Bacteriology for the Progressive Veterinarian

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Many progressive Veterinarians are becoming aware of the great value of office bacteriology as a tool in their practices. A simple bacteriology lab in a practice situation can serve two very useful purposes. First and most important, it can be an aid in making the correct diagnosis of animal infections. Much can be learned about infective processes and antibiotic sensitivities by applying practical microbiological procedures. Only a minimum amount of equipment is needed to perform most of the needed procedures.

Secondly, it helps you practice modern “Progressive Veterinary Medicine” and not “Shot-gun medicine”. Lay-men, feed salesmen and farmers themselves can use shot gun therapy without years of schooling. With use of bacteriology, Veterinarians with a minimum amount of effort can prescribe a specific treatment. Clients will realize that you are putting forth an extra effort and are interested in doing your best to find a solution to their problem. This will build up the client’s confidence in you as a Veterinarian and therefore will serve as a practice builder.

The number of products available to a Veterinarian to treat animal infections is decreasing. The combination or “shot gun” products are being taken off of the market thus putting the Veterinarian on the spot. In the near future he may have to pick a specific drug for specific infections and this may be complicated by the appearance of more and more resistant strains of organisms. The choice of the correct antibiotic may mean the difference between success and failure.

In many practice situations, Veterinarians feel they are too busy to make proper use of a bacteriology lab. A solution to this problem would be training lay help to run the lab leaving you the Veterinarian with only the job in interpretation of the lab results. A new manual, Identification of Veterinary Pathogenic Bacteria by Dr. D. L. Harris and Dr. Kathryn A. Burds is now available from the ISU Bookstore and is a “cook book” approach to Veterinary Bacteriology. Part of this manual will be published in the AVMA Journal. It presents a simple easy to follow method of identification of pathogenic bacteria and also explains the why and how of lab procedures tests. This manual could easily be used to train lab help to do routine lab work.

**Equipment and Media Needed**

Only a minimum amount of equipment and media is needed to isolate and identify the more common pathogens. The lab should be kept simple so it can and will be used on a day-to-day basis.

1. 37°C incubator (May be purchased or an old refrigerator may be used by removing the refrigerator unit and adding a heating element and thermostat.)
2. Microscope with oil immersion lens.
3. Gas Burner. (Portable propane torch is satisfactory.)
4. Gram stains. (Commercially available)
5. Microscope slides.
6. Culture tubes (10 cm x mm; screw caps 13 cm x 13 mm)
7. Petri plates (Plastic disposable eliminate washing and sterilizing.) 100 x 15 mm
8. Transfer loops.
9. Scale (weight in grams)
10. Sterilizing apparatus. (an ordinary household pressure cooker works very well.)

The pressure cooker is used just as it would be in the kitchen. A small amount of water is placed in the cooker, the material to be sterilized added, the lid closed, and heat applied. The exhaust valve is left open until steam escapes. The exhaust valve is then closed and the cooker held at 15 lbs. pressure for 15–20 minutes depending upon the material to be sterilized. The cooker is then removed from the heat, the pressure allowed to come down slowly if liquids are being sterilized, or can be rapidly exhausted if no liquids are involved.

11. Common media used (all available commercially)
   a. Blood Agar plates
   b. S.S. agar, Selenite Broth
   c. Trytose Broth
   d. Kliger's Iron Agar
   e. SIM
   f. EMB

The equipment and media can be obtained from some of the following companies. 1

1. Gentec Hospital Supply Company
   107 South West 9th Street
   Des Moines, Iowa, 50309
   a. Sterile plastic disposable Petri dishes
   b. All kinds of plastic disposable labware
   c. Dehydrated media
   d. Sensitivity discs
   e. Sensitivity disc dispensers
   f. Sterile swabs

2. Scientific Products
   4700 West Chase Avenue
   Chicago, Illinois, 60646
   All kinds of laboratory equipment such as microscopes, slides, immersion oil, transfer loops, incubators and laboratory scales.

3. Difco Laboratories
   Detroit, Michigan, 48201
   a. Dehydrated media
   b. Gram's stains
c. Sensitivity discs

4. Mid-West Instrument Company
   1924 Fourth Avenue South
   Minneapolis, Minnesota, 55404
   a. Prepared plate media
   b. Prepared tube media

5. Wallerstein Company
   Division of Travenol Laboratories, Inc.
   Morton Grove, Illinois, 60053
   a. Prepared plate media
   b. Prepared tube media

One of the most important factors in clinical bacteriology is the proper collection of specimens. Collection should be done with sterile technique to help insure that the organism cultured is the pathogen and not just a contaminant. Several steps are involved in the process of obtaining good specimens and isolation of the causative organisms.

Collection of Specimens for Aerobic Bacteria

1. Obtain specimens with sterile techniques
   a. Fluids—sterilize surface if possible, aspirate with syringe and needle or lance and collect on swab.
   b. Solid exudate or tissue—sterilize surface, lance and swab or collect small piece with sterile scissors and forceps
   c. For blood, see below.

