Establishing and Managing an In-House Mastitis Lab

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Abstract

Milk culture is another mastitis detection tool that has been used for numerous years in udder health management. It is usually considered the gold standard for diagnosing subclinical and clinical intramammary infections in dairy cattle. Private veterinarians should seriously consider setting up a milk microbiology laboratory in their practice for the benefit to the clients, the diversification of their services and the economic return to their practice.

Résumé

La culture de lait est un outil de détection de la mammite qui a été utilisé depuis plusieurs années pour la régie de la santé du pis. Cette méthode est communément considérée comme l'étalon-or dans le diagnostic des infections intramammaires sous-cliniques et cliniques chez les bovins laitiers. Les vétérinaires en pratique privée devraient sérieusement considérer l'établissement d'un laboratoire de microbiologie du lait dans leur pratique pour le bénéfice de leurs clients, la diversification de leurs services et les retombées économiques pour leur pratique.

Introduction

Culture results are important in making therapy choices, understanding specific herd problems and guiding selling decisions on individual cows. The author advocates culturing clinical cows, bulk-tank milk and fresh cows. Culturing clinical cows is paramount to managing udder health. Bulk-tank milk cultures can demonstrate where breakdowns in udder health and hygiene are occurring: environmental pathogens point towards bedding and milking hygiene, and contagious pathogens demonstrate poor biosecurity or milking hygiene. Bulk-tank cultures provide insight into the udder health management on a farm which includes teat skin sanitation and cleanliness and effective machine wash-up. Culturing fresh cows evaluates dry cow therapy, provides insight into the udder health management and hygiene of the dry cows, and screens fresh cows and heifers for contagious pathogens.

Milk Microbiology

The National Mastitis Council recommends that a minimum of 10 microliters of milk be used when individual cow milk samples are cultured. Sears *et al* advocated using 100 microliters of milk to increase the sensitivity of milk cultures.[6] *Staphylococcus aureus* infections demonstrate cyclical patterns of shedding. Low-shedding quarters can shed 0 cfu/mL on one day, and the next day that quarter may be shedding 1800 cfu/mL.[6] The work by Sears *et al* demonstrates that a single sample has a sensitivity of 74.5 % for the detection of *S. aureus*. Sampling a quarter two or three consecutive times increases the sensitivity to 94.0% and 98.0%, respectively. *Streptococcus* species infections have higher sensitivities from a single sample, due to their nature of shedding higher cfu/mL consistently.

Culturing milk samples on non-selective trypticase soy agar (TSA) with 5% bovine or sheep blood has the highest sensitivity for isolating gram-positive or gram-negative mastitis pathogens, but some simple ancillary tests are required to determine the genus or species of bacteria that is isolated. If three or more organisms are grown from a milk sample, the sample is considered contaminated. The inoculated plates need to be incubated for 24 to 48 hours in an ambient air incubator at 95° to 98.6° Fahrenheit (35° to 37° Celsius). When an organism is isolated, the Gram status of the organism must be determined with either a Gram stain or 3-5% potassium hydroxide.

The catalase test is performed on gram-positive isolates to differentiate staphylococci from streptococci. Catalase-positive organisms are staphylococci. The coagulase test is used to determine if the staphylococci are *S. aureus* (coagulase-positive) or coagulase-negative staphylococci. Catalase-negative organisms are streptococci. The streptococci are differentiated from each other using the CAMP test and the esculin test. *Streptococcus agalactiae* is CAMP-positive and esculin-negative, *S. uberis* is CAMP variable and esculin-positive, and *S. dysgalactiae* is CAMP-negative and esculin-negative.[3] If a herd with good treatment protocols for streptococci has poor cure rates, API testing of the streptococcal isolates needs to be performed to rule out the presence of enterococci, which are indistinguishable

from streptococci with standard culture techniques.

Gram-negative isolates are differentiated using MacConkey plates. *Escherichia coli* and *Klebsiella* species have characteristic morphologies on these plates after 12 to 24 hours of growth. Other gram-negative organisms can only be differentiated using API test strips.

Culturing *Mycoplasma* species is not difficult, but it does require special media and a carbon dioxide enriched environment. In Biddle's study,[1] greater than 78% of the milk samples from 60% of the cows in the study were shedding greater than 1 million *Mycoplasma* organisms per mL. Without enrichment, *Mycoplasma* species should be identified in infected animals in 77% of samples. In Biddle's study,[1] cows with *Mycoplasma* species infections that were not found using standard culture techniques (broth enrichment was used to identify these animals) were infected with *Mycoplasma bovigenitalium*. Cows infected with *Mycoplasma* species may not yield growth on up to 29% of composite milk samples using standard *Mycoplasma* species culture techniques,[1] and cattle that have cultured positive for *M. bovis* can cease shedding the bacteria for up to 56 days.[2] Therefore, it is recommended that more than one sample be obtained from cows suspected of having a *Mycoplasma* species infection. If you are isolating *Mycoplasma* species for the first time from a farm, the species should be definitively diagnosed at a regional laboratory with polymerase chain reaction (PCR) techniques.

