Veterinary Technician Program

Moderator: Dick Wiley

Basic Mastitis Microbiology for Veterinary Technicians

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Abstract

Mastitis culturing programs provide a wealth of information to dairy farms for their milk quality control and treatment programs. Labs providing this information can be in-house at veterinary clinics or on-farm. Laboratories must have established protocols for collecting and handling proper samples, identifying bacterial isolates, reporting results and quality control. Veterinary technicians can be integral in the setting up and day-to-day operation of these labs, both in-house and on-farm.

Résumé

Les programmes de mise en culture pour la mammite donnent aux fermes laitières une foule d'information sur le contrôle de la qualité du lait et sur les programmes de traitement. Les laboratoires qui fournissent ces informations se retrouvent dans des cliniques vétérinaires ou à la ferme. Les laboratoires doivent avoir des protocoles établis pour recueillir et manipuler les échantillons correctement, pour identifier les isolats bactériens, pour rapporter les résultats et pour le contrôle de la qualité. Les techniciens vétérinaires font partie intégrale de la mise en place et de l'opération au jour le jour de ces laboratoires à la fois à la ferme ou à la clinique.

Introduction

Mastitis is one of the most common diseases of dairy animals, with most farms having 25% or more of their milking herd experiencing mastitis each year. Effective treatment and control programs require a culturing program to be in place. Fortunately, most of the pathogens that cause bovine mastitis are bacterial in nature. This allows veterinary clinics to set up in-house or even on-farm culture labs to assist in treatment and control programs.

Individuals performing microbiological procedures need to have a basic understanding of microbiology and microbiological procedures. Veterinary technicians can play an integral role in not only the actual in-house culturing, but also in sample collection, processing, and reporting results back to producers. In addition, technicians could help set up, train personnel and oversee quality control on dairies that wish to utilize on-farm culturing.

Training in Mastitis Microbiology

Initial training in mastitis microbiology can come from many sources. In addition to training received in the veterinary technician programs, many diagnostic labs and large mastitis labs offer programs for technicians interested in receiving additional education on microbiology and microbiological techniques.

NMC (formerly National Mastitis Council)[a] publishes the *Laboratory Handbook on Bovine Mastitis*,[2] the most comprehensive manual available for bovine mastitis microbiology. This manual not only describes laboratory procedures necessary to identify different microorganisms, but also provides epidemiological information about the pathogens. Additionally, NMC has a website with various fact sheets and other information for individuals working in the mastitis field.

Sample Collection and Processing

To achieve the highest quality results from a mastitis culture lab, milk samples must be acquired utilizing aseptic technique. Contaminated samples result in diagnostic error with all types of diagnostic samples, but probably more so with milk samples because the common contaminants (fecal bugs, common skin inhabitants) can also cause mastitis in the dairy cow. Attention should be paid to train personnel and clients in proper techniques and methods to clean and disinfect teats, with special attention to teat ends (pre-dip with a teat dip and thoroughly dry teats and ends, followed by cleaning teats and ends with an alcohol pledget). Also, collecting proper sample volumes quickly into the proper sterile container is critical.

When collecting milk samples, time should be taken to assure that all vital information is included on the sample tube or submission form. Important items would include the cow ID, date the sample was collected, and

affected quarter(s) or whether it was a composite sample, and type of analysis requested (i.e. aerobic only or aerobic and mycoplasma). While this information may be of little value to the diagnostician, it becomes vitally important in the subsequent analysis of culture results.

After samples have been collected, they should be cooled down in a refrigerator or on ice or frozen as soon as possible. Allowing samples to sit at room temperature will only allow bacteria that are present in the sample to incubate. Environmental Streptococcus and *E. coli* numbers can double in less than 20 minutes if allowed to incubate at ambient temperature. Most mastitis pathogens will survive refrigeration for several days and freezing for several weeks. Freezing can also improve sensitivity in detecting some organisms (particularly staphs and streps) due to releasing bacteria inside somatic cells (white blood cells). In some cases, however, bacterial numbers will be altered negatively by freezing, especially long term. For example, *E. coli* numbers will be significantly reduced.[1,2]

Samples need to arrive at the laboratory as quickly as possible. If the samples need to be shipped, they should be shipped frozen or packed on ice and the sender must utilize overnight shipping to prevent bacterial incubation. Upon arrival at the laboratory, the condition of the samples should be noted and they should be plated on the appropriate media as soon as possible.

