

Tutorial Article

Diagnosis of bacterial infections. Part 1: Principles of sample collection and transportation

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Summary

Bacterial infections are common in equine practice, therefore it is important that diseases caused by these organisms are diagnosed accurately. Collection of appropriate samples in a correct and timely manner is fundamental to determining the causative agent. Furthermore, transportation of samples to the laboratory must ensure that the agents survive and can be identified. This paper outlines a recommended diagnostic approach when bacteria are suspected, the samples that may be obtained in order to confirm their presence, and the transportation requirements to facilitate their isolation in diagnostic laboratories.

Introduction

Major technological advances have changed the way in which bacteria can be detected, identified and their susceptibility to antimicrobial agents determined. However, there is no value in these new technologies unless the clinician accurately diagnoses a potential bacterial infection, specifically localises this infection, obtains the appropriate sample in an appropriate manner, sends the sample to the diagnostic laboratory using approved procedures, and correctly interprets the results from the laboratory. All too frequently in practice poor samples are collected for bacterial isolation. These are sent to the laboratory for identification and susceptibility testing, the results of which are then applied to the case in question. However, this scenario often results in the wrong bacteria being cultured, the results misinterpreted, and the incorrect antimicrobial agents prescribed.

This article will reiterate the basic microbiological principles that should be applied to all cases where samples are collected for culture and susceptibility testing. The article will not cover

sample collection in circumstances when susceptibility testing is rarely required, such as isolation of bacteria from *post mortem* specimens. The paper will review also the principles required for transportation of samples to the laboratory. In an adjunct article (Hodgson *et al.* 2008), the techniques for culture and susceptibility testing will be outlined and interpretation of results discussed.

Principles of sample collection

Despite the best intentions there are a number of common pitfalls associated with sample collection that can adversely affect the outcome of this procedure. These include multiple and often poorly accessible sites for collection of samples, contamination of samples with indigenous (normal) flora, failure to consider the diversity of bacterial agents that may be involved, and the ever present temptation to sample incorrect sites such as draining tracts. The implementation of a simple, logical, step-wise process that is consistently followed when collecting samples for culture and susceptibility testing will help avoid many of these possible pitfalls.

Step 1: Determine if the patient has a treatable microbial infection

A key decision that should be made early in the diagnostic work-up is whether the patient's condition is due to an infectious aetiology. Evidence of inflammation is central to this process, but inflammation may have a variety of causes, which require different treatments. For example, noninfectious aetiologies are commonly treated with corticosteroids. However, these drugs are usually contraindicated for treatment of conditions with an infectious cause (Hirsh *et al.* 1999), where antibiotics are often indicated. In contrast, using an antimicrobial agent in a patient 'just in case' an infection is present usually makes it improbable that the agent(s) will be chosen on the basis of a rational approach (Aucoin 2007). It is important to consider that there are no inexpensive, broad

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spectrum antimicrobials that are effective against most bacterial organisms that cause infections in horses. Further, antimicrobials with an extended spectrum of efficacy and/or efficacy against resistant bacteria are not appropriate for primary antimicrobial use (Morley *et al.* 2005). Therefore, if an infection is suspected, the best way to confirm the diagnosis and to treat with the most appropriate antimicrobial agent is to localise the site of infection, choose an appropriate method for sample collection and identify the causative agent.

Clinical signs suggestive of, but not pathognomonic for, bacterial infections include fever, pain, heat, swelling and discharge (Byrne 2007). Clinicopathological findings consistent with bacterial infection include increased total WBC count with neutrophilia, possible left shift with toxic changes, increased fibrinogen and other acute phase protein concentrations, and hyperglobulinaemia. However, these changes will not occur in all cases, and their absence does not rule out the possibility of infectious disease. In addition, these clinical and/or clinicopathological changes can be present in the absence of microbial infection.

Step 2: Determine if bacterial isolation and identification is required

Isolation and identification of the causative bacteria are not always indicated and empirical therapy is appropriate in cases where a known organism is likely, especially if these bacteria have a predictable susceptibility pattern (Hodgson *et al.* 2008). In addition, certain infectious diseases may be more efficiently diagnosed by direct visualisation (microscopy) of the organism, or detection of antigens, nucleic acids or bacterial toxins (Jones 2006). For example, diagnosis of clostridial diseases such as botulism and enterocolitis are routinely undertaken by detection of toxin rather than relying solely (or at all) on isolation of the bacteria. Additionally, DNA probes and PCR are useful for identification of microorganisms for which culture and serological methods are difficult, extremely expensive or unavailable (e.g. *Lawsonia intracellularis*) and direct or dark field microscopy may be helpful for the rapid, presumptive diagnosis of infection with some bacteria, e.g. *Dermatophilus congolensis* or *Leptospira*.