There are basically three things required for *Mycoplasma* species culturing: media, incubator and magnification. The media plates cost about $2.50 each, including shipping. You can easily put four samples per plate. The author inoculates the plates with 100 microliters of milk per cow, and spreads the milk with cotton-tipped swabs.

The plates need to be incubated in a carbon dioxide-enriched environment at a temperature of 98.6° Fahrenheit (37° Celsius) for seven to 10 days. The carbon dioxide needs to be about 5-10% concentration in the plate environment. Candle jars can typically achieve about 5% carbon dioxide, and they are used widely in private labs. If you are using candle jars, you inoculate the plates, and put them in a wide-mouth jar. Place a candle on the top of the plates, light it, and screw the lid on tightly. The candle should burn out, which elevates the carbon dioxide in the microenvironment of the jar. A moist piece of cloth placed in the jar will supply humidity to the jar environment. If you are purchasing candles, be certain to purchase unscented, plain wax candles, because toxic products from some of the candles may inhibit mycoplasmal growth.[5] A carbon dioxide incubator costs about four times what a regular incubator costs, but you get better reliability, ease of use, and more quality control.

Ten times total magnification is needed to look at the mycoplasmal media plates at three days and seven days after inoculation. A dissecting microscope or a compound microscope with a very low-power objective can achieve this goal. After you have looked at the plates, the candle will need to be lighted, tighten the lid of the jar, and place the jar back into the incubator. If you are using a carbon dioxide incubator, you simply place the plates back into the incubator.

Neuder *et al*[4] has brought a new culture and treatment approach to the mastitis world. In this study, all cows were cultured before they were treated. The samples were taken from clinical cows on day 1 and plated on both standard blood agar with 1% esculin and MacConkey agar plates. On day 2 the cultures were interpreted, and only the cows that had a gram-positive infection were treated with intramammary amoxicillin twice a day for three days. While culture results were pending, the cows were placed in a health observation pen, and any cow with systemic illness was given supportive therapy, but no antibiotics. The cows that were not treated were returned to the milking herd when the milk returned to normal. Non-responsive quarters were removed from production. In this study, 80% of quarters that were cultured resulted either no growth (55%) or a coliform infection (25%). This approach reduced the lost days of production, decreased the amount of antibiotic used and did not jeopardize the animal's health and well-being.[4] This approach is very promising for dairies that have rapid culture results, but rapid results are not readily available to all dairy farms. More studies are needed to determine the amount of time that can lapse between detection and treatment of mastitis without detriment to the affected quarter and cow.

Instead of using standard bi-plates with blood agar and MacConkey agar, most people are using bi-plates with Factor media (selective for gram positive) and MacConkey media. With these plates, the organism's Gram status is determined without any additional testing, and treatment protocols are based off this information. Another option for rapid culture results without additional diagnostics involves using three different types of media, which are called tri-plates. These plates have Factor media, MacConkey media, and TKT media (selective for streptococci). These selective media work well, but *S. aureus* and *S. agalactiae* can sometimes be missed on this media. The author recommends periodic, full diagnostic workups on the clinical cases of mastitis and the bulk-tank samples in these herds to prevent contagious problems from sneaking into these herds.

Conclusion

These culturing techniques should increase the

amount of information that you can bring to your dairy clients, and provide another economic generator to your practice.

References

1. Biddle MK, Fox LK, Hancock DD: Patterns of *Mycoplasma* shedding in the milk of dairy cows with intramammary *Mycoplasma* infection. *J Am Vet Med Assoc* 223: 1163-1166, 2003.
2. Gonzalez RN: *Mycoplasma* mastitis-What is new in the northeast? *NMC Regional Meeting Proceedings* 56-66, 2005.
3. Hogan JS, Gonzalez RN, Harmon RJ, *et al*: *NMC Laboratory Handbook on Bovine Mastitis.* Verona, WI, NMC, 1999.
4. Neuder LM, Hess JL, Sears PM: Rethinking clinical mastitis: culture and treatment. *NMC Regional Meeting Proceedings* 36-39, 2003.
5. Oliver SP, Gonzalez RN, Hogan JS, *et al*: *Microbiological Procedures for the Diagnosis of Bovine Udder Infection and Determination of Milk Quality,* 4th ed, Verona, WI, NMC, 2004.
6. Sears PM, Smith BS, English PB, *et al*: Shedding pattern of *Staphylococcus aureus* from bovine intramammary infections. *J Dairy Sci* 73: 2785-2789, 1990.