

MICROBIOLOGIC SAMPLING



BASICS

OVERVIEW

- This chapter is designed to aid in selection of optimal samples for bacterial culture or virus isolation from live animals and necropsy specimens, and describe appropriate transport to the laboratory.
- Organisms that have special culture characteristics (such as viruses or anaerobic bacteria), fastidious growth requirements, or require enrichment procedures need special handling either for transport or in the laboratory to enhance or select for their isolation. Examples of organisms which require special culture procedures can be found at the end of this chapter.

SAMPLE COLLECTION FOR BACTERIAL ISOLATION

- Tissue or fluids are the best samples to collect for bacterial isolation. Tissues should be collected as aseptically as possible and placed in a sterile container such as a Whirl-pac™ sampling bag (Nasco, Fort Atkinson, WI).
- Swabs and other transport media designated for viral use frequently contain antimicrobial agents and are not suitable for transport of samples for bacterial isolation.
- If transport to the laboratory will take more than a day or is likely to dry during transfer to the laboratory, a transport medium, such as Cary-Blair (Fisher Scientific, Pittsburgh, PA) could be used to prolong survival of bacteria.
- Swabs are not the best choice for sampling because they only hold a small amount of material and some swabs are made of substances that inhibit bacterial growth. Swabs are often not appropriate for fecal or intestinal cultures due to an inadequate sample volume. They are also inappropriate for sampling for anaerobes because these organisms cannot survive within the swab due to trapped air.
- Tissues received in the microbiology laboratory are often seared on the outside to eliminate contaminating surface bacteria; therefore, the samples should be large enough to withstand this treatment yet allow bacteria to survive deep within the tissue. Ideally they should be 1 cm² or larger.
- Samples should be placed in individual containers to eliminate the possibility of cross-contamination.

SAMPLING FOR VIRAL DISEASES

- Sampling for viral diseases is often very similar to sampling for bacterial diseases. Samples should be collected using a sterile technique, kept cool, and packaged in individual bags. Often the same sample can be used for detection of viruses and bacteria if ample material is available. A single swab will NOT be adequate for testing for multiple agents such as bacteria and viruses.
- Sampling early in disease (acute phase) is essential to optimize the likelihood of viral isolation/detection. Virus may only be present early in the disease process.
- Tissues from animals that have only very recently died should be used for virus isolation.

- Collection of tissues for histologic examination in formalin can help to determine if a viral disease is present. Immunohistochemistry of formalin fixed tissues can also be used to detect viral antigen in infected tissues. Examples of these viruses are bovine viral diarrhea virus and infectious bovine rhinotracheitis virus.
- The polymerase chain reaction (PCR) can be used to detect viral DNA from fresh and sometimes formalin-fixed samples.
- Serology using both acute and convalescent samples from affected animals or the dam in the case of abortion can be used to determine recent infection/exposure to a viral agent. A 4-fold rise in antibody titer indicates recent exposure.
- Electron microscopy can also be used to identify viral particles in feces or tissues. This technique can be very helpful when performed on feces of young animals when coronavirus or rotavirus infection is suspected.

HISTORY AND NECROPSY FINDINGS

- The type of bacterial or viral detection methodology used by the laboratory is determined by the species of animal, age, history, and clinical findings entered on the submission form and the tissues received. Thus, it is important that the clinician provide a limited history and any lesions or clinical signs detected.
- If a particular bacterial or viral agent is suspected in the case, it should be indicated on the submission form. The laboratory can then use this information to establish any specialized culture conditions needed to detect suspected agents.

SAMPLE TRANSPORT

- Samples are best shipped cool, not frozen, with ice or frozen gel pack by overnight delivery in a sealed container to prevent leakage according to regional or local regulations. Some bacteria and viruses might survive freezing but their number in the tissue will be decreased, thus diminishing the likelihood of isolation. Remember that in hot weather, samples will warm up quickly so additional cold packs may be needed.
- For isolation of obligate anaerobic bacteria, the sample should be placed in a transport medium capable of supporting an anaerobic environment.
- If possible, avoid shipping over the weekend because samples may sit in warm locations prior to delivery to the laboratory on Monday.

SAMPLING TO DETERMINE IF SEPTICEMIA IS PRESENT

- In the live animal, blood culture is the optimal method to detect septicemia. Blood should be collected aseptically and transferred immediately into blood culture vials.
- At necropsy it is best to sample an organ from each body cavity, optimally spleen and lung, to determine whether an animal is septicemic.
- If there is gross evidence of an infectious process in other organs, such as a joint, these tissues should also be submitted.
- Additional tissues can be collected and refrigerated or frozen for future isolation if deemed necessary after the histopathologic or cytologic examination.

