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INTRODUCTION

Laboratories are the practitioner's link to a wide variety of services that are essential for accurate diagnosis and that facilitate cost-effective treatment of diseases of livestock and companion animals. An accurate diagnosis is also required if a zoonosis is suspected or to ensure that pets are monitored for the presence of potential zoonoses before they enter environments of the aged or immunosuppressed individuals. The most important aspects of specimen collection for subsequent laboratory examination are the correct choice, method of collection and submission, and requests for appropriate tests.

![Diagram showing the relationship between a laboratory and a clinical diagnosis]

**SPECIMEN SELECTION**

**Selecting the animal**

- **The best animal for diagnostic examination is an untreated, acutely ill animal.**
- **Live animals.** If the clinical signs are seen in more than one animal of a group, it is useful if samples of e.g. blood, faeces, and urine are taken from a representative number of clinically affected animals and some "healthy" in contact animals. If the groups are large, it is considered sufficient to collect samples from 10% of the clinically ill animals.
- **Dead animals.** If the case warrants it, and the owner permits it, sacrificing an animal *in extremis* in order to get good quality specimens may be preferable. Second best is a recently dead animal. Post mortem invasion of tissues by intestinal and other bacteria can be rapid, particularly in warm weather. Animals with long hair or obese animals tend to cool slower and thus autolysis of especially the abdominal viscera will proceed quickly. This hampers identification of the causative agent as well as histopathological examinations where cell structure will be lost.
Choosing tissues for submission

Live animals. Samples should be taken from easily accessible affected site(s) as early as possible following
the onset of clinical signs. Examples include blood in anticoagulant for bacterial isolation; faeces when
diarrhoea is present; and urine if cystitis is suspected.

Dead animals. Samples from tissues or organs with lesions are preferred.

Here are some tips:

- It is best to collect samples from the edge of lesions, including some healthy tissue. This is usually the
  site of active replication of organisms.
- If there are no apparent gross lesions, select tissues from organ systems to which the clinical signs are
  referable, i.e. transtracheal aspirate in animals with clinical signs of pneumonia. If a particular disease
  is suspected, choose tissues targeted by the causative organisms.
- If there are no gross lesions and if clinical signs do not narrow the disease process to a particular organ
  system, collect samples from several organs. Appropriate specimens include lung, liver, kidney, spleen,
  brain, heart, duodenum, jejunum, ileum, colon and body fluids. The bone marrow can be sent, if no
  other suitable samples are available, as it is less accessible to putrefactive organisms. Send 50-100
  mm of rib stripped of muscle and periosteum in a sterile well-sealed container.
- In the case of aborted foetuses, neonatal animals, birds and other small pets such as rats and canaries,
  the whole carcass can be sent to the laboratory.
- If uncertain as to which specimens and how to take them, contact the diagnostic laboratory and enquire
  if the laboratory can perform the relevant tests.

TISSUE HANDLING AND PACKAGING

Samples should be collected as aseptically as possible. This will prevent the aetiological agent from being
overgrown by contaminating bacteria. Handle specimens gently. Rough handling of tissues exacerbates
tissue artefacts and autolytic changes.

Fixed specimens for histopathological and immunocytochemical examination

- Take thin tissue samples about 5 mm thick, so that the formalin can penetrate the tissues, and place in
  10% buffered formalin. The formalin to tissue ratio should be at least 4:1. Do not freeze these
  specimens.

