

University of Tennessee College of Veterinary Medicine
Clinical Bacteriology and Mycology Laboratory
Specimen Collection and Submission Guidelines

I. Submitting specimens for culture

Specimens from Live Animals

- Aspirates - Joint fluid, CSF, abscesses, collect into a syringe, cap tightly, refrigerate and transport on ice. Joint fluid may require collection in a syringe that contains a small amount of sterile heparin to prevent clotting. Alternatively, to avoid clotting, joint fluid specimens may be put in a Wampole Isolator™ blood culture tube (pediatric 1.5 ml draw and adult 10 ml draw) for transport. New Monovette™ devices from Starstedt also provide a convenient and safe means for collection and transport.
- Biopsies - Collect surgical biopsies aseptically. Transport on ice in a sterile container, eg. tube, Whirl Pak™ bag. For small samples it is advisable to add one or a few drops of sterile saline to keep the sample from drying during transport. Anaerobic organisms can be maintained in small samples by immersing them in a suitable transport medium such as Portacult™ tubes from BD Biosciences.
- Blood - An appropriate volume of blood proportionate to the size of animal and tube being used is drawn and injected aseptically into a Wampole Isolator™ blood culture tube. After mixing, tubes from patients not receiving antimicrobial drugs at the time of collection may be held at room temperature for up to 8 hrs while subsequent samples are drawn. Samples from patients receiving antimicrobials at the time of collection should be transported (on ice if by mail) to the laboratory immediately. Mail in samples may alternatively be sent in two commercial blood culture bottles, one aseptically vented with a needle for aerobes (remove needle before transport) and the other left unvented for anaerobes. Blood cultures for “*Brucella canis* only” may be mailed in sterile tubes containing sodium citrate or heparin.
- Feces - Submit 5-10 grams in a sterile screw cap specimen container. Avoid the use of swabs if possible. Swabs do not always contain sufficient numbers of organisms and do not protect anaerobic or microaerophilic organisms well. Refrigerate and transport specimen on ice. Feces is acceptable for culture of *E. coli*, *Salmonella*, *Yersinia*, *Campylobacter*, *Citrobacter rodentium*, *Rhodococcus equi*, *Clostridium perfringens*, and *Clostridium difficile*. Cultures for *Mycobacterium paratuberculosis* should be sent to the TN state veterinary diagnostic lab (Kord Lab) in Nashville.
- Milk - Milk is a good cryoprotectant for bacteria but also is a good growth medium and often harbors organisms that are adapted to grow at low temperatures. Samples should be transported on ice immediately or frozen after collection.

- Swabs - Collect swabs from eyes, ears, nose, throat, and skin. Clean and disinfect surface prior to collection. Use appropriate transport media. BBL™ CultureSwab™ Plus and similarly designed commercial culture swab systems with Amies semi-solid transport medium work well for Mycoplasma, fastidious respiratory pathogens and anaerobes. When using systems containing liquid transport medium, remember to crush the ampule to moisten the swab. General purpose cotton swabs contain substances that are inhibitory for some bacteria and are not suitable for specimen transport. Remember that aspirates and biopsies are almost always preferable to swabs.
- Trans-tracheal washes or broncho-alveolar lavages - Place wash fluid in a sterile tube or vial. Recovery rate for fastidious organisms is higher than with oropharyngeal or nasal swabs. Transport and lab set-up should be ASAP as saline is not a good preservative, especially for fastidious respiratory pathogens. For mail-in samples, refrigeration and use of a suitable transport medium is recommended.
- Urine - The lab needs to know when and how it was collected. It should be collected into a sterile container NOT on a culture swab. Transport specimen on ice. Urine is a good growth medium. Specimens should be refrigerated within 1 hr of collection and processed by the lab within 72 hrs of collection. If refrigeration is not reliable, commercial transport devices containing boric acid may be used.
- Uterine swabs - Use guarded swabs for collection AND place swab in an appropriate transport medium for recovery of fastidious organisms.
Mares – routine aerobic culture is usually sufficient
Cows, ewes, sows – multiple culture methods may be required, eg. aerobic,
anaerobic, *Campylobacter*, *Mycoplasma*, *Ureaplasma*, *Brucella*, etc.
Culture results from pre-breeding vaginal swabs in dogs must be interpreted with caution. Beta hemolytic *Streptococcus spp* and *Mycoplasma spp* are commonly recovered in such specimens from healthy, reproductively sound dogs.

Special Instructions

- Inform the lab if the animal is being treated with antimicrobial drugs. There are means that can be taken to dilute or remove antimicrobials from some specimens to enhance chances of recovery. Also, please indicate 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 at least two forms of identification. For in-house patients, order # and patient clinic # is sufficient. For referral patients use patient name, clinic # or owner, body collection site, and date on container and include accompanying history and patient demographic information on referral form.
- Results of Gram stains or other microscopic exams may be requested for rapid overview of microbial profile. Microscopic exams may be requested as separate tests or are performed routinely on most culture requests. Note that smears prepared at the time of collection and submitted with a specimen for culture are often better than those prepared in the lab, especially those from swabs after cultures have been set-up.

