

Veterinary Medical Center

Bacteriology/Mycology Specimen Collection and Submission Guidelines

1. Submitting Specimens for Culture

General instructions

- Select sample from a site that is expected to be most representative of the infection
- Aseptically prepare cutaneous collection sites with a quick acting and tissue penetrating antiseptic, eg povidone-iodine, followed by a 70% alcohol swab/wipe. Avoid use of chlorhexidine or benzalkonium chloride for this purpose, especially from multiuse containers, as they may become contaminated.
- Transport samples to the laboratory, with appropriate containment and packaging, as soon as possible.
- Inform the lab if the animal is being treated with antimicrobial drugs and if patient is immuno-compromised.
- Inform the lab of the animal's age, production type and species. This may affect culture interpretation and selective reporting of antimicrobial susceptibility results.
- All specimen containers must be labeled with the patient name, clinic # or owner, body collection site, and date on container. Include history and patient demographic information on referral form.
- Commercial culture swab systems containing transport media provide convenient means to sample mucosal surfaces or wound sites. For small volume samples such systems prevent drying in transit and help maintain viability of aerobes and anaerobes. However, in general, swabs are the least preferred samples for culture purposes; aspirates, fluids and solid tissue samples are best. In addition to having small sampling volumes that might reduce detection of organisms present in low numbers, swabs may also interfere with the release and viability of some types of organisms. Such effects of the swabs and diluting effects of the transport media make it difficult to accurately quantitate samples submitted on swabs. In no case should liquid samples be added to a culture swab system in volumes that exceed the absorbing capacity of the swabs. The presence of excess liquid in such a system results in a safety hazard for laboratory personnel who receive the samples and increases chances for contamination of the samples.
- Results of Gram stains or other microscopic exams can often be made available the same day the specimen is received, if requested. Microscopic exams may be requested as separate tests or are performed routinely on most culture requests.
- Short-term refrigeration is acceptable (and preferred) for many organisms. Those that do not survive freezing or refrigeration well include some anaerobes, fastidious respiratory pathogens, zygomycetous fungi, and *Pythium insidiosum*.

Specimen type

- Aspirates of Fluid – Joint fluid, CSF, abscesses, and other body fluids may be collected in a syringe and transferred to a sterile, tightly capped tube. Do not pour liquid samples into culture swab containers. Samples should be refrigerated for transport.
- Biopsies – Transport on cold packs in a sterile tube or Whirl Pak™ bag. Add 1 or 2 drops of sterile

saline to small biopsies to avoid drying during transport. Avoid transporting samples in syringes. Fine needle tissue aspirates should be flushed with a minimal amount of sterile saline into a sterile tube or onto a culture swab for transport. If absolutely necessary, tissue lodged inside a needle or syringe hub may be submitted inside another sterile, puncture-proof container, again making sure that the sample will not dry out during transport. Anaerobic organisms can be maintained in small samples by immersing them in a suitable transport medium. Small samples of tissue can be imbedded in the gel portion of the culture swab.