Conversely, horses with serious or life-threatening infections, infections in sites that are difficult to treat, or where the suspected causative agent is known to have unpredictable and likely antimicrobial resistance (e.g. *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) should routinely have samples collected for culture and susceptibility testing. In addition, the information derived from these tests enables the clinician to monitor common isolates from infectious processes and the potentially changing trends in their antimicrobial susceptibility patterns.

Step 3: Determine optimal time for sampling

Acute vs. chronic disease

Samples should be collected as early in the course of the disease process as possible (Jones 2006). As the disease

progresses and necrosis of tissues occurs, some microorganisms may die or be overgrown by other bacteria. It may also be useful to collect samples from in-contact animals, which may be at the earlier stage in the infection with an associated greater chance of shedding substantial numbers of microorganisms. An example of this would be in the case of an outbreak of strangles (due to *Streptococcus equi ssp. equi*).

Prior to initiation of antimicrobial therapy

Whenever possible, specimens should be obtained before the administration of antimicrobials. However, prior antimicrobial use does not necessarily preclude the recovery of bacteria from selected tissues in which low antimicrobial concentrations are achieved or from those animals infected with antimicrobial resistant bacteria (Jones 2006). In addition, if broth culture media are to be used for cultivation (e.g. blood cultures), these dilute, adsorb and/or inactivate some antimicrobial drugs administered prior to sampling.

If it is not possible to collect the sample before antimicrobials have been administered, then the sample should be collected immediately before the next dose is given (when trough concentrations of antibiotic should exist). If antimicrobials are concentrated at the sampling site, such as in urine, it is best to wait at least 48 h after the last dose before collecting a specimen.

Step 4: Specifically localise site of infection

One of the most critical steps in sample collection is to specifically localise the site of infection and to collect a sample from that site - near enough is not good enough! If this step is not undertaken meticulously, interpretation of culture results may be impossible.

Many infectious processes arise subsequent to the contamination of compromised tissues by microorganisms that are also a part of the normal flora; these being present on a contiguous mucosal or skin surface (Hirsh *et al.* 1999). In other words, microorganisms isolated from an infected site are often similar (if not identical) to those found as part of the normal flora of the patient. Therefore, the clinician must sample the actual site of infection and not the nearby site containing normal flora, in order to identify the actual bacteria causing disease. The more thorough the clinician is at localising the site of infection and collecting only from that site (and not contaminating the sample from adjacent tissues, organs or secretions), greater confidence can be placed in the interpretation of the significance of any bacteria isolated.

Step 5: Determine if the site has indigenous flora or is normally sterile

Once the site of infection has been specifically localised, it is necessary to establish if this site is normally considered sterile or has an indigenous bacterial flora. Although the definition of a sterile site is often simplified to one where microorganisms are never present, this definition is not useful in clinical

practice. An alternate definition, and one the authors find more effective, is that sites with normal flora are those which are adapted to having bacteria present and may even require their presence to have normal function. Alternatively, sites that should be considered sterile are not adapted to the presence of bacteria and have elaborate defence mechanisms to remove any bacteria that are introduced. This definition is preferred as sites such as the trachea, the urinary bladder and the uterus are thus considered to be sterile even though bacteria may be isolated from these areas. However, as these sites are not adapted for the presence of bacteria, any organisms gaining access to these areas are normally quickly and efficiently removed. As such, few if any bacteria are routinely isolated from these sites in normal horses. Therefore, collection of samples from these sites should use the same principles and techniques as for sterile sites, but interpretation of the presence of bacteria will be assisted by quantitation and identification of the bacteria isolated. **Table 1** outlines sites with a normal flora, the bacteria that may be isolated from these sites, and the sites that should be considered 'sterile'. It is important to note that a number of bacteria present as normal flora may opportunistically cause disease in these or adjacent tissues.

Step 6: Determine if anaerobes are likely to be involved

Before samples are collected and submitted to the laboratory, it is important to determine whether anaerobes are likely to be present so that the sample can be collected and handled appropriately. Clinical features which are consistent with anaerobic infections include gas and/or black discolouration of tissues, foul smell, and infections near sites where anaerobes are part of the normal flora (e.g. gastrointestinal tract, respiratory tract, urogenital tract). In addition, it must be remembered that anaerobes cannot survive in normally

oxygenated tissues (i.e. normal body tissues) and anaerobic infections are only possible in sites where sufficient necrosis has occurred to generate conditions with a redox potential that will allow these organisms to survive (<-100 mV). Therefore, anaerobes are more commonly isolated in severe, chronic infections such as tooth root or foot abscesses, and pleuropneumonia.

Samples that may include anaerobes must be inoculated into suitable transport media and sent to the laboratory as soon after collection as possible (see below) as many of these bacteria do not survive exposure to the oxygen in air for more than 20 min. It is also important not to contaminate the samples by contact with adjacent mucosal surfaces as these frequently have a resident anaerobic flora.