Fresh tissues for microbiological examination

- For microbial isolation, individual tissues must be placed in separate sterile containers.
- Do not allow specimens for culture to come into direct contact with disinfectants or antiseptics.
- For microbiological examination, a generous amount of the sample should be submitted, such as blocks
  of tissue (approximately 40 mm³), biopsy material, or several milliliters of pus, exudate or faeces.
• When possible, submit together with the microbiological specimens, a few air-dried smears of exudates, urine sediments, and tissue impressions. These may provide a picture of the process within the body and could aid in a rapid presumptive diagnosis. If indicated, these smears could be used for a direct fluorescent antibody test.
• Appropriate containers for organ specimens are sterile plastic screw top jars, “Whirl-pak” plastic bags or plastic bags that are well sealed. Do not select zipper seal bags as they tend to leak. If glass jars are used they should be well packed to avoid breakage.
• If swabs are used, they should preferably be placed in an appropriate non-nutritive transport medium to prevent desiccation. Swabs with transport medium are commercially available and are suitable for aerobic and fungal cultures.
• Do not allow specimens to dry out. If the sample size is small, it is best to place it in an appropriate transport medium.
• Different transport media are available for aerobic and anaerobic bacteria, viral and mycoplasma isolation. It can usually be obtained from the diagnostic laboratory.
• To slow autolysis and prevent unchecked proliferation of contaminants, fresh tissues samples should be submitted in a container containing adequate refrigerant and sufficient insulation to maintain them at a cold temperature for the entire shipping process. A small polystyrene container with at least two frozen ice packs that are well wrapped is usually sufficient. If transportation is expected to take longer than 24 hours, these packs can be substituted with dry ice. Because cold air is heavier than warm air, samples should always be packed at the bottom of the container. Most samples should not be frozen.
• Fixed and fresh specimens should be separated as formalin leakage will render fresh tissues unusable.
• Ensure that parcels sent to a laboratory are watertight and correctly labeled.
• As the laboratory personnel have not examined the animals, they are essentially uninformed. Therefore a complete history should be provided. Include animal species, age, sex, numbers affected and any relevant clinical and management information. Recent treatment with antimicrobials as well as vaccination history should be included as these can affect the interpretation of results. If the samples were taken during necropsy, the necropsy findings should be included. All this information will aid the laboratory personnel in interpreting the results as well as performing any additional relevant tests.
• Forms containing all the relevant information of the samples/s should be placed in a separate zipper sealed plastic bag.

SPECIMENS REQUIRING SPECIALISED TREATMENT

Anaerobic specimens for bacterial culture

• As strict anaerobes survive for only a short period in air, great care must be taken when collecting for isolation of anaerobes.
• Specimens should be taken aseptically to prevent contamination with normal mucosal microflora, of which anaerobes form a part.
• Samples from animals that have been dead for longer than four hours are usually unsuitable because of the rapid post-mortem invasion of the body by anaerobes from the intestinal tract.
Suitable samples include blocks of affected tissues (40 mm³), placed in a commercial "anaerobic specimen collector". An alternative method is to spray carbon dioxide gas into the container containing the specimen so that the air is replaced. The container is then well sealed.

If the samples are sufficiently large and collected aseptically, the centre of the tissue often remains anaerobic without taking special precautions. In such cases the sample should reach the laboratory with 2 to 3 hours.

In the case of exudates, the sample can be placed in a disposable syringe. Air is expelled from the syringe and the needle bent back on itself or plugged. Alternatively, fluids can be injected through the cap into a red-capped vacutainer without anticoagulant. It is preferable not to send these specimens on ice as oxygen dissolves better in fluids at low temperatures.

**Blood for bacterial isolation**

If a bacteraemia or septicaemia is suspected, blood should be submitted for bacterial cultures. It is best to collect blood during a fever peak. Strict aseptic procedures should be taken when collecting the blood. The area over the blood vessel puncture site must be shaved, cleaned thoroughly with detergent, dried and 70% alcohol applied to the skin and allowed to act for at least 30 seconds. As a bacteraemia can be intermittent, it is advisable to take three to four blood cultures within a 24 hour period. About 10 ml of blood should be added aseptically and without delay into a commercial blood-culture bottle and then sent to the laboratory. If blood culture bottles are not available, blood should be collected using heparin as an anticoagulant and submitted to the laboratory within 2 to 3 hours. Blood culture bottles are usually available from most veterinary or medical diagnostic laboratories.

