General Guidelines for Sample Submissions

(See the Client Handbook for specific test requirements.)

HISTORY

The history of the animal is very important, including treatments, vaccinations and feeding, as well as general information such as the environment, conditions of other animals from the same place, etc. Including the number of sick, dead and total animals at a facility is also very important. Such information can provide clues to the pathologists and laboratory technicians performing tests to identify causes of illnesses and deaths. The accession form has a place to record this pertinent information.

TIMING OF SPECIMEN COLLECTION FOR MICROBES

Specimens should be collected as soon as possible in the course of an illness and before antimicrobial therapy begins, if at all possible. The likelihood of recovering most viruses and many bacteria diminishes markedly >72 hours after symptom onset and after the initiation of appropriate antimicrobial therapy.

Viral studies often require convalescent specimens to confirm active disease, submitted 3 to 6 weeks after the initial specimen. Very young animal tests may be altered due to maternal antibodies. Vaccinations may also be measured in some tests due to circulating antibodies.

Click the following topic links for details:

- AEROBIC CULTURE AND SENSITIVITY
- ANAEROBIC CULTURES
- BACTERIAL SPECIMENS FROM HAIR/SKIN
- BLOOD SPECIMENS
- BVD SPECIMENS TESTING FOR PERSISTENT INFECTION
- FUNGAL SPECIMENS FROM HAIR / SKIN
- PCR SPECIMENS
- SLIDE SUBMISSIONS
- SHIPPING
- TRICHOMONAS POUCHES
- VIRAL SPECIMENS

AEROBIC CULTURE AND SENSITIVITY

*Several specimen types are acceptable for aerobic culture.*

1. Specimens can be collected using culturettes (sterile swab with transport media).
2. Biopsy specimens or tissue sections for culture must be sent in a sterile container. Sterile normal saline may be added to keep the tissue from drying out.
3. Foreign bodies for culture should be submitted in a sterile container – no saline required.
4. Specimens collected by syringe should be aseptically transferred to a sterile tube or cup. Do not send a syringe with a needle attached.
5. Specimens in formalin or EDTA are *not* acceptable for culture.
6. Swabs that contain mostly pus may not grow on cultures due to the toxic effects of white blood cells. For best results, swab deep into an infected wound or draw fluids off infected tissue.
7. Most Aerobic Culture swabs (culturette) can be refrigerated.
ANAEROBIC CULTURES

Anaerobic cultures should be submitted on a separate culturette (sterile swab with anaerobic transport media). All anaerobic cultures should be processed within 48 hours of collection. After collection, keep the specimen at room temperature as anaerobes are fastidious organisms easily killed by exposure to environmental variation – heat, cold, air, sunlight, etc.

** Proper collection of anaerobic samples is critical as contamination by normal flora can quickly overgrow anaerobes and prevent growth.

Methods that minimize contamination include:

1. aspiration using a needle and syringe
2. cystocentesis
3. thoracocentesis
4. curettage
5. tissue biopsy

BACTERIAL SPECIMENS FROM HAIR/SKIN

Impression smears of pustules and vesicles can show acantholytic cells, bacteria and granulocytes. The sample site must be chosen with care, preferably unruptured lesions. Secondary bacterial infection is common, and smears from ruptured and crusted lesions are often unsatisfactory. The majority of bacterial skin infections are caused by a coagulase positive *Staph* although other organisms such as *E.Coli* can cause secondary infections.

FUNGAL SPECIMENS FROM HAIR / SKIN

The toothbrush method works well for collecting fungal specimens. Use a new toothbrush and gently comb the hair onto paper or into an envelope. Submit the brushings and toothbrush in a non-air tight sealed container, such as an envelope. The container should not allow moisture to build up as that can encourage bacterial overgrowth.

Skin scrapings and sections can also be submitted for fungal isolation. Clean the skin gently with alcohol to remove debris. Collect the sample from the periphery of the lesion and adjacent skin.

Skin impression smears can be used to diagnosis yeast infections. Press the slide directly onto the lesion and allow it to air dry. Submit the slide for cytology.
**BLOOD SPECIMENS:** *(Check Client Handbook for individual test requirements)*

Protect blood specimens from heat. Heat causes hemolysis, which may be severe enough that specimens are not suitable for processing. Make sure tubes are clearly identified.

When using EDTA tubes, make sure the blood and anti-coagulant are thoroughly mixed and keep the specimens out of direct sunlight.

If serum is required, the preferred method would be to separate the cells from the serum. Separation can be done by centrifugation or by sitting the tube upright in a rack until clear serum appears. Remove the serum and place it in a leak-proof transport tube.

Samples on the left are normal compared to the samples to the right:

- **Normal serum & plasma**
- **Hemolyzed**
- **Too hemolyzed to use**
- **Jaundice - dark yellow**
- **Lipemic – white layer may form when refrigerated**

In the summer, even a few minutes in a hot vehicle can cause hemolysis. In the winter, freezing temperatures can cause hemolysis. Transportation can lead to hemolysis, particularly if the travel time is several days. For best results, separate the serum from the clot. For whole blood samples, use a gentle rocking motion to mix the blood and anti-coagulants immediately after filling.
**BVD SPECIMENS: TESTING FOR PERSISTENT INFECTION**

BVD specimens for ELISA testing: For calves less than 3 months old, the specimen must be an ear notch. For cattle over 3 months old, ear notches or serum samples are acceptable.