Plating Milk Samples and Identifying Mastitis Microorganisms

Traditionally, aerobic mastitis microbiology starts by plating the sample on bovine blood agar. Ovine blood agar plates are often substituted as they are more readily available, but may give different hemolytic patterns. Most labs utilize either cotton swabs or 0.01 – 0.1 mL inoculating loops to plate milk onto the agar plates. Cotton swabs may be quicker, but loops allow quantifying bacterial numbers if important. Samples should be swabbed or looped across the entire available plate area. Plates should then be inverted and placed into an aerobic incubator at 98.6°F (37°C) for 24-48 hours. After 24 hours, observe plates for growth. If growth is present, the identification process can begin. Plates should be placed back in the incubator for an additional 24 hours regardless of whether growth was present after the first day or not. It is critical to have a good incubator with proper temperature and humidity control. Old, dry ovens or light bulbs often lead to poor incubation temperature control and false-negative or slow-growing cultures.

Colony characteristics such as length of time for growth to occur, size, appearance and color of the colonies should be noted, as well as changes in the agar, such as hemolysis. Bacterial isolates are first differentiated by identifying their gram reaction using gram's stain or the potassium hydroxide (KOH) test. Once bacteria are identified as gram-positive or negative, the bacteria are identified to the appropriate level using a series of biochemical tests and/ or special media and plates. Determination of the level of identification (genius only or genius and species) depends on what information is necessary for identifying control procedures on the dairy. For example, labs will always key out *Staphylococci aureus* to the specie level, but all other staphylococci are generally lumped into the coagulase-negative staph (CNS) group.

When analyzing samples, the diagnostician needs to properly recognize contaminated samples. All common mastitis pathogens, including *S. aureus* and *Streptococcus agalactiae* can result from contaminated samples. However, if *Streptococcus agalactiae* or *S. aureus* are found in contaminated samples, one may suspect those as infections or have those animals re-sampled due to the contagious nature and urgency in identifying those animals. Laboratories need to establish protocols for the proper identification of contaminated samples and insignificant growth based on patterns of dissimilar growth and numbers of colonies present. Rarely will mammary quarters be infected with more than one (occasionally two) organisms, so media with 2^+ different colony types or organisms should indicate contamination. Failure to properly control and identify contaminated samples will only lead to inaccurate diagnosis, improper treatments and much time and money being wasted.

If analysis for mycoplasma species is desired, milk samples must also be plated on mycoplasma agar and incubated at 98.6°F (37°C) in a 10% CO_2 environment. Mycoplasma growth is very slow and requires use of a stereomicroscope to visualize the colonies. Growth may appear on the plate after 48 hours of incubation, but plates should not be declared negative before they have incubated for 7 to 10 days. Mycoplasma isolates should be identified to the species level to be certain that the isolate is pathogenic. Speciation utilizes PCR, fluorescent antibody, or other specialized biochemical technology, and is often referred to diagnostic labs or larger mastitis microbiology labs.

Currently, many mastitis labs will take short cuts to identify bacteria more rapidly and economically. These shortcuts can be as simple as utilizing a MacConkey agar plate in addition to blood agar to help differentiate the gram reaction (MacConkey grow gram- negative only), utilizing blood agar plates containing esculin to help key out streptococci, or use of differential agar plates. These differential plates come in all sorts of configurations (bi, tri, or quad plates) with different combinations of selective media that will only allow certain types of bacteria to grow on the individual compartments.

