs to insure a quality sample when it arrives to our lab.

Department of Transportation Hazardous Materials Regulations Section:

New Department of Transportation regulations regarding shipment of diagnostic specimens went into effect on January 1, 2007. According to 49 CFR 173.134 exception #11, given that most serum samples sent to our lab are for routine diagnostic testing not related to the diagnosis of an infectious disease (i.e. Category B), they are considered “Exempt Animal Specimen”. Therefore shipments of serum samples sent to the BIVI HMC do not need a “Biological Substance Category B” & “UN3373” label. Please note that “Diagnostic Specimen” is no longer compliant under the new regulations and that if you are shipping a Category B specimen it must be labeled as “Biological Substance Category B” with the “UN3373” label.

*According to 49 CFR 173.199(e), Category B substance is defined as an infectious substance that is not in a form generally capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs.

Please note: This does not exempt you from the triple packaging method. All shipments should still be packaged in the triple packaging method to ensure that leakage does not occur in transit.

Contamination:

Samples can become contaminated by many things, including bacteria, molds or other subsequent samples. Any of these types of contamination can affect the results by giving false positive or false negative results.

Some antibodies are more unstable than others and degrade rapidly in a blood sample. This degradation increases when the sample is stored under non-optimal conditions (such as high or fluctuating temperatures), frozen/thawed several times, or kept as whole blood. Bacteria can produce proteases that will 'chew up' proteins, including the antibodies. Bacterial contamination in a sample may also result in an inaccurate result due to degradation of the antibodies. Samples kept at 4°C (normal refrigeration temperature) for a prolonged period (over 7 days) run the risk of bacterial colonization and subsequent spoilage.³

The IDEXX Technical Guide recommends that serum samples be refrigerated at 2-4°C for no more than 3-5 days to avoid possible contamination. If the samples need to be stored longer they should be frozen to a minimum of -20°C (normal freezer temp).²

Remember that good sample results start with sterile technique.

Detection Methods:

The tests run at the HMC can be broken up into 3 categories: Antibody Detection, Antigen Detection, and Virus Isolation. ([See Table 1 for instructions](#))

- **Antibody Detection:** (ELISA, IPMA) According to one study, antibodies are stable at various temperatures (-40°C up to room temperature) and that the shelf life of antibody is considerably longer than the 3-year period studied as long as precautions are taken to minimize bacterial contamination³. Because of the possibility of contamination, serum samples should be kept on ice immediately following collection. Process according to General Guidelines in this document, then ship on ice. Timing of collection and shipment should be such that they are refrigerated or on ice for no more than 7 days before testing (see our test schedule). Recommendation: Serum samples should be kept at 4°C (for only a couple of days due to contamination) and at -20°C or colder (can remain for several years) as long as repeated freeze/thaw cycles (most detrimental) are kept less than 5. ([See contamination section](#))
- **Antigen Detection:** (PCR)
 - PRRS virus in serum samples should be immediately chilled following collection and during shipment. PRRS is heat-labile meaning it degrades rapidly at room temperature or warmer. If received warm the results may be unreliable because the virus may be inactivated.
 - Lawsonia intracellularis samples are recommended to be **frozen at -20°C if not submitted to our lab immediately following collection**. Fecal swabs and infected tissues samples most likely contain many confounding variables that inhibit *Lawsonia intracellularis* detection using PCR, for example, other bacteria and viruses, host cellular debris, degradative enzymes to DNA, etc.¹ At 4°C, the quality of a fecal swab or tissue sample

degrades so that the chances of detecting Lawsonia in these samples are decreased after storage of 2-3 days.¹

- **Virus Isolation:** Isolation of the PRRS virus. The quality of samples and handling of samples is paramount to PRRS virus isolation success.
 - Half-life viability rapidly decreases at room temperature.
 - As stated in “Antigen Detection/PRRS Virus” PRRS virus is stable at 4°C for at least 48 hours (sources vary)
 - Very stable at ultra-low (-80°C) temperatures

Serum has a sparing effect on virus viability in the suboptimal time and temperature conditions that may occur during shipment—therefore serum is an excellent sample for PRRS Virus Isolation.⁴

Table 1

Test	Detection Method	Sample Submission	Handling/ Shipping and Comments
PRRS ELISA	Antibody	Serum	Ship with Ice packs overnight.
Ileitis ELISA	Antibody	Serum	Ship with Ice packs overnight
IDEXX Mhyo ELISA	Antibody	Serum	Ship with Ice packs overnight
Ileitis PCR	Antigen	Fecal/ Intestine	Ideally, swab early in the week to test on Wednesday and keep at 4°C or colder. Otherwise, freeze samples until you can send. Ship overnight on ice packs. Note: Lawsonia fecal swabs/tissues samples may degrade after a couple of days at 4°C
Salmonella ELISA	Antibody	Serum	Ship with Ice packs overnight
PRRS PCR or PCV2 PCR	Antigen	Serum	Chill immediately following collection. Keep serum at 4°C but not frozen. Ship overnight on ice packs. Note: This test requires a large volume of serum, please send extra.
PRRS Strain Diff	Antigen	Serum	Must be tested positive by PRRS PCR, refer to PRRS PCR comments.
PRRS Virus Isolation	Virus Isolation	Serum	Collect aseptically and transport immediately on ice packs. If cannot send immediately, keep at 4°C for no more than 2 days, longer storage must be kept at -70°C but NEVER at -20°C (regular freezer temp). ⁵

HMC Test schedule located at www.bi-hmc.com

Key Summary Points:

- Use aseptic technique during collection
- Samples must be a minimum of 1mL of blood (0.5mL serum) per test requesting

- Sample chilling process must begin immediately after collection (use cold packs & coolers for transport from farm to clinic)
- Separate serum ASAP, use snap-cap tubes, and ship only serum to lab
- Properly identify samples
- We recommend less than 7 days cumulative refrigerated storage time from sample collection to sample testing
- Comply with IATA/Dangerous Goods Guidelines
- Refer to the 'Handling Guidelines for Specific Tests' table for specific comments

References:

1. BIVI Biologic Research and Development (Kroll J., Vaughn E.)
2. IDEXX Laboratories. 2003 Poultry and Livestock Catalog and ELISA Technical Guide.
3. Middletown BA, Morgan LM, Aherne GW, Marks V. The effect of storage temperature and physical state on the performance of antisera in radioimmunoassay after long-term storage. *Ann Clin Biochem* 1988; **25**:89-95.
4. Val Alstine W.G., Kanitz C.O., Aherne GW, Marks V. Time and temperature survivability of PRRS virus in serum and tissues. *J Vet Diagn Invest* 1993;**5**:621-622.
5. Yoon-K.-J., Christopher-Hennings J., Nelson E.A Section 3.1 Diagnosis. 2003 PRRS Compendium.

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