Step 7: Determine which samples are required

There are a number of infections that require specific samples to be collected in order to identify the causative agent and perform susceptibility testing. These infections and the appropriate samples are summarised in **Table 2**.

Step 8: Choose an appropriate method of sample collection and number of samples to collect

There are a number of suitable ways to collect samples for bacterial cultivation and identification. The underlying principle of these techniques is to choose a method that will collect a sufficient volume of sample, whilst avoiding contamination from nearby sites with a normal flora.

In general, an adequate quantity of sample involves several millilitres of liquid or grams of tissue. All too frequently, an inadequate amount of material is obtained with a swab, making it nearly impossible for the laboratory to make appropriate smears and inoculate adequate culture material. A good rule is to assume that a swab should never be submitted

TABLE 1: Tissue sites with normal bacterial flora, the bacteria commonly isolated from these sites in normal horses, and sites which are considered 'sterile'

Sites with normal flora	Bacterial flora	'Sterile' sites
Skin	<i>Staphylococcus aureus</i> Coagulase negative <i>Staphylococcus</i> spp. <i>Micrococcus</i> spp. <i>Corynebacterium</i> spp.	Intradermal, subcutaneous, muscle
Oral cavity and gastrointestinal tract	Enterobacteriaceae (e.g. <i>E. coli</i> , <i>Enterobacter</i>) <i>Actinobacillus</i> spp. Anaerobes including <i>Actinomyces</i> spp., <i>Bacteroides</i> spp., <i>Fusobacterium</i> spp., <i>Clostridium</i> spp.	Peritoneal cavity, other abdominal organs (e.g. liver, spleen, kidneys)
Nasal cavity (to larynx)	<i>S. equi</i> ssp. <i>zooepidemicus</i> <i>Streptococcus</i> spp. (β - and nonhaemolytic) Pasteurellaceae (i.e. <i>Pasteurella</i> and <i>Actinobacillus</i> spp.) <i>Actinomyces</i> spp.	Trachea and lungs, pleural cavity
Distal urethra (proximal urethra is not colonised by bacteria)	<i>Streptococcus</i> spp. (β - and α -haemolytic) Enterobacteriaceae (i.e. <i>E. coli</i> , <i>Enterobacter</i>) Anaerobes including <i>Bacteroides</i> spp.	Bladder
Vagina and external cervix	As above for distal urethra	Uterus Blood Bone, joints, tendons, ligaments CSF

in lieu of an aspirate, biopsy material, fluid or surgically removed tissue.

Multiple specimens should be submitted when lesions are large, present at several sites, or when more than one laboratory procedure is requested. Multiple samples may also be necessary for isolation of bacteria present in low numbers (e.g. in blood, joints, faeces).

Appropriate collection devices and specimen transport systems are needed to ensure survival of the microorganism, prevent overgrowth, and allow for optimal isolation and identification of the causative agent. Various containers are

commercially available, ranging from simple swabs and plastic tube combinations (**Fig 1**) to more complicated specimen collection devices.

Swabs

Swabs are rarely the best choice for sampling bacterial infections because they collect a small amount of material, bacteria can be adsorbed to swab fibres further decreasing the effective sample volume, and they can be made with substances that inhibit bacterial growth (Songer and Post

TABLE 2: Preferred techniques for collection of samples for microbial culture from different body sites