Petrifilm™ plates[b] are ready-made selective culture medium systems that can be used to differentiate and enumerate bacteria from food products. Petrifilm™ products have been widely adopted by labs that enumerate bacte-

ria numbers for quality analysis as they are convenient to use and the plates are very thin, thus reducing space needs to house incubators. Petrifilm™ products that potentially could be used in mastitis microbiology labs include the Petrifilm™ Aerobic Count Plate, Petrifilm™ Coliform Count Plate and Petrifilm™ Staph Express Count Plate that, when used along with the Petrifilm™ Staph Express Disk, can differentiate *S. aureus*. Siva *et al*,[3] evaluated the Petrifilm™ Staph Express Plates and found similar positive and negative predictive values as standard microbiological methods for the identification of *S. aureus*.[3] Petrifilm™ uses a larger inoculum volume so it may increase sensitivity, but may also lead to more false positives or contamination. Petrifilm™ products have not been widely adopted by mastitis microbiology labs.

Recording and Reporting of Results

Ideally, all microbiological samples would be plated from Monday to Wednesday so that all reading of plates would not have to be done on weekends. Unfortunately, dairying is a 24/7 business and mastitis occurs everyday. Therefore, mastitis microbiology labs must have plans in place to deal with samples that arrive at the lab late in the week. This is especially true if dairies are waiting for results to initiate mastitis treatments on individual animals.

Mastitis culturing results need to be reported not only in a timely manner, but also in a form that can be utilized by the dairy, both immediately and retrospectively. Many labs provide results via standardized forms that are sent back to the dairy by e-mail, fax, or regular mail or have them available on a website. Unfortunately, after an initial review by the dairy, most results never get entered into management software systems that are usable for future analysis. Dairy Comp 305[c] now has a system that will create a submission form that can be transferred electronically to the laboratory. Once the samples have been processed at the lab, personnel can enter the results electronically and ship the results back to the dairy electronically for import into Dairy Comp 305. This allows dairy management and their consultants to easily analyze culture results and success of mastitis management programs. Additionally, it has allowed milk quality labs to adopt a paperless system which improves employee efficiency and accuracy.

It is important for individuals working in mastitis microbiology to have an expectation of what their results will be. These results and expectations can vary based on the sample population (clinical vs. subclinical individual cows vs. whole herd cultures). In many situations, samples that yield "no significant growth" are the most common result. This is usually 30-40+% of the results. The second most common clinical mastitis isolate is usually coliform organisms, typically around 25-35%, while targeted herd cultures (subclinical) may give the prevalent herd organism, and whole-herd cultures result in high levels of CNS. The high level of "no significant growth" results is one of the most common reasons on-farm culture systems fail to continue. Therefore, when setting up culture labs, both on-farm and in-house, it is important that individuals doing the culturing be informed on the expected outcomes.

Quality Control

Mastitis microbiology labs need a quality control program in place to assure that all equipment is functioning correctly, agar plates and other supplies are not outdated and that lab technicians are correctly identifying microorganisms. Currently, there are low cost data loggers available that can monitor temperature and humidity over time and provide computer printouts of the results. These products or some other method of monitoring temperatures should be utilized in all equipment that is used by the microbiology lab (incubators, refrigerators, freezers, etc).

To monitor the performance of lab technicians, a series of bacterial unknowns should be evaluated on a regular basis by all technicians who perform microbiological evaluations. These unknowns can be supplied by state diagnostic labs or other large mastitis labs.

Conclusion

Mastitis microbiology labs can provide a vast amount of information to dairies that utilize their services. Unfortunately, with all the demands on veterinarians, these labs often get neglected during busy times of the year. Veterinary technicians can play an integral role in managing these labs as well as collecting and processing samples and reporting results back to the dairies in a timely, effective, informative and interpretive manner to provide necessary information for optimizing milk quality and mastitis treatment programs.

Endnotes

[a]NMC, Madison, WI
[b]3M Microbiology, St Paul, MN
[c]Valley Ag Software, Tulare, CA

References

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