2. Place specimen in sterile container and seal tightly.
   (Do not place in transport media).

3. Store in refrigerator and/or inoculate isolation media as soon as possible.

Isolation

1. Third-streak specimens on blood agar plate (BAP) with loop. Rub swab or loop, use one edge of loop and streak from the inoculated portion of the plate to another 1/2 of the plate, use the other edge of the loop and streak the final 1/2 of the plate.

   Streak nurse colony on portions b and c for Hemophilus (optional). Incubate at 37° C, observe and pick after 24 hours of incubation, reincubate for 2 more days.
2. Make a smear of specimen (loop of material in small amount of water), Gram stain, and record all morphotypes present and relative numbers of each.

3. Place swab or portion of specimen in tryptose broth (TB). Incubate at 37°C.

4. (Optional) Inoculate specimen into various selective media. This will depend on and vary with the type of specimen.

5. If no growth on BAP [I-1] after 24 hours, third-streak TB on second BAP.

6. After incubation of plates, observe colonies by visual inspection and/or with a dissecting microscope.
   a. With sterile cooled loop or needle, pick each colony type to TB.
   b. Whenever possible, pick a portion of the colony to a drop of water on slide for Gram stain. Determine whether all morphotypes originally present are represented by the isolated colonies.
   c. If all morphotypes were not isolated, do Gram stain of TB [I-3]. If all morphotypes are not observed, consider a second collection of specimen for anaerobic bacteria.
   d. If types not observed in original specimen are isolated (allowing for some morphological differences in medium vs. tissue), suspect contamination during sampling or culture.

7. Gram stain from TB to make sure all types of cells present are of the same morphology and Gram reaction.

8. Inoculate a BAP (optional) and all differential media.
   a. Check BAP after incubation to be sure all colonies are of the same morphology.
   b. Read reactions in differential media for determination of Genus and/or species. These reactions are only valid if a pure culture was inoculated.

Blood Specimens

1. Inoculate 5 ml of an aseptically collected sample into 5-50 ml of Beef Heart Infusion.

2. Incubate at 37°C until growth is apparent. Allow one week before discarding.

3. Stain, streak for isolation of types, as above.

Techniques and Procedures using your Bacteriology Lab

I. Drug Sensitivity Testing

Drug sensitivity will be an important procedure in your bacteriology lab. Results can be used to determine which drug should be used to treat a specific infection.

Pick an isolated colony into tryptose broth and incubate 4 to 24 hours (culture may be used after growth turbidity is present). Aseptically dip a sterile cotton swab into the culture. Press the swab against the top of the tube to remove excess liquid and streak the swab onto blood agar or Mueller-Hinton Agar (Difco) for confluent growth. Allow the plate to dry for 10 to 15 minutes, and apply sensitivity discs. Press the sensitivity discs gently against the agar with a sterile forceps. Disc dispensers are available commercially. Incubate the plate inverted. (agar up) for 16-24 hours at 37°C. Determine the zone diameter of inhibition in millimeters. Sulfonamide sensitivity of organisms should only be connected on Mueller-Hinton Agar.

II. Mastitis Control

A method of controlling mastitis which is used by some Veterinarians in progressive dairy herds is outlined below:

1. Periodically throughout the year each quarter on each cow in the milking herd is tested for mastitis using the California Mastitis Test.

2. Each positive quarter is cultured.
   a. Each teat is thoroughly cleaned with alcohol being especially careful to clean the end of the teat.
   b. As aseptic as possible collect a few ml. of milk in a sterile tube from each positive quarter.
   c. The causative organisms are then isolated and drug sensitivities testing done. Results of the drug sensitivity testing can

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help determine the best possible therapy for each cow.

III. Bordetella Culturing

Collecting Nasal Swabs:

For Bordetella rhinitis herd status testing, a random sample of 15 pigs 4 to 10 weeks of age is adequate. For establishment of a Bordetella rhinitis-free breeding herd, test all animals in the breeding herd about once a week until those retained have passed three consecutive negative tests.

Animals to be swabbed should be confined to a clean and dust-free area at least 2 hours prior to nasal swabbing. Pasture or clean concrete is recommended. The nasal opening should be thoroughly cleaned. Insert swab at a slight angle with the cotton tip directed towards the nasal septum and hold as loosely as possible without dropping. Roll stick between fingers, at the same time exerting gentle forward pressure until the area of the 2nd premolar tooth is reached. Plastic applicator stemmed swabs are highly recommended.

It is best to streak culture plates as soon as possible after swabs have been taken. Refrigerate swabs if they must be held over two hours post-collection.

Isolation and Identification.