Site of infection	Samples required
Endometritis	Guarded uterine swab (Fig 1) or culture from endometrial biopsy. Swabs may be less diagnostic than biopsies (Lu and Morresey 2006).
Septicaemia and bacteraemia	Multiple blood samples are often required as bacteraemia can be intermittent and frequently only low numbers of bacteria are present. Collect one sample before administration of antibiotics if possible or at the onset of fever. Ideally 2–3 more samples should be collected within 24 h from different collection sites, with the final sample(s) collected when trough antimicrobial concentrations occur (i.e. just prior to administration of the next dose). Ten ml of blood should be collected to enhance the chance of isolation of causative agents. Special media is required for transportation and cultivation of these samples (Fig 8).
Cystitis and pyelonephritis	Urinary tract infections in horses are rare (Frye 2006) and usually result from structural or functional inhibition of normal urine flow. Urine samples are usually best collected by catheterisation of the bladder (Fig 5), but mid-stream voided samples may also be used. Quantification of urine cultures and concurrent cytological analysis are required for interpretation.
Pneumonia and pleuropneumonia	Tracheal wash samples should be collected using either the transtracheal technique or with a guarded catheter passed (Fig 4) through an endoscope (Hodgson and Hodgson 2006). It is important to ensure the wash solution does not contain a bacteriostatic preservative e.g. EDTA. Quantification of tracheal wash cultures and cytological analysis are required for optimal interpretation. Bronchoalveolar lavage samples are rarely, if ever, indicated for bacterial isolation in suspected cases of pneumonia. Samples of pleural fluid should be obtained by thoracocentesis in addition to tracheal wash if concurrent pleuritis is suspected. Both sides of the thoracic cavity should be sampled as different bacteria may be isolated from each hemithorax.
Peritonitis	Samples of peritoneal fluid should be collected using techniques to avoid enterocentesis. A portion of the sample should be immediately placed in fluid (blood) culture media to enhance isolation of the causative organism(s).
Abscesses	Samples from the centre of an abscess may be sterile, particularly if the process is chronic. Up to 3 ml should be collected from early lesions, if possible, together with a scraping from the wall of the abscess.
Diarrhoea	Multiple, fresh faecal samples (3–5 g) are preferred over rectal swabs as bacteria may be shed in low numbers and therefore missed on swabs. The laboratory should be specifically asked for identification of <i>Salmonella</i> through selective enrichment and/or PCR. Rectal biopsies may increase sensitivity of isolation, but are invasive and carry more risk to the horse (Feary and Hassel 2006).
Dermatitis	In suspected cases of dermatophilosis, swab samples should be collected from underneath crusted lesions. The microscopic appearance of the bacteria (<i>Dermatophilus congolensis</i>) is pathognomic. All other cases of dermatitis should have a sample obtained by skin biopsy and/or aspiration of unruptured pustule/vesicles.
Ulcerative keratitis	Gentle scraping of tissues at the edge of a corneal ulcer will provide the optimal sample for culture and susceptibility testing. Alternatively, a fine swab (Fig 1), moistened with sterile saline, may be applied to this area.
Septic arthritis	Isolation of bacteria from joints can be difficult as bacterial numbers can be low, especially in adults (Byrne 2007). Samples should be inoculated into the same media used for blood cultures. Biopsies of synovial membranes may enhance bacterial isolation, but these samples are difficult to obtain as they require invasive procedures.
Wounds	Culture of fresh wounds is rarely warranted, but culture of established wounds with signs of infections (heat, swelling, discharge) is clearly indicated. Aspiration of deep tissues or a biopsy of the affected tissues is most likely to lead to identification of the causative bacterium. NEVER collect samples of the surface discharge or from draining tracts in these cases as they will invariably be contaminated with local flora, which may include potential pathogens. These samples are impossible to interpret correctly.
Meningitis	Culture of cerebrospinal fluid (CSF) should be attempted in cases of suspected bacterial meningitis, but is frequently unrewarding (Seino 2007). Cytological evidence of inflammation (neutrophilic pleocytosis) in the CSF, together with possible visualisation of the organism, is suggestive of bacterial meningitis.

2005). In addition, samples submitted on swabs are prone to desiccation and the relevant pathogen may not be viable on arrival at the laboratory. Therefore, swabs are only acceptable for sample collection if samples are sent to a laboratory in a humidified transporting chamber or placed in a transport medium (see below).

Swabs should never be used to collect samples from the surface of wounds (**Fig 2**) or from draining tracts. Samples



Fig 1: Swab collection devices used to collect samples for bacterial isolation and identification in horses: (1) Guarded swab for collection of uterine samples; (2) Swab collection device including media that will support growth of aerobic and anaerobic bacteria; (3) Swab collection device with small (fine) swab suitable for collection of samples from the cornea or clitoral fossa (for isolation of *Taylorella equigenitalis*); (4) Swabs without transport media are rarely suitable for use in equine practice.



Fig 2: Samples should NOT be collected by swabbing the surface of wounds or inserting a swab into a draining tract. Samples obtained from deeper tissues (e.g. subcutaneous tissues) in cases of open wounds, or from the specific, localised site of infection (e.g. muscle, bone) in cases of draining tracts, should be obtained. These samples should be obtained by fine needle aspiration or biopsy.

obtained from deeper tissues (e.g. subcutaneous tissues) in cases of open wounds, or from the specific, localised site of infection (e.g. muscle, bone) in cases of draining tracts, should be obtained. These samples should be obtained by fine needle aspiration or biopsy. Swabs are also usually of little value in collecting samples from sites with normal flora (e.g. upper respiratory tract) unless detection of specific bacteria is the goal of cultivation (e.g. in the case of strangles where isolation of *S. equi* ssp. *equi* is diagnostic). In these cases, the surface of the mucous membrane can be gently wiped of any discharge and a swab used to collect the sample.

Fine needle aspirates and catheterisation

Samples collected by fine needle aspirate (**Fig 3**), by catheterisation (**Figs 4 and 5**), or with a biopsy are good specimens for bacterial isolation as they are relatively easy to collect and the larger sample size will increase the likelihood that a causative pathogen will be isolated. In addition, samples collected using these techniques are usually obtained in a more



Fig 3: Fine needle aspirates, after a site has been surgically prepared, are a preferred method of sample collection for microbial cultivation and susceptibility testing.



