Microbiology

Microbiology encompasses the study of disease caused by microbes (bacteria, viruses, fungi and protozoa). As a general rule, to associate disease with an organism it is important to demonstrate the presence of the organism in diseased tissue, and to demonstrate changes within the tissue consistent with an infection by that organism.

This section deals mainly with bacteria and fungi. Viral and protozoal diseases are mainly dealt with elsewhere (e.g. serology).

Bacteria can be found in large numbers in the external environment, and in areas of the body connected to that environment e.g. GIT, vagina, upper respiratory tract; skin. Other tissues are generally sterile in a healthy animal, and there are numerous mechanisms to keep them that way. These may sometimes be by-passed so that normal commensal bacteria and environmental saprophytes (opportunistic pathogens) gain admittance e.g. through trauma or suppression of the immune response. There are some obligate pathogens with virulence factors that allow invasion of the body in the absence of predisposing conditions and some bacteria fall between these 2 extremes.

Bacterial invasion generally results in tissue destruction and inflammation resulting in disease. Additional tests such as histopathology and cytology can be used to support the results of culture and without these it may be difficult to be sure of the significance of a particular isolate.

SAMPLING AND SUBMITTING MATERIAL

The aim is to provide the laboratory with a sample that contains the same types and numbers of bacteria that were present in the sampled site.

In order for microbiological examination to be useful, the submitted material must:

- Be the appropriate type
- Be collected and stored in as sterile a manner as possible.
- Reach the laboratory as soon as possible so there is minimal multiplication of organisms in transit.
- Be in good condition by the time it reaches the laboratory (kept moist and cool).

To achieve this:

- Always use sterile containers (and try to avoid contaminating the outside of them).
- Use clean and preferably sterile scissors and forceps for collection of samples.
- Ensure the pottle/jar lids are threaded correctly for tight leak proof seal.
- When using swabs, collect as much of the relevant material as possible on them and place in sterile transport media (dry swabs are not recommended for microbial culture, but are required for PCR).
- Keep specimens cool in transit (but avoid freezing) e.g. use a chilli-bin in the car, refrigerator in the clinic, and coolant silica pads in the courier bag.
- Try to get the samples to the laboratory within 24 hours of collection, or if this is not possible (e.g. over a weekend), store in the refrigerator until they can be sent.

Additional Information on sampling in specific circumstances:

- **Fluids** - Use sterile containers. Blood collection tubes are NOT guaranteed sterile. An aliquot can also be placed in EDTA to prevent clotting and allow cytology if required.
- **Urine -** Collect aseptically, preferably by cystocentesis, alternatively by catheter or, if necessary, mid-stream sample. There are several different collection tubes designed for collecting urine samples

depending on the testing required refer to this guide (see urinalysis is documented elsewhere in this handbook).

- **Pus -** from abscesses should be collected together with scrapings from the wall of the abscess if possible as pus at the centre of an abscess may be sterile. Recent abscesses are preferable to old ones. Anaerobic bacteria can often be isolated from abscesses.
- **Faeces** - A freshly voided sample or one collected from the rectum is preferable to a rectal swab which often does not have enough faecal matter for agent detection.
- **Solid tissue -** Fresh tissue (e.g. from necropsy or biopsy). If possible, cut a block of tissue approximately $2cm^{3.}$ It can be difficult to avoid contamination with necropsy samples. If possible take samples from solid organs before opening the GIT.
- **Anaerobic culture -** Many anaerobes do not survive exposure to air for more than a few minutes. Avoid contact of samples with adjacent mucosal surfaces which may have resident anaerobic flora. Specimens from animals dead more than 4 hours are usually unsuitable due to rapid post-mortem invasion of tissues by anaerobes from the GIT. Bone marrow appears to be one of the last tissues to be invaded by contaminating bacteria. Other acceptable samples include blocks of tissue, several millilitres of fluid in a sterile container or as a last resort, a swab in appropriate transport medium. Samples for anaerobic culture should arrive at the laboratory as soon as possible after collection.
- **Abortion culture -** Panels are available. Samples of choice are fetal stomach contents, fetal lung and finally cotyledons/placenta if these are in good condition. There are also PCR tests and panels available for sheep and cattle in particular.
- **Blood and joint culture -** Blood culture is indicated in animals with pyrexia of unknown origin and in disease processes where bacteraemia or septicaemia is expected (e.g. discospondylitis, endocarditis). Sterile blood (free of anticoagulant) is injected directly into a blood culture bottle (available from Gribbles Veterinary) – paediatric blood culture bottles only require 1-3mL blood. Aseptic collection of the sample is critical with the site of venepuncture being shaved and prepared as for surgical procedures. A single contaminating bacterium will multiply and produce erroneous results. Once the blood culture media has been inoculated DO NOT refrigerate, keep at room temperature until submitted to the laboratory. Courier to the laboratory in the usual way.

  It is recommended that joint fluid from suspected cases of septic joint disease be inoculated into blood culture bottles as this greatly increases the sensitivity of joint cultures.

