Veterinary Technician Sessions

Moderators: Doug Hostetler, Kent Ames

Milk bacteriology: Basics and pitfalls

R.J. Erskine, *DVM, PhD*
Michigan State University, East Lansing, MI 48824

Abstract

Bacteriological culture of milk provides fundamental information to veterinarians, their support staff, and dairy producers who want to improve milk quality on dairy farms. In-clinic or on-farm culture cannot achieve the degree of sophistication of an accredited diagnostic laboratory. Nonetheless, non-diagnostic laboratory culture of milk samples can help identify opportunities in farm management, as well as therapeutic decisions. The fundamentals of milk bacteriology are not difficult, and numerous resources are available to help veterinary personnel establish basic milk bacteriology laboratories. Care should be taken to avoid common pitfalls in interpretation and application of the culture results.

Key words: dairy, mastitis, bacteriology, milk quality

Résumé

La culture bactériologique du lait apporte une information fondamentale aux vétérinaires, à leur personnel de soutien et aux producteurs laitiers qui veulent améliorer la qualité du lait dans les fermes laitières. La culture à la clinique ou à la ferme ne permet pas d'atteindre le niveau de sophistication d'un laboratoire de diagnostic accrédité. Néanmoins, la culture d'échantillons de lait par des laboratoires non spécialisés peut aider à cibler des options de gestion à la ferme et à formuler des choix thérapeutiques. Les principes de base de la bactériologie du lait ne sont pas compliqués et plusieurs sources d'information sont disponibles afin d'aider le personnel vétérinaire à établir un laboratoire de bactériologie du lait de base. Il faut prendre soin d'éviter des écueils bien connus dans l'interprétation et l'application des résultats de culture.

Introduction

Bacteriological culture of milk is a cornerstone of a quality milk program in dairy herds. Milk bacteriology helps veterinarians and their dairy farm clientele to better decide housing and bedding options, vaccination strategies, and culling selections, as well as identify potential reservoirs of infection, and mastitis therapy protocols.[2] Bacteriological support for therapy protocols is particularly important because mastitis is the most common cause of antimicrobial drug use for cows on US dairy farms.[14] Antimicrobial therapy benefits animal health and well-being, but economic losses associated with mastitis therapy include drug and veterinary expenses, extended labor, and most importantly, discarded milk.[9] Unwarranted antimicrobial use in dairy cattle can also result in residue violations in milk and meat, and decrease consumer confidence in the safety of dairy foods.

This paper will discuss bacterial culture of milk in a non-diagnostic laboratory setting, such as in veterinary clinics or "on-farm". Potential benefits and pitfalls of milk bacteriology will be presented. The reader should be aware that this application of laboratory methods is not intended to replace in-depth diagnostic support and quality control that is provided by an accredited laboratory.

Milk Bacteriology Basics

It cannot be emphasized enough that the quality of milk culture begins with properly collected, aseptic samples. If veterinary personnel or farm employees are careless in the cleaning of teat ends