Fig 4: Collection of samples from the lower respiratory tract for bacterial cultivation must involve use of a guarded catheter if collection is through the biopsy port of an endoscope.

aseptic manner than for swabs. For all specimens obtained by fine needle aspiration skin decontamination should be performed as for surgery. Briefly, after clipping the site of collection, the area should be thoroughly disinfected by repeated application of 10% povidone-iodine or other suitable skin antiseptic solutions and allowed to dry. A final application of 70% isopropyl or ethyl alcohol should be applied to the skin and allowed to dry for at least 30 s.

Aspirates may also be used to collect samples from the lower respiratory tract through a catheter that is passed

either transtracheally or through a guarded system using an endoscope (Hodgson and Hodgson 2006). Guarded catheters (**Fig 4**) must be used in these cases to prevent contamination of the sample with bacteria from the upper respiratory tract. These normal flora cover the external surface of the endoscope during passage through the nasopharynx, therefore care also must be taken to avoid prior placement of the tip of the endoscope on the mucosa at the site of sampling.



Fig 5: Urinary tract infections are rare. However, when suspected, samples for cultivation and sensitivity testing may be collected from the bladder by catheterisation.

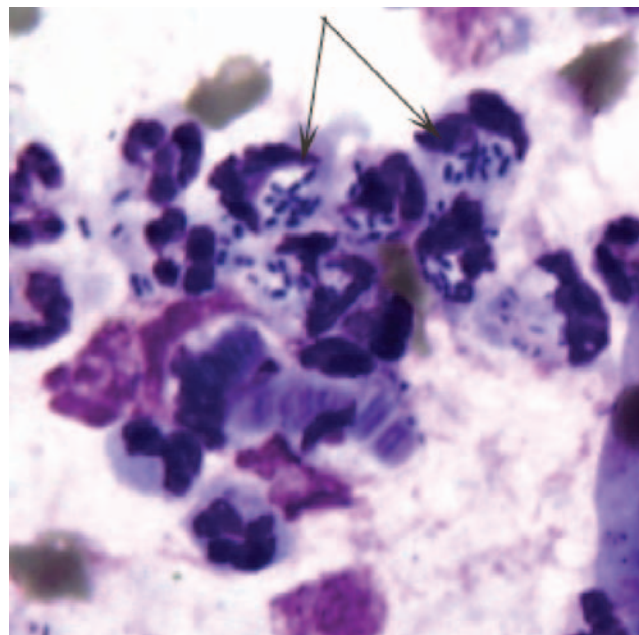


Fig 6: Neutrophilic inflammation and intracellular bacteria (arrows) provide cytological evidence that the sample collected has been obtained from a site with a bacterial infection. (Reprinted from *Equine Respiratory Medicine and Surgery*, 2006, Elsevier, with permission.)

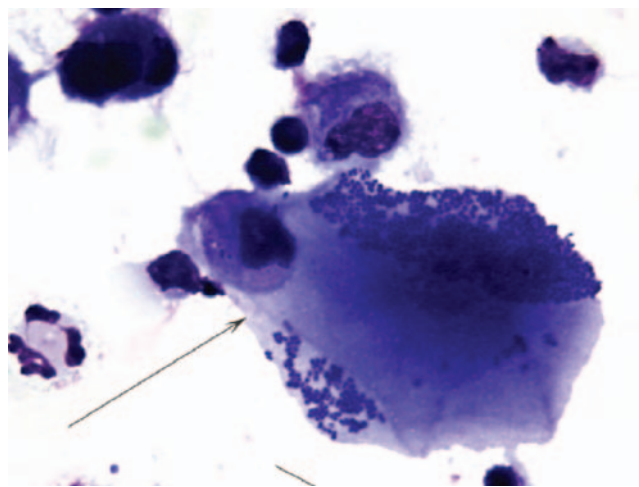


Fig 7: Large squamous epithelial cell with numerous surface bacteria (arrow), and no cytological evidence of inflammation (few neutrophils) are consistent with contamination of sample with normal flora from a mucosal or skin surface. (Reprinted from *Equine Respiratory Medicine and Surgery*, 2006, Elsevier, with permission.)

Catheterisation of the bladder is preferred for collection of urine in cases where urinary tract infections are suspected (Fig 5). However, contamination of these samples with normal flora of the vagina or prepuce still occurs and quantification of colony forming units should be performed to assist interpretation (Hodgson *et al.* 2008).

Biopsy or tissue samples

Surgical specimens or biopsies are often the preferred sample as they may represent the entire pathological process (Byrne 2007). However, they are usually obtained at a considerable expense and some risk to the patient. If these procedures are performed, a portion of the tissue, rather than a swabbed specimen, should be submitted and they should be handled carefully to avoid contamination or desiccation. Biopsies, tissue samples or aspirates are the preferred samples for the attempted isolation of anaerobes. These tissues should be placed in commercially available transport systems for anaerobes, such as Port-a-Cul transport jars¹, which are inexpensive and easy to use.