  Refer to our ***How To*** Take a Blood Culture guide for further information.
- **Milk sampling:** Prepare as follows:

  - Wash the teats with clean running water or water containing disinfectant such as cetrimide.
  - Dry the teats and udder with paper towel.
  - Wipe the teat orifice with a gauze wad soaked in 70% ethanol, using a separate wad for each teat.
  - Dry the side of the teat with a clean gauze swab, using one motion and moving away from the teat orifice.
  - The teats on the side of the udder opposite to the collector should be cleaned first.
  - It is essential that NO disinfectant or ethanol drip into the collection container.

  Milk collection method:

  - The collector should wear gloves or wash their hands in a solution of disinfectant. Change gloves or wash between taking samples from different animals.
  - Reduce contamination by any existing milk in the streak canal by NOT collecting the first squirt of milk.
  - Collect 10-20 ml of milk from each quarter into a sterile screw top container
  - Samples should be collected from teats nearest the collector first.
  - Ensure no milk runs off the collector's hand into the container.
  - Do not let the teat touch the container.

- Containers should be clearly labelled with the animal's identification, owner's name and the quarter that has been sampled.

- **Fungal examination of the skin:** In cases where lesions are present, pluck as many hairs as practicable from the peripheral areas for mycology examination. In suspect infections (no obvious visible lesions, negative to Wood's lamp illumination), brush the animal with an unused toothbrush. Collect the hairs and scurf into a container (e.g. by having paper under the animal during brushing). Submit the container with samples and the entire toothbrush to the laboratory for fungal examination. **DO NOT use paraffin oil** to aid in the collection of these types of specimens. Please note that absence of positive KOH test on hairs and scale does NOT rule out ringworm. Culture is required and this can take up to four weeks.

CAMPYLOBACTER FETUS SUBSP. VENEREALIS AND TRITRICHOMONAS FOETUS EXAMINATION AND CULTURE

These organisms can establish on the preputial mucosa of largely asymptomatic bulls, and be transmitted during coitis, resulting in infertility and abortion. Samples require the use of a "Tricamper" collection tool, and the sample collected can be used for culture or PCR.

Preputial samples: Insert the tool into the prepuce and move back and forth to scrape across preputial mucosa and the surface of the penis. Block end (e.g. with finger) to prevent loss of material then remove from prepuce. Holding just off horizontal, insert tip into saline tube and remove block from end. Rinse smegma using 5 ml saline from a syringe, or cut off the black head of tricamper into saline.

Vaginal mucus: Clean perineum. Introduce tricamper in a dorsocranial direction to avoid urethra until anterior end reaches cervix, and move gently back and forth. Block end (e.g. with finger) to prevent loss of material then remove. Holding just off horizontal, insert tip into saline tube and remove block from end. Rinse material off.

Note: Cultures should be initiated at the clinic. Culture media (for Tritrichomonas and Campylobacter) and Tricampers can be obtained using our consumable order form. Further advice on sampling for these pathogens can be obtained by calling Gribbles Palmerston North.

CHLAMYDIA

Chlamydia is found commonly in birds and cats. Chlamydia are intracellular organisms so epithelial cells need to be collected in the specimen. This requires fairly vigorous scraping of the tissue. Although cats can carry chlamydia asymptomatically in their conjunctiva, birds carrying chlamydia usually have conjunctivitis. It is therefore recommended that conjunctival scrapes from birds only be done on those showing clinical conjunctivitis. The best samples to submit are conjunctival scrapings, cloacal scrapings, or whole fresh spleen or liver (from necropsy).

Chlamydia infection is diagnosed by ELISA or PCR: Please refer to other sections of this Handbook for more details. If submitting swabs for PCR remember to submit a dry swab which is more suitable.

Special handling/shipping requirements: Ship chilled, double bagged and in a leak proof container, mentioning in your history that Chlamydia is on your differential list, as this is a zoonotic organism.

LEPTOSPIROSIS

Leptospirosis is one of the world's most widespread zoonotic diseases. Native, feral and domestic animals may serve as reservoirs, with rats and other rodents recognised as the most important maintenance hosts. Leptospirosis affects a range of domestic animals including cattle, pigs, dogs, sheep, horses, goats and deer.

Classically the serovar linkages are cattle with serovar Hardjo and pigs with serovars Pomona and Tarrasovi. There is evidence for the infection of sheep, particularly with serovar Hardjo. Infection of horses may occur and dogs are occasionally infected.

The clinical signs of infection can range from inapparent to severe, and be influenced by factors such as species, inoculation dose, immune status and age. Clinical signs may include fever, jaundice, haemoglobinuria, pulmonary congestion and death, and in more chronic infections infertility/reproductive failure/ agalactia. In pigs at slaughter, visible kidney lesions ('white spotting') are often used as an indication that a group of pigs carries leptospirosis infection but this is of limited value in identifying infection in individuals.

Leptospirosis is diagnosed by PCR, histology and serology: Fresh urine, body cavity fluid or fresh post mortem tissue (e.g. kidney, liver) and/or serum for serology are useful samples. Serology indicates whether an animal has been infected in the past. PCR determines if the agent's genetic material is currently present in the sample.