MILK

- Milk samples should be collected as aseptically as possible into a sterile container.
- Milk samples can be refrigerated, or if needed, frozen after collection and for transport to the laboratory.

SAMPLING FOR ENTERIC INFECTIONS

- Potential pathogens in intestinal or fecal contents vary according to the species of origin and the age of the animal; thus, the laboratory will focus its efforts to identify likely pathogens for a given signalment.
- Samples for culture of intestinal contents should be collected from the site of the lesion at necropsy rather than merely collecting fecal material. This is especially important for suspected clostridial enteritis.
- When salmonellosis is suspected, inclusion of gall bladder and mesenteric lymph nodes may aid diagnosis.
- When *Mycobacterium avium* ssp. *paratuberculosis* is suspected, ileum and ileo-cecal lymph nodes should be included.
- Depending on the laboratory, *Campylobacter* or *Mycobacterium* cultures may be performed only if they are specifically requested or if numerous vibrio-like or acid fast organisms, respectively, are seen on smears. Be sure to indicate what pathogen(s) are likely to cause the lesions seen. Don't simply ask for aerobic and anaerobic cultures on feces.

SAMPLING FOR CLOSTRIDIAL ENTERITIS

- Anaerobic cultures from fecal material on food animal cases are not routinely done at all microbiology laboratories. Therefore, it is important to state that a *Clostridium* is suspected on the bacteriology submission form.
- A loop of small intestine from an affected region is the preferred sample.
- *Clostridium perfringens* is considered to be normal flora of ruminants, thus, isolation of *C. perfringens* without detection of toxin or toxin genes is of doubtful significance. The sample should be collected as soon after death (within 1 to 2 hours) as possible because clostridial organisms will rapidly proliferate throughout the intestine. Thus, in cases long post-mortem interval, isolation of *Clostridium perfringens* is of doubtful significance.
- Isolation of *C. perfringens* should be correlated with the presence of appropriate histologic lesions.
- Few labs offer detection of *C. perfringens* toxins or toxin genes for typing. However, isolates can be sent out to a specialty laboratory for genetic testing and identification of toxin or toxin genes.

ANAEROBIC INFECTIONS

- Decide prior to submission whether you think anaerobic culture is necessary and be selective regarding the tissue submitted.
- Anaerobic infections are common in abscesses, peritonitis, pneumonia, and pleuritis.
- Anaerobic culture of tissues from an animal that has been dead for many hours may not be worthwhile because many anaerobes will proliferate and disperse throughout the carcass.
- Most anaerobes are highly sensitive to oxygen. Therefore, they should be kept in an environment which minimizes their exposure to the atmosphere. Tissues or a generous volume of fluid (several milliliters) are the best samples for anaerobic culture. Tissues should be at least 1 × 1 cm.
- If only very small tissue or fluid samples are available for sampling, they should be placed in some sort of transport media such as Anaerobic Transport Medium (Anaerobe Systems, Santa Maria, CA), Port-A-Cul tubes™ (swabs or tissues) or Port-A-Cul vials™ (fluid samples) (BD Biosciences, Franklin Lakes, NJ).

EXAMPLES OF SAMPLES TO BE SELECTED FOR DISEASES IN EACH ORGAN SYSTEM

- Respiratory: If the animal is alive, tracheal secretions from transtracheal aspiration are best. Lung tissue is preferred in when the animal is dead..
- Gastrointestinal: Feces should be collected in the live animal; in the dead animal, select contents or an intestinal loop from the lesion (e.g., small intestine, colon, mesenteric lymph node, etc.).
- Genitourinary (including abortion): Abortion—multiple samples are needed and submission of an entire selection for culture and microscopic examination is best to optimize identification of the etiology: fetal stomach contents, liver, lung, heart, fetal heart blood, spleen, placenta, dam serum (for serology).
- Neurologic: Cerebral spinal fluid, brain, spinal cord.
- Integument: Skin biopsy following a sterile preparation. Unopened lesions or pustules are preferred. Dermatophytes—skin flakes and plucked hairs from advancing edge of lesion.
- Musculoskeletal: Joint fluid, bone, affected muscle (e.g., blackleg). Anaerobic culture will be needed for isolation of *Clostridium* spp.