- Blood - The venipuncture site should be shaved and free of hair and debris. The site should be disinfected first with a quick acting and tissue penetrating antiseptic, eg povidone-iodine followed by a 70% alcohol swab/wipe. Avoid use of chlorhexidine or benzalkonium chloride for this purpose, especially from multiuse containers. Surface should be allowed to dry briefly before performing venipuncture with an appropriately sized sterile hypodermic needle and syringe (or Vacutainer needle and tube holder). A volume of blood (small dogs and cats – 1 to 5 mLs; larger animals – 10 to 20 mL) is drawn and injected aseptically into a Wampole Isolator™ blood culture tube or alternative commercial blood culture tube or bottle. Fill containers with volume specified for each container; do not over fill (usually the vacuum will fill to the appropriate volume). Isolator™ tubes from patients not receiving antimicrobial drugs at the time of collection may be held at room temperature for up to 8 hrs. Samples from patients receiving antimicrobials at the time of collection should be transported to the laboratory immediately. The anticoagulant with greatest stability and least overall inhibitory effect on different types of bacteria is sodium polyanethol sulfonate (SPS), a relative of heparin. Some blood collection containers also contain blood cell lysing agents. With advanced notice, collection bottles and tubes optimally designed for culturing blood and containing this anticoagulant may be obtained from laboratories or medical supply distributors. For culture of some aerobic, facultative and aerotolerant organisms, tubes normally available in veterinary clinics, eg. sterile Vacutainer™ tubes containing sodium citrate or heparin anticoagulants, may suffice (eg. blood cultures for *Brucella canis*).
- Feces – Submit 5-10 grams in a clean specimen container with a secure lid for best yield of enteric pathogens (*E. coli*, *Salmonella*, *Yersinia*, *Campylobacter*, *Citrobacter rodentium*, *Rhodococcus equi*, *Clostridium* spp.). Rectal/cloacal swabs are acceptable for small animals or animals from which feces are difficult to obtain. Refrigerate and transport on ice. Cultures for *Mycobacterium paratuberculosis* should be sent to the TN state veterinary diagnostic lab (Kord Lab) in Nashville.
- Milk – Samples should be transported immediately on cold packs or frozen after collection.
- Swabs – Swabs from eyes, ears, nose, throat, and skin should be put in transport media. BBL CultureSwab Plus™ or other culture swab systems with Amies semi-solid transport medium work well for *Mycoplasma*, fastidious respiratory pathogens and anaerobes. General purpose cotton swabs contain substances that are inhibitory for some bacteria and are not suitable for specimen transport. Aspirates and biopsies are preferable to swabs.
- Trans-tracheal washes or broncho-alveolar lavages – Place wash fluid in a sterile tube or vial. Transport on cold packs to lab as soon as possible.
- Urine – The lab needs to know when and how it was collected (cystocentesis, catheter, voided). It should be collected into a sterile container, NOT on a culture swab. The specimen should be refrigerated within 1 hour of collection, if it cannot be immediately processed by the lab. Ideally, specimens should be transported on ice and processed by the lab within 24 hours of collection. A longer holding period may be acceptable, if necessary, but should be considered when interpreting results. If refrigeration is not reliable, commercial transport devices containing boric acid may be used. Indicate if animal has received antibiotics within 72 hours of collection.
- Nails, skin scraping, hair – Clean to remove superficial debris. Use sterile blade to scrape active border of lesion. Hairs should include follicular end for culture of dermatophilic fungi.

- Uterine swabs – Use guarded swabs for collection and place swab in transport medium for recovery of fastidious organisms.
 - Mares: Routine aerobic culture is usually sufficient. Testing is not performed in-house for CEM.
 - Cows, ewes, sows: Multiple culture methods may be required, eg. aerobic, anaerobic, *Campylobacter*, *Mycoplasma*. Testing for brucellosis in livestock is performed at Kord Laboratory in Nashville.
 - Dogs: Pre-breeding vaginal swabs must be interpreted with caution. Beta hemolytic *Streptococcus* spp and *Mycoplasma* spp are commonly recovered from healthy, reproductively normal dogs.

2. Submitting Specimens for Tests Other than Culture

Special staining and microscopic examination (Call lab)

- Microscopic examination may be requested as a separate test or is included with a culture.
- Dried impression smears of tissue on glass slides are suitable for some microscopy procedures (eg. Gram stain) while fresh tissue is preferable for others (eg. Calcofluor stain).

Nucleic Acid detection assays (Call lab)

- Benefits may include: identification of culture isolates that are not readily identifiable by routine biochemical methods, specific toxin or virulence gene detection in isolated organisms, detection of organisms that are difficult to culture or are slow growing, and epidemiological comparisons.
- Assays may be performed in some cases on fixed tissues or smears; however, efficiency is best if organisms have been first microscopically visualized in the tissues. Fixed specimens should not remain in formalin for more than 10 days if PCR is to be performed on them.

Direct fecal ELISA for Clostridial toxin detection

- *Clostridium difficile* enterotoxin (toxin A) and cytotoxin (toxin B) – fresh, liquid fecal specimen is preferred. Specimen may be frozen for storage prior to testing.
- *Clostridium perfringens* enterotoxin (cpe) – known primarily as a source of food poisoning in humans, has a potential association with disease in several animals.

ELISA for *Cryptococcus* capsular antigen

- Often performed on serum but may also be performed on CSF or BAL fluids for diagnosis. Indicate if test is being requested to monitor response to therapy (needs sequential samples for comparison).

ELISA for other fungal antigens (*Blastomyces*, *Histoplasma*, *Coccidioides*, *Aspergillus*)

- These are send-out tests. May be performed on urine, serum, CSF, BAL fluid; inquire for recommendations. (Call lab)

Serology tests (tests performed on serum for detection of antibodies to specific agents)

- Requires separated, hemolysis-free serum in volumes of 0.5 to 1.0 mL. May be refrigerated.
- Tests available for *Blastomyces*, *Histoplasma*, *Coccidioides*, *Aspergillus fumigatus* and *Brucella canis* antibodies.

Questions?

If you have any questions about sample collection or handling, please call the Clinical Bacteriology/Mycology Laboratory at 865-974-5639.