**Table 1: Samples recommended for confirming bacterial infection in sick animals or carcasses**

<table>
<thead>
<tr>
<th>Predominant clinical sign or post-mortem lesion</th>
<th>Bacteria</th>
<th>Recommended samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septicaemia, toxemia, sudden death, haemorrhages, subcutaneous oedema, gas gangrene</td>
<td><em>Bacillus anthracis</em>, <em>Clostridium</em>, <em>Pasteurella</em>, <em>E. coli</em>, <em>Salmonella</em>, <em>Erysipelothrix</em>, <em>Staphylococcus</em></td>
<td>Blood, body fluids, parenchymatous organs, bone marrow, lymph node</td>
</tr>
<tr>
<td>Enteritis, dysentery</td>
<td><em>E. coli</em>, <em>Salmonella</em>, <em>Mycobacterium paratuberculosis</em>, <em>Clostridium</em>, <em>Campylobacter</em>, <em>Brachyspira hyodysenterica</em></td>
<td>Tied off portions of affected intestine, mesenteric lymph nodes</td>
</tr>
<tr>
<td>Pneumonia, coughing</td>
<td><em>Pasteurella</em>, <em>Mannheimia</em>, <em>Bordetella</em>, <em>Histophilus</em>, <em>Mycoplasma</em></td>
<td>Affected lung, transtracheal aspirates,*nasal swabs, mediastinal lymph nodes.</td>
</tr>
<tr>
<td>Nervous signs</td>
<td><em>Clostridium botulinum</em>, <em>Clostridium tetani</em>, <em>Listeria monocytogenes</em>, <em>Acanobacterium pyogenes</em>, <em>E. coli</em></td>
<td>Serum, contents of the rumen and large intestine, wound swab or tissue, brain, liver, spleen, CSF</td>
</tr>
</tbody>
</table>
### Skin conditions

**Abscesses**

- Recently formed abscesses should be selected.
- If the abscess is intact, attempt to aspirate pus into a syringe.
- Take a swab of the pus from the edge of the abscess, where the likelihood of viable organisms is greatest. The centre of an abscess is often sterile.
- Make air-dried smears of the pus and edge scrapings.
- Aerobic culture is usually requested, but if the abscess is deep-seated or malodorous, anaerobic bacterial culture should also be considered.

**Lumps and vesicles**

- Resect the lump and send half in formalin for histopathology and the other half on ice for bacterial or fungal isolation. Samples taken with biopsy needles or punches can also be used.
- Fine needle aspirates can be made from soft lumps or intact vesicles, especially if the lumps appear soft. Send a portion for cytology and another for culture.
- In animals common causes of lumps and bumps, if not neoplasms, are abscesses or granulomas due to pyogenic bacteria, or mycetomas caused by either bacteria (actinomycetes) or fungi.

**Open lesions e.g. ulcers/ erosions/ suppurrative lesions/ alopecia**

- A biopsy is best, half for culture, the other half for histopathology. In open lesions secondary infection is common which can mask the original aetiology.
- Before sending specimens to the laboratory, examine deep skin scrapings under the microscope for the presence of fungal hyphae and spores at the base of hair shafts.
- Skin scrapings, hair plucks or sterile toothbrush brushings from the edge of affected areas are taken for dermatophyte culture. Send the sample in a clean sealed envelope or cotton wool plugged test tube. Saprophytic fungi will frequently proliferate rapidly in a sealed tube due to moisture build up.
- If *Dermatophilus congolensis* infection is suspected, impression smears should be made of the base of crusts or of the skin exudate. Crusts can be sent in a sterile container for bacterial isolation.
Otitis externa in dogs and cats

Bacteria and the yeast *Malassezia pachydermatis*, tend to be secondary to other causes of ear inflammation. In dogs, bacteria that have most commonly been implicated in otitis externa are *Staphylococcus pseudintermedius*, *Pseudomonas* spp., *Proteus mirabilis* and beta-haemolytic streptococci. *Pasteurella multocida* may also be isolated from cats. Prior to collection of specimens for bacterial culture, it is recommended that heat fixed smears of the ear exudate be examined. If a bacterial infection is present, leucocytes and phagocytosed bacteria are usually seen. *Malassezia pachydermatis* is seen as yeasts with broad based budding. More than ten yeasts per microscope field are considered significant. Bacterial culture and susceptibility testing are useful when the tympanic membrane is ruptured and the clinician feels that systemic therapy is warranted or when mainly rods are seen in an exudate smear. For culture a sterile swab should be inserted deep into the ear canal, then placed in bacterial transport medium and submitted to the laboratory for aerobic culture.