DISEASES WITH SPECIAL CONSIDERATIONS

- Botulism: Not all laboratories have the capability to detect botulinum toxin or *C. botulinum*. The best way to diagnose botulism is detection of toxin in blood or intestinal contents of the animal; however, many tests have low sensitivity. Sampling the suspected origin of the toxin (feed, water) for toxin and/or *C. botulinum* culture is best. These samples can be sent to your microbiology laboratory and forwarded from there, or better still, contact your laboratory to determine where samples should be sent to speed analysis.
- Tetanus: *C. tetani* is difficult to grow and frequently is not isolated from affected animals. However, the laboratory can perform smears to identify typical bacilli that could be causative. Generally, it is a clinical diagnosis.
- Anthrax: Collect blood for a smear and a stat Gram stain. The blood can also be cultured to detect *Bacillus anthracis*. Pigs do not have the same overwhelming terminal bacteremia as cows, sheep, and horses, so detection of the bacillus in smears may be difficult. Remember that animals dead for some time may have many cadaver bacilli and after several days these organisms may overwhelm *B. anthracis*. Do not open carcass until anthrax has been ruled out.
- Rabies: The entire brain should be submitted to the state laboratory for rabies testing. Request that additional tissues be held by the laboratory until rabies testing on the brain has been completed and shown to be negative before additional testing occurs.

EXAMPLES OF FASTIDIOUS ORGANISMS OR THOSE REQUIRING SPECIALIZED MEDIA/CONDITIONS OR ARE DIFFICULT TO IDENTIFY

- Generally, the average ruminant practitioner will not attempt to culture these organisms in their own laboratory because of the specialized media and/or equipment needed:
 - All viruses
 - *Mycobacterium avium* spp. *paratuberculosis*
 - Other mycobacteria including *M. avium*
 - *Mycoplasma/Ureaplasma*
 - Anaerobes including clostridial organisms—differ according to species, especially *C. difficile*. FA available on fixed tissues (e.g. liver, muscle)
 - *Campylobacter*
 - *Leptospira*

- *Listeria*—can be readily isolated in laboratory but cold enrichment for neural tissues can require additional reagents and time commitment
- *Salmonella* (selection/enrichment)—can be readily isolated but requires enrichment in the laboratory
- *Helicobacter*
- *Yersinia*
- Fungi



MISCELLANEOUS

ZOONOTIC POTENTIAL

Notify the laboratory if you are suspicious of a disease that is highly infectious for humans. Submit all samples in a sterile container that is sealed such as a Whirl-pac™ sampling bag. Examples of such diseases: rabies, anthrax, plague, tularemia, dimorphic fungi (*Coccidioides*, *Histoplasma*, *Blastomyces*), *Chlamydia*, and mycobacterial infections.

BIOSECURITY

See the above section on zoonotic potential. If bacterial culture and isolation are carried out in the practice setting local regulations for handling and disposal of infectious material should be followed.

ABBREVIATIONS

- FA = fluorescent antibody
- PCR = polymerase chain reaction

Suggested Reading

[Caswell, J.L.](#), [Hewson, J.](#), [Slavić, Đ.](#), [DeLay, J.](#), [Bateman, K.](#). 2012. Laboratory and postmortem diagnosis of bovine respiratory disease. *Vet Clin North Am Food Anim Pract.* Nov;28(3):419-441.

Dinsmore, R.P., English, P.B., Gonzalez, R.N., Sears, P.M., Schulte, H.F. 1991. Evaluation of methods for the diagnosis of *Streptococcus agalactiae* intramammary infections in dairy cattle. *J Dairy Sci.* May; 74(5):1521–6.

Feldman, S.H., Feldman, S. 2001. Diagnostic molecular microbiology in laboratory animal health monitoring and surveillance programs. *Lab Anim* (NY). Nov; 30(10):34–42.

Hyatt, D.R., Weese, J.S. 2004. [Salmonella culture: sampling procedures and laboratory techniques](#). *Vet Clin North Am Equine Pract.* Dec;20(3):577-585

Knowles, D.P. Jr, Gorham, J.R. 1990. Diagnosis of viral and bacterial diseases. *Rev Sci Tech.* Sep; 9(3):733–57.

Rodriguez, J.M. 1997. Detection of animal pathogens by using the polymerase chain reaction (PCR). *Vet J.* May; 153(3):287–305.

Washington Animal Disease Diagnostic Laboratory web site: <http://www.vetmed.wsu.edu/depts/waddl/general.asp> Includes excellent guidelines regarding sampling for various disease processes.

Author Barbara A. Byrne

Consulting Editor Christopher C.L. Chase