Necropsy Derived Specimens

- Ideally, necropsy should be performed as soon after death as possible to limit post mortem changes.
- Specimens collected more than 6 hours after death are frequently heavily contaminated with gastrointestinal contaminants
- If uncontaminated, the survival of pathogens in animal tissues is usually quite good. Short-term refrigeration and freezing is acceptable (and preferred) for many organisms. Some organisms do not survive freezing or refrigeration well (eg. some anaerobes, fastidious respiratory pathogens, zygomycetous fungi, *Pythium insidiosum*).

II. Specimen Selection

- Submit tissues consistent with gross lesions and history
- List carefully considered differentials or reasons for submitting for bacteriology/mycology testing
- Submit a representative number of specimens from appropriate sources when diagnosing disease in large populations (herds, flocks, etc.)
- Use freshest tissues possible for microbial culture
- Ideal specimen size is 2-4 cm³ of parenchymal tissue, centered on the margin of a lesion. If larger samples are submitted or if margins are not apparent, the lab may require assistance to recognize the lesion or preferred sampling orientation. Smaller specimens may not be sufficiently treated in the lab to eliminate surface contamination prior to culture. If surfaces of a parenchymal organ are inadvertently contaminated prior to sample collection, they may be quickly rinsed with sterile saline, sterile water or 70% ethyl alcohol to minimize subsequent sample contamination.
- Send ligated intestinal loop approximately 8-10 cm long, NOT intestinal contents for culture as many GI pathogens are adherent to the mucosa. Site (duodenum, ileum, jejunum, colon) may vary depending on pathogen being sought.
- Spleen, heart blood or bone marrow are preferred over liver to detect septicemic infections as livers are often contaminated by retrograde spread of intestinal flora from the bile duct. On the other hand, some intestinal pathogens may be more readily detected in liver than in intestine (eg. *Clostridium colinum*).
- Tissue just ahead of the advancing lesion is usually the most rewarding for lung culture. Airways are best for *Mycoplasma spp.*

III. Collecting Tissues

- Attempt to collect specimens aseptically. Use sterile instruments and gloves. Consider collecting specimens for microbial culture prior to handling viscera.
- Collect normally sterile/minimally contaminated sites first (heart, spleen, brain) and progress to sites with low to moderate chances of contamination (lung, liver, lymph nodes, kidneys, uterus, etc.) and collect tissues known to contain high numbers of microorganisms last (GI tract, integument).
- Do not serially section specimens looking for lesions before submitting them to the microbiology lab
- Avoid using band saws to collect specimens for microbiology as they may drive contaminated particles deep within tissues
- Collect samples into pre-labeled containers and check to see that they match the specimen and patient.
- Make sure that specimens, whether being transported in-house or being packaged for shipping, are placed in an appropriate leakproof/spillproof outer container that is free of contamination before they leave the primary collection location.
- Be sure when shipping diagnostic specimens that the packaging and accompanying documentation meets all applicable regulatory requirements.

IV. Submitting specimens for special services other than culture

Special staining and microscopic examination

- Microscopic examination may be requested as a separate test or if included with a culture, preliminary results may be requested for rapid initial assessment of microorganisms present
- Gram stain – gives a broad overview of microbial profile
- Giemsa stains – aid detection of intracellular pathogens and other tissue-associated organisms
- Acid Fast stains – aid in differentiation of *Mycobacterium* and *Nocardia*
- Calcofluor stain – can be applied to large pieces of fresh tissue to rapidly visualize fungi in tissue
- Darkfield microscopy, modified Gram stain, Victoria Blue 4R, Warthin- Starry silver stain – enhance detection of spiral-shaped bacteria

IV. Submitting specimens for special services other than culture (cont.)

Fluorescent antibody assays for agent detection

- Used on fresh tissue, frozen sections, impression smears or occasionally on fixed tissue sections
- Time consuming and requires technical expertise to localize fluorescence to appropriate morphological architecture
- Specific antibodies are sometimes difficult to obtain
- Fast turnaround
- Muscle impression smears for *Clostridium chauvoei* (UT Bacteriology lab)
- Direct FA on urine sediments for *Leptospira* and tissue smears for *Chlamydomphila* (UT Virology lab)

Nucleic Acid detection assays

- Detection of organisms that are difficult to culture or are slow growing (eg. PCR)
- Direct molecular detection of nucleic acid does not require viable organisms but closely parallels the presence of viable organisms in tissues. Assays may be performed in many cases on fixed tissues or even 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.
- Detection of virulent strains of common organisms (PCR)
- More rapid and sensitive detection of organisms present in low numbers (eg. PCR)
- Identification of organisms by DNA sequencing of portions of conserved genes or intergene regions (eg. 16S rRNA, ITS)
- Molecular subtyping of groups of organisms to determine sources (eg. PFGE, RAPD)
- Some molecular diagnostic assays are offered internally through the UT Bacteriology/Mycology, Virology and immunology laboratories; specimens are also sent to reference laboratories that specialize in individual tests.
- Common uses include:
 - Clostridium perfringens* toxin genotyping
 - Mycobacterium* and *Mycoplasma* species identification
 - Leptospira*, *Mycoplasma hemofelis*, *Neorickettsia risticii*, and *Bartonella* detection

Direct fecal ELISA for Clostridial toxin detection

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