**PCR SPECIMENS**

PCR specimens collected on swabs must be on Dacron or synthetic fiber swabs with plastic sticks. Do not use swabs with cotton tips or wooden sticks. Swabs should be placed in a sterile tube with 2 – 3 drops of sterile water or sterile saline to keep them moist for transport. DO NOT use the microbiology transport media used for culture and sensitivity. Submission in viral transport media is acceptable.

**VIRAL SPECIMENS**

**General Guidelines**

For short periods (≤ 72 hours), most specimens should be held at 2-8°C rather than frozen. For delays exceeding 72 hours, freeze specimens at -70°C as soon as possible after collection (with exceptions noted below). Label each specimen container with the patient’s ID number, specimen type and the date collected.

For swab samples, use a sterile Dacron or rayon swab with a plastic shaft to collect the sample and place the swab into a sterile container of 2-3 ml of viral transport media. One swab per container. Do not use swabs with wooden shafts or calcium alginate as they may inhibit viruses.

When blood testing for viral pathogens, serum is preferred and must be removed from the clot before freezing. Whole blood in EDTA tubes can be used for some tests but cannot be frozen and must be protected from heat.

CSF is collected using sterile techniques and must be clean as blood may compromise results. Place specimen into a sterile, leak-proof container. No transport media is required.

For tissue samples, place into a sterile, leak-proof container with 1 – 3 ml of viral transport media. Only one specimen per container. If tissue samples are intended for viral isolation, freeze tissues immediately after collection and ship frozen as quickly as possible. Cold chain is important for recovery of virus.

**SLIDE SUBMISSIONS: (Slides should be individually labeled.)**

Slides for cytology exams must be air dried. Do NOT heat fix cytology slides as heat damages cells.

Transporting the slides in slide containers prevents damage to the smears. If shipping slides with tissues in formalin, place the slide container in a separate ziplock bag to protect it from the formalin fumes.
TRICHOMONAS

Trichomonas samples require special handling as the organisms are very sensitive to cold and heat. All samples for trichomonas analysis should be placed into the InPouch TF media within two hours of collection. For feline fecal submissions, use a swab to transfer a small amount of feces into the liquid by swishing and then removing the fecal swab. For bovine submissions, place the prepucial wash or the prepucial swab directly into the media. For the wash, immediately push the wash to the bottom of the pouch. For the swab, squeeze the liquid to the top to submerge the swab during transport to the laboratory. It is best to keep the samples at room to body temperatures and deliver to the lab as quickly as possible. Insulated shipping containers may prevent extremes in temperatures if prompt delivery is not possible. PCR testing is still available if the organisms are not viable, however incubation to ensure sufficient quantities for testing and visual confirmation of positive PCR’s will not.

SHIPPING:  (Ensure all samples are identified and paperwork is complete.)

All specimens must be pre-packed to prevent breakage and spillage according to DOT / CDC guidelines. Links for shipment guidelines can be found on the BVC website under Submission Policies.

Properly packaged samples: Carefully labeled, individually bagged samples with the paperwork in a separate bag to protect it in case of leaks, all packed in a suitable cardboard box.

Specimen containers should be sealed and placed in ziplock bags. Place enough absorbent material in the ziplock bag to absorb the contents of the Primary Container(s) should it break or leak in transit.

Separate and cushion the Primary Containers if needed to prevent breakage. (Packing containers designed specifically for transporting specimens are available. Follow the manufacturer’s instructions.)
Send specimens with cold packs or other refrigerant blocks that are self-contained, not wet ice. One ice pack will only keep a specimen cold for one day. For longer deliveries, use more than one ice pack or request 2nd day delivery to ensure specimen quality.

When large numbers of specimens are being shipped, they should be organized and identified.

**Properly identified:**

- Labels on samples
- Multiple milk samples with labels on lids, in leak proof containers, inside a secondary container

**Unsuitable Conditions:**

Foam or bubble envelopes are not sturdy enough or safe for shipping blood tubes.

Unidentified containers and incomplete or missing paperwork delay testing.

Leaking containers result in unacceptable specimen conditions.

- Glass tubes need stronger protection than a padded envelope alone provides.
- Unidentified specimen
- Urine collection cups are not leak proof.

**Notes:**

- Do not place any dry ice in the Primary or Secondary Container, foam envelopes, ziplock bags, cryovial boxes, or hermetically sealed containers.
- Do not place any paperwork inside the containers or ziplock bags with the specimens. Place the paperwork in a separate ziplock bag or outside the secondary container to prevent damage and contamination.

Created using information from CDC, DOT, OSHA, USPS, IATA, the Mayo Clinic and other relevant sources.