1. MacConkey's agar with crystal violet is modified for use as the selective medium.
   a. One percent dextrose is added.
   b. After autoclave sterilization and cooling to 45°C, 50 units of mycostatin are added to each ml. of medium.
   c. Furaltadone (NF–260) is added to the medium at the level of 0.02 mg. per ml. The Furaltadone is added to the medium prior to autoclaving.

2. The inoculated plates are incubated for 48 hours at 37°C in an aerobic atmosphere.

3. *Bordetella bronchiseptica* is recognized by the grayish tan colonies which produce a slight alkalization of the medium surrounding the colony. Such suspicious colonies are transferred into Tryptose Phosphate Broth.

4. The broth is incubated 18–24 hours at 37°C.
   a. By this time the culture has developed moderately heavy growth, considerable sediment, and a faint, incomplete pellicle.
   b. They are gram negative coccoid rods.
   c. They are motile by means of peritrichous flagella.

5. Cultures showing characteristic growth are pipetted into Simon's citrate agar slants, urea agar slants, and dextrose broth. Lactose broth and litmus milk may be used as additional tests.

6. *Bordetella bronchiseptica* produces the following reactions:
   a. Dextrose broth is alkalinized.
   b. Rapid alkalinization of urea agar. The reaction is completed in less than 24 hours.
   c. Citrate is utilized.
   d. Lactose broth is alkalinized.
   e. Litmus milk is slowly alkalinized. (About 3 days)

7. Mueller-Hinton agar is used as the medium for sulfa sensitivity testing.
   a. Sterile bland concentration discs saturated in sulfamethazine solutions containing 5–10–20–30 mg./ml. of sulfamethazine can be used. These discs will contain approximately 0.15–0.30–0.60–0.90 mg. sulfamethazine per disc.

IV. Baby Pig Scours

Baby pig scours is a constant problem which Veterinarians are confronted with. A bacteriology lab could be a valuable tool for diagnosis and treatment. It is critical in baby pigs that the efficacious drug be used as soon as possible. Guessing may loose valuable time and valuable pigs. Drug sensitivities can be used to direct the Veterinarian toward the most efficacious drug on each individual farm.

The three most common bacterial agents which cause scours in baby pigs are all common inhabitants of the GI tract of normal pigs. Therefore, a fecal swab is of little or no value in diagnosing the cause of scours. Swabs should be taken directly out of the anterior small intestines at post mortem. At this time a di-
rect smear should be made and Gram stained to determine the predominant organism. If it is a Gram positive rod one should consider both aerobic and anaerobic culturing since Clostridium perfringens Type C is an anaerobe.

Anaerobic culturing is not complicated, but one does need the proper equipment. A Gas Pak (Bioquest), pressure cooker or a tupperware container can be used. A H₂-CO₃ generator envelope is placed in the container to which 10 ml. of water is added. The generator envelope produces H₂ which combines with O₂ to form water, the reaction is catalyzed by a palladium catalyst. This type of system is adequate to grow Clostridium.

E. coli must be present in high numbers in the anterior area of the small intestine. They do not have to be hemolytic on blood agar plates to be pathogenic. It is important to run drug sensitivity tests on the E. coli isolates because of the many drug resistant strains now being encountered.

Salmonella is usually a problem in older pigs and will cause a septicemia, therefore Salmonella can be cultured out of other tissues such as liver and spleen.

A well run Bacteriology Lab will help Veterinarians serve this new era of Agribusiness and practice “Progressive Veterinary Medicine”

BIBLIOGRAPHY

3. Switzer, W. P., Spear, M. L., Farrington, D. O.: Swine Bordetella bronchiseptica Rhinitis Fact Sheet. II. Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University, Ames, Iowa.

Book Review

by John H. Greve, D.V.M., Ph.D.*
BOOTS AND FORCEPS
by Willet J. Price, D.V.M.,
as told to Hazel Heckman
The Iowa State University Press
Ames, 50010 1973 $5.95

Do you like to swap anecdotes with your colleagues? Stories about the “good old days” in college, about funny things that happened to you? If so, you will certainly enjoy “Boots and Forceps,” a most entertaining, and informative book, written by a veterinarian about his experiences—the Dustbowl, TB testing, cavalry horses, and so on. Dr. Price gives us a picture, both humanistic and historic, of governmental and private practice during a period when veterinary medicine was undergoing rapid progress and growth.

Many of the episodes will leave you chuckling. Like the time rustlers tossed the remains of a calf down a well. To identify the brand, Dr. Price was lowered into the well by the calf’s owner. But then the rancher sped off in a dither to catch the rustler, leaving Dr. Price in the well with the remains. Or the time a naive woman found her in-season bitch with a male attached. Well, read about it for yourself. Probably many of the episodes will remind you of things that have happened to you, but have been forgotten.

“Boots and Forceps” offers delightful, light, but also informative, reading that really is a must for history buffs, nostalgics, and lovers of good tales.

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