Musculoskeletal infections

Gangrenous myositis in cattle and sheep

Six impression smears are made from the cut surfaces of incisions into affected muscle groups for Gram’s staining and direct fluorescent antibody tests. Fresh tissue samples of lesions are submitted in a sterile container for clostridial isolation.

Vertebral osteomyelitis in dogs and cats

Bacteria usually associated with discospondylitis are coagulase-positive staphylococci, *Streptococcus* spp. and in some countries, *Brucella canis*. Rarely, *Aspergillus terreus* causes discospondylitis in German Shepherd dogs. Blood cultures should be attempted as up to 75% of cases may yield positive cultures. If surgical intervention is done, bone samples for culture can be obtained.

Septic arthritis

If an infectious agent is suspected, a joint fluid aspirate or, preferably a synovial biopsy, should be taken and sent for bacterial (aerobic and anaerobic), mycoplasmal and fungal cultures. Blood cultures should be performed if systemic infections are suspected.
Applied Veterinary Bacteriology and Mycology: Bacteriological Techniques → Chapter 1: Practical aspects of specimen collection and transport for meaningful and optimum laboratory results

Diarrhoea

Living animals

- Fresh faecal smears should be stained to visualize the bacteria flora and detect presence of acid-fast mycobacteria. An overabundance of large Gram-positive rods may suggest a clostridial infection.
- For microbiological examination faeces collected from the rectum is best. More than one sample taken at intervals may be necessary as a diarrhoea can dilute the organisms. From chronic cases of diarrhoea, faeces should also be submitted for the isolation of Campylobacter and Mycobacterium spp.

Dead animals

- Portions of affected intestine and mesenteric lymph nodes must be submitted. In addition, submit a long portion (approximately 100 mm long) of affected intestine tied off at both ends. This will allow for anaerobic culture and if Clostridium perfringens or C. difficile is suspected, enterotoxin identification.
- Parenchymatous organs, i.e. liver, spleen, kidney and lung are collected if septicaemia is suspected.
- If a clostridial infection is suspected only fresh carcasses (less than 6 hours old) should be used for sampling.
- Impression smears of the large intestine can be stained with fluorescent antibodies to detect Lawsonia spp. in pigs and foals. In pigs, samples of affected small intestines can be submitted in 10% formalin to detect Brachyspira (Serpulina) hyodysenteriae.
- If paratuberculosis is suspected, submit duplicate samples of the small intestine and mesenteric lymph nodes. One set in 10% formalin for histopathology and the other in a separate sterile container for Mycobacterium isolation and identification.

Respiratory disease

Upper respiratory tract infections

- A laboratory diagnosis is usually only requested if the infection is unresponsive to therapy.
- Nasal discharges or swabs for bacterial isolation usually yield very poor results as many potential pathogens normally reside in the nasal cavity.
- Nasal biopsies if a fungal infection is suspected i.e. Aspergillus fumigatus. The samples should be submitted for both histopathology and fungal isolation. A nasal flush or swab is less satisfactory as air-borne fungi are often inhaled yielding false positive results, and invasive fungi are surrounded by fibrous tissue and thus not shed into the nasal cavity giving false negative results.
- Conjunctival scrapings or smears may be helpful for fluorescent antibody tests for the presence of Chlamydia psittaci in cats.
Lower respiratory tract infections

Living animals

- Transtracheal aspirates or bronchioalveolar lavages are collected for both cytological examination and bacterial culture. If the pneumonia is long standing, the sample should be submitted for both aerobic and anaerobic bacterial isolation.
- Although nasal swabs are not ideal due to the presence of normal commensal bacteria, they can be used to detect obligate pathogens, such as *Mycoplasma mycoides* subspecies *mycoides* (small colony) in cattle.
- Tonsillar swabs from pigs enable the detection of carriers of *Mycoplasma hyopneumoniae*.
- If there is a pleural effusion, fluid should be withdrawn and submitted for aerobic, anaerobic and mycoplasma isolation. A few air-dried smears should also be submitted.

