# Clinical Microbiology In Bovine Practice

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The major equipment needed to establish a clinical microbiology laboratory in veterinary practice includes: microscope, incubator and autoclave. Consumable supplies and small items include: a flame (alcohol burner or Bunsen burner with natural or bottled gas), scale, plastic petri plates, test tubes and racks, bottles, chemicals, dehydrated media and stains. Most useful media for primary plating includes: blood agar for general purpose, mannitol agar (1) for mastitis, MacConkey agar for coliforms and salmonellas. Mueller-Hinton agar is used for susceptibility testing.

Preliminary identification can be done using: Gram's stain [a], catalase test [b], and TSI [a] slants. These are simple to run and results are available as plates are received except for TSI slants, which require 24 hours incubation. I recommend CAMP-esculin strips (2) for identification of mastitis streptococci and commercial systems [c] for identification of coliforms and other gram-negative organisms.

In order to be successful in clinical microbiology in a general practice setting at the beginning, the organ systems cultured should be limited to the respiratory tract (bacterial pneumonia), mammary gland (mastitis) and gut (bacterial enteritis in calves). Culture and identification of *Pasteurella multocida* and *hemolytica* from bacterial pneumonia is useful because these organisms have highly variable antibiotic susceptibility patterns and are the cause of most bacterial pneumonia in cattle. Culture at the onset of shipping fever or other serious respiratory disease is the most useful. Culture from the live animal must be done by tracheal aspiration. The head and neck are extended and restrained. The skin (2″ x 2″ area) over the trachea is surgically prepared. Using a large-size, commercial, intravenous catheter set, a 12 or 14 gauge needle is inserted through the skin into the trachea. While pointing caudal, the polyethylene catheter is passed into the trachea to the bifurcation. The needle is withdrawn leaving the catheter in place. Ten ml of sterile 0.9 per cent saline solution is injected rapidly. Coughing results. Suction is immediately applied, and tracheobronchopulmonary secretions and exudates are aspirated into the syringe. Culture aspirate on blood agar. Pasteurellas are tiny gram-negative short rods and from translucent (smooth, tiny or mucoid) colonies. Inoculation of TSI slant (stab and streak) produces slight acid slant and butt (orange) in 24 hours.

I suggest the use of mannitol agar (1) or blood agar for mastitis diagnosis. Milk samples should be obtained using aseptic technique. Four samples can be streaked onto each of four quadrants of a single petri plate using a 0.01 ml loop. A platinum calibrated loop [d] is preferred because a standard inoculum size permits determination and comparison of number of colonies. Plates are read after 24 hours incubation at 35°C. On mannitol agar pathogenic staphylococci appear as medium opaque colonies with yellow zones in media around colonies. Nonpathogenic staphylococci are somewhat smaller opaque colonies with red zones in media. Staphylococci are catalase [a]-positive. Streptococci from translucent small colonies are catalase-negative. Pathogenic species (*S. agalactiae, S. dysgalactiae, S. uberis,* and group G) will produce > 20 colonies (average 50-70) when 0.01 ml inoculum is used. *S. agalactiae, S. dysgalactiae* and group G streptococci have red zones (no color change) around colonies and are eradicable from herds. *S. uberis* forms yellow zones and is not eradicable. Coliforms produce large (>2 mm diameter) colonies with yellow zones and are gram-negative rods. **When culturing milk for coliforms, it is important to sample cows early in the disease and to culture milk soon after sampling because coliforms are killed by milk bactericidal factors.**

I suggest use of MacConkey agar for culture of gut contents or feces of calves. Scours caused by *Escherichia coli* can be diagnosed by appearance of large numbers of brick red colonies. Test for enteropathogenicity by agglutination with K99 antiserum [c]. Only 20 per cent of colonies will be positive on K99 testing, therefore, test >5 colonies before calling negative. Salmonellas will appear in large numbers (in active cases) of grey colonies. Final identification of gram-negatives (coliforms, Salmonellas, etc.) can be performed in small laboratories using commercial media [c].

An important reason for cultural diagnosis of bacterial disease is to properly select antimicrobial therapeutic agents

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through susceptibility testing. Standard methods must be used in performing susceptibility tests in order to have the results applicable to the clinical setting. These include: use of pure cultures, use of proper media, selection of proper disc concentration of drug, inoculation of standard concentration of organisms, and measurement of zones of inhibition. These techniques have been well developed and are available from Difco.[a] Use of standard methods will assure that susceptible organisms are likely to respond to drugs given in therapeutic doses.

**Integration of the clinical microbiology laboratory into bovine practice encourages the veterinarian to establish disease control programs. Through education, the veterinarian can teach the cattleman to allow the veterinarian to select drugs for major problems on the basis of susceptibility testing. This method of drug selection should reduce drug costs since many older less expensive drugs will be found effective once "shot-gun" therapy is reduced. A record system, which tabulates according to organ system, results of previous susceptibility testing will indicate the best drug to begin therapy before susceptibility testing is complete on the case at hand. For these reasons it is important to do culture and susceptibility testing early in disease outbreaks and to keep tabulation of susceptibility results.**

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[a] *Difco Laboratories, P.O. Box 1058A, Detroit, MI 48232*

[b] *Catalase test is by mixing colony in 3% $H^2O^2$. Bubble formation is a positive reaction.*

[c] *Commercial gram negative systems available are:*

*API*

*Analytab Products*
*Division of Ryerst Laboratories*
*200 Express St - Plainview, NY 11803*

*Micro-ID*

*General Diagnostics*
*Division of Warner-Lambert Co*
*Morris Plains, NJ 07950*

*r/b*

*Corning Medical Microbiology Products*
*25 Lumber Road - Roslyn NY 11576*

*entrotube*

*Roche Diagnistics*
*Division of Hoffman-LaRoche Inc*
*340 Kingsland St - Nutley, NJ 07110*

[d] *Scientific Products, 1430 Waukegan Rd, McGraw Park, IL 60085*

[e] *K99 antiserum from: P. J. Gtanz, Vet. Sci. Dept., Penn State U, Univer. Park, Penn.*

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**References**

1. Ward, G. E. *et al.* Mannitol agar for the microbiologic diagnosis of bovine mastitis. JAVMA in press. - 2. Farnsworth, K. J. *et al.* CAMP-esculin strips for the identification of mastitis streptococci. JAVMA in press.