Free catch

Samples collected by free catch (e.g. urine, milk) are more rarely collected in horses than other domestic species. However, if these samples are collected, procedures that minimise contamination should be used, such as collection of mid stream samples and careful cleaning of the udder prior to sample collection.

Step 9: Evaluate the sample for suitability prior to submission - cytological evaluation

Direct microscopic examination of the collected samples is a simple and cost-effective procedure that should be performed on every sample collected, preferably before sample submission. Microscopic examination gives an indication of the likelihood of the presence of infection, the presence of a host inflammatory response, the possible pathogens, the predominant organisms in cases of mixed infection, and finally the suitability of the specimen for culture. This information also may be used for implementation of initial therapy and as a basis for interpretation of the significance of subsequent culture results. Finally, smears may provide the only information about the sample collected in cases where the causative agent cannot be cultured.

Smears should be made of the samples obtained using routine techniques (Tyler *et al.* 2002). Simple staining methods are suitable for most samples collected from equine infections and include Gram and Diff Quik² stains. Gram stained smears allow evaluation of the presence and type of bacteria (Gram reaction, number, size, shape) and Diff Quik stained smears allow evaluation of the type and intensity of inflammatory response, as well as the presence of toxic or degenerative changes in neutrophils consistent with a septic process. At least 10⁴–10⁵ microorganisms/ml of fluid or gram of tissue must be present in order to be detected microscopically; therefore, failure to observe bacteria on a stained sample does not rule out an infection. The presence of intracellular bacteria is highly

TABLE 3: Gram stain, morphology and arrangement of bacteria commonly involved in equine infections

Organism	Arrangement	Comment
Gram-positive cocci		
<i>Staphylococcus</i> spp.	Diplococci or small clumps	MUST differentiate between likely pathogens (i.e. <i>S. aureus</i>) and probable contaminant (i.e. coagulase negative <i>Staphylococcus</i> spp.).
<i>Streptococcus</i> spp.	Cocci in chains	Need to differentiate the β -haemolytic <i>Streptococcus</i> spp. in suspected cases of strangles.
Gram-positive rods		
<i>Rhodococcus equi</i>	Short rods, cocco-bacilli	Gram-positive rods in samples obtained from cases of pneumonia in foals are highly suggestive of <i>Rhodococcus equi</i> ('Rattles').
<i>Corynebacterium pseudotuberculosis</i>	Short rods	
<i>Actinomyces</i> spp.	Rods (short or long) which may be branching	Most commonly observed in mixed infections involving anaerobes and extension from a site with normal flora. Branching morphology is characteristic of <i>Actinomyces</i> or <i>Nocardia</i> spp.
<i>Clostridium</i> spp.	Large, spore-forming rods	Presence of spore-forming, Gram-positive rods in smears from normally sterile sites is highly suggestive of clostridial infection, e.g. clostridial myonecrosis.
<i>Dermatophilus congolensis</i>	Gram-positive branching rods, cocci	Rods have characteristic 'railroad' morphology. This morphology may facilitate presumptive diagnosis.
Gram-negative rods		
Enterobacteriaceae e.g. <i>E. coli</i> , <i>Klebsiella</i> spp., <i>Salmonella</i> spp., <i>Enterobacter</i> spp., <i>Proteus</i> spp.	Rods	Members of the Enterobacteriaceae family cannot be distinguished by morphology in a Gram stain.
Pasteurellaceae e.g., <i>Pasteurella</i> spp., <i>Actinobacillus</i> spp., <i>Pseudomonas</i> spp.	Rods	Members of the Pasteurellaceae family cannot be distinguished by morphology in a Gram stain.
Anaerobes e.g. <i>Bacteroides</i> spp., <i>Fusobacterium</i> spp.	Rods	Usually present in mixed infections and may also involve Gram-positive rods (e.g. <i>Actinomyces</i> spp.) and Gram-positive cocci (e.g. <i>Peptostreptococcus</i> spp.) <i>Fusobacterium</i> spp. have distinctive tapered ends.

suggestive of a bacterial infection rather than mere contamination, especially in conjunction with cytological evidence of inflammation (**Fig 6**). Samples obtained from sites with normal flora will have many bacteria present, but these should not be accompanied by inflammatory cells if tissues are healthy (**Fig 7**). However, contamination of samples from sites of inflammation with normal flora will confuse interpretation, as inflammation will be present as will many bacteria. In these cases it is impossible to interpret cytological or culture results and emphasises the need for meticulous sample collection.

The Gram reaction, bacterial morphology and the arrangement of the bacteria may be used to identify some bacteria and to develop a list of possible aetiologies that be used for empiric antimicrobial drug selection (**Table 3**). In addition, the observation of bacteria with specific morphological and staining characteristics is consistent with the presence of anaerobic bacteria and indicates that the sample should also be incubated anaerobically. For example, large, Gram-positive, spore-forming rods or long, tapered Gram-negative rods are reflective of *Clostridium* spp. or *Fusobacterium* spp. infection, respectively.

Transportation

The major goals of sample transportation are to prevent further contamination, to maintain viability of pathogens, and if more than one type of microorganism is present, to maintain them in approximately the same ratio as occurred *in vivo*. Sample drying (all microorganisms), exposure to noxious atmosphere (oxygen for anaerobes) and excessive time delays are the major dangers in sample transportation.

Various types of transport devices containing media (e.g. Stuart's medium)¹ may be used for transporting specimens to prevent desiccation and maintain viability of organisms. Essentially these contain buffered, non-nutritive salt solutions usually in a gelled matrix. As these media do not contain any nutrients, microorganisms multiply poorly if at all and thereby preserve the relative numbers and ratio of the original sample



Fig 8: An example of a commercially available, conventional broth-based culture system which may be used for transportation and cultivation of fluid samples e.g. blood, joint fluid.

and minimise overgrowth by rapid-growing bacteria that may be present. However, bacteria do not remain viable in these media indefinitely, and the more fastidious bacteria e.g. β -haemolytic *Streptococcus* spp., will not survive as long as *E. coli* or *P. aeruginosa*.

Swab transport systems

Swabs should always be placed in transport medium, regardless of the expected time lapse between collection and processing. There are a number of different swab transport systems (**Fig 1**), which are best obtained from the diagnostic laboratory to which the sample will be sent. In addition, a number of newer systems have been developed, such as Copan Vi-Pak Amies agar gel collection¹ and transport swabs (Hindiyeh *et al.* 2001), which maintain the viability of more fastidious organisms for longer (up to 24 h). These newer systems may also support anaerobes, though swabs are usually considered inferior for isolation of anaerobes. If submitting multiple samples, individual containers should be used to eliminate the possibility of cross contamination.

Broth transport systems

Nutrient broths are recommended for transportation of some samples (e.g. blood and synovial fluid) to help overcome bactericidal components of these fluids and to maintain viability of the bacteria present during transportation (Byrne 2007). These systems also may be useful for transportation of fluids from the peritoneal or thoracic cavity in cases where low numbers of bacteria are anticipated (e.g. *Actinobacillus equuli* peritonitis). In general, the same systems are used for transportation as for cultivation of blood in the laboratory (**Fig 8**). However, it is important to note that these fluids will support the growth of any organisms within the sample, and therefore can only be used when aspirates are collected from normally sterile body sites, and when great care has been taken to avoid contamination at the time of sampling. In addition, these fluids are rarely of use when polymicrobial infections are suspected, due to rapid overgrowth by less fastidious bacteria. Therefore, their use should be restricted to cases where a single organism is likely involved, and when meticulous care has been used during sample collection.

Anaerobes

Samples that may include anaerobes must be inoculated into suitable transport media and sent to the laboratory as soon after collection as possible as many of these bacteria do not survive exposure to the oxygen in air for more than 20 min. If a swab is used for sampling sites with suspected anaerobes, it should be placed in semi-solid transport medium such as the Port-a-Cul¹ to minimise air exposure. Alternatively, newer systems such as the Copan Vi-Pak Amies agar gel collection¹ and transport swabs (Hindiyeh *et al.* 2001) may be used, which has been modified by nitrogen gas flushing to keep an ideal (low) redox potential and to prevent oxidation of the transport

medium. These media can support anaerobes and other fastidious bacteria for up to 24 h. If tissues or fluid samples are sampled, they should be placed in similar transport systems to those for swabs, and are also commercially available (e.g. Porta-Cul jars and vials)¹. Many of the blood culture systems can support the growth of anaerobes, but the conventional broth-based method (Hodgson *et al.* 2008) will more reliably allow detection of anaerobic bacteria probably because the large volume of liquid preserves the anaerobic environment and allows proliferation of anaerobes that could be present in very low numbers (Byrne 2007). Aerobic swabs are unacceptable for isolation of anaerobes and should not be submitted to the laboratory. However, because reduced oxygen is not lethal for aerobes and facultative anaerobes, anaerobic transport devices may be used for transportation of these bacteria in cases where mixed infections are suspected.

Media for transportation of specific pathogens

Swabs and discharges submitted for the isolation of *Taylorella equigenitalis* should be placed in Amies transport medium containing charcoal¹. Cary Blair medium is routinely used for transportation of faecal samples where *Salmonella* spp. are suspected. These samples should not be cooled during transportation.

Packaging and temperature

Samples should always be submitted individually in separate leak-proof containers. A second container around the primary containers should be used in case leakage occurs – this should be packed with absorbent materials. Each container should be labelled with the identity of the animal, the type of specimen, and the date of collection. If transportation to the laboratory is delayed, most samples should be refrigerated at 4°C or stored on ice, but not frozen until the samples can be sent. Exceptions to this rule are samples obtained from cases where *Salmonella* spp. or anaerobes are suspected as these species do not tolerate cool temperatures. Samples should be shipped to the laboratory as soon as possible after collection (e.g. overnight). Frozen gel packs or ice can be used during transportation to maintain cool conditions without freezing. Obviously if the weather is warmer, more packs may be required. If possible samples should not be sent over the weekend, as there is an increased likelihood of delays during which temperature variations occur and bacterial viability is compromised.

If time of transportation is short, a fluid sample may be kept in the syringe used for collection, but the excess air should be expelled, the needle removed and the syringe capped with a sterile luer bung (stopper) to avoid inadvertent injury and introduction of bacteria to personnel. However, because of occupational health and safety issues laboratories will often not accept samples presented in such a manner. Therefore, it is wise to check prior to attempting sample submission in this way.

Information regarding signalment, case history and a tentative diagnosis should be submitted to the laboratory with

the specimens to help laboratory staff to decide on the range of possible agents and thus select the appropriate media and procedures to identify the pathogen(s). It is recommended to always send an accompanying prepared smear for cytological evaluation as these represent a snapshot of the bacteria present at the time of sampling.

Manufacturers' addresses

¹BD Diagnostic Systems, Oxford, UK.

²Baxter, Deerfield, Illinois, USA.

References

- Aucoin, D. (2007) *The Antimicrobial Reference Guide to Effective Treatment*, 3rd edn., North American Compendiums Inc. Port Huron. pp i-xiv.
- Byrne, B.A. (2007) Laboratory diagnosis of bacterial infections. In: *Equine Infectious Diseases*, Eds: D.C. Sellon and M.T. Long, W.B. Saunders, St Louis. pp 236-243.
- Feary, D.J. and Hassel, D.M. (2006) Enteritis and colitis in horses. *Vet. Clin. N. Am.: Equine Pract.* **22**, 437-479.
- Frye, M.A. (2006) Pathophysiology, diagnosis and management of urinary tract infection in horses. *Vet. Clin. N. Am.: equine Pract.* **22**, 497-517.
- Hindiyyeh, M. and Acevedo, V. and Carroll, K.C. (2001) Comparison of three transport systems (Starplex StarSwab II, the new Copan Vi-Pak Amies Agar Gel collection and transport swabs, and BBL Porta-Cul) for maintenance of anaerobic and fastidious aerobic organisms. *J. clin. Microbiol.* **39**, 377-380.
- Hirsh, D.C., Zee, Y.C. and Castro, A.E. (1999) Laboratory diagnosis. In: *Veterinary Microbiology*, Blackwell Publishing, Oxford. pp 15-27.
- Hodgson, J.L. and Hodgson, D.R. (2006) Collection and analysis of respiratory tract samples. In: *Equine Respiratory Medicine and Surgery*, Eds: B.C. McGorum, P.M. Dixon, N.E. Robinson and J. Schumacher, Elsevier, Edinburgh. pp 119-150.
- Hodgson, J.L., Hughes, K.J. and Hodgson, D.R. (2008) Diagnosis of bacterial infections. Part 2: Bacterial cultivation, susceptibility testing and their interpretation. *Equine vet. Educ.* In Press.
- Jones, R.L. (2006) Laboratory diagnosis of bacterial infections. In: *Infectious Disease of the Dog*, Ed: C. Greene, W.B. Saunders, St Louis. pp 267-273.
- Lu, K.G. and Morresey, P.R. (2006) Reproductive tract infections in horses. *Vet. Clin. N. Am.: Equine Pract.* **22**, 519-552.
- Morley, P.S., Apley, M.D., Besser, T.E., Burney, D.P., Fedorka-Cray, P.J., Papich, M.G., Traub-Dargatz, J.D. and Weese, J.S. (2005) Antimicrobial drug use in veterinary medicine. *J. vet. Int. Med.* **19**, 617-629.
- Seino, K.K. (2007). Central nervous system infections. In: *Equine Infectious Diseases*, Eds: D.C. Sellon and M.T. Long, W.B. Saunders, St Louis. pp 46-57.
- Songer, J.G. and Post, K.W. (2005) General principles of bacterial disease diagnosis. In: *Veterinary Microbiology: Bacterial and Fungal Agents of Animal Disease*, Eds: J.G. Songer and K.W. Post, W.B. Saunders, St Louis. pp 10-20.
- Tyler, R.D., Cowell, R.L., MacAllister, C.G., Morton, R.J. and Caruso, K.J. (2002) Introduction. In: *Diagnostic Cytology and Haematology of the Horse*, 2nd edn., Eds: R.L. Cowell and R.D. Tyler, Mosby, St Louis. pp 1-18.