Dead animals

- For bacteriological examination, the most caudal portion of the affected lung tissue (less contaminated) and mediastinal lymph nodes are preferred. These should be submitted for aerobic culture and, if indicated, mycoplasma, anaerobic bacteria and fungal cultures.

Nervous system infections

Living animals

- In the living animal, the cerebral spinal fluid can be used for cytology, microbiological examination and antibody detection.

Dead animals

- If the brain is macroscopically affected submit lesions for histopathology and on ice for bacterial or fungal isolation.

Urogenital tract infections

Abortions

- The whole fresh foetus with foetal membranes should be submitted on ice. Failing this, submit portions of the placenta, abomasal contents, foetal liver, kidney, lung and any gross lesions ice to the laboratory. Impression smears of these organs as well as of the stomach contents can be made, air-dried and sent to the laboratory.
- If no placenta is available, the uterine discharge of the dam can be submitted.
- If a leptospira-induced abortion is suspected, 20 ml of mid-stream urine from the dam preserved with 1.5 ml of 10% formalin should be submitted for dark-field microscopy.
Cystitis

- Urine samples may be submitted for urine analysis, bacterial microscopy and culture or for a viable bacterial count to establish whether a clinical bacteriuria is present.
- Collection by cystocentesis is best, as it less likely to have contaminating bacteria.
- Catheterised urine samples are also acceptable.
- Mid-flow urine. Bacteriological counts should be performed on this urine. A count of $10^5$ CFU/ml or more is considered significant. These samples should be cultured within one hour of collection or cooled to 4°C and cultured within four hours after collection.

Endometritis

Endometritis is recognized more commonly in mares.

- Two guarded swabs are taken of the endometrial discharge, one for cytology and, if that one is positive for bacteria, the other for bacterial and fungal culture.
- For the isolation and identification of *Lawsonia intracellularis* from stallions, samples are taken twice with a seven day interval. Cotton-tipped, dry bacteriology swabs without transport medium are used. Separate sterile gloves for each stallion are used and swabs taken under sedation. Three swabs from the following sites are collected: urethra, urethral fossa and sinus (insert swab into the sinus) and penile sheath (lamina interna). Submit within 48 hours – cooling not necessary.
- For the isolation of *L. intracellularis* from mares, swabs of the clitoris and clitoral fossa are taken.

Infections of the male genital tract

- Sheath-washes or preputial scrapings are done to detect *Campylobacter foetus* subsp. *Venerealis* in bulls.
- Semen should be submitted for aerobic culture and bacterial counts.

INTERPRETATION OF LABORATORY RESULTS

The following points are pertinent when interpreting results from a diagnostic microbiology laboratory:

- A negative diagnostic report does not necessarily mean that the suspected micro-organism is not the aetiological agent of the condition. There may be many reasons for failure of the laboratory to isolate or identify the pathogen, such as a bacterium being overgrown by contaminants, fragile micro-organisms having died on the way to the laboratory, or the animal may have stopped excreting the micro-organisms when the sample was taken. Another very important reason is treatment with antimicrobials prior to sample collection.
- Many bacteria, such as the members of the family *Enterobacteriaceae* are ubiquitous. Isolation of micro-organisms may represent contamination of the sample by faeces or soil, or their presence is due to post-mortem invasion.
- Apparently healthy animals can be subclinical shedders of micro-organisms such as salmonellas in faeces and leptospires in urine. Even if these potential pathogens are isolated and identified, the illness or death may be due to another cause.
- Some bacteria such as *Salmonella* spp. or leptospires may be shed intermittently. One or more repeat samples following a negative examination report may be worthwhile.
- It is necessary to demonstrate the fungal hyphae actually invading the tissue for a positive diagnosis of an opportunistic fungal disease such as that caused by *Aspergillus fumigatus*.

**TIME CONSTRAINTS IN THE LABORATORY**

The laboratory endeavors to get the results to you as soon as possible. There are however, constraints, which include:

- The time it took for samples to reach the laboratory.
- The time required for sample processing.
- If a culture is required, this can be especially long and is often dependent on the speed of growth of the organisms, contaminants and number of tests required
- The time for results to reach the clinician.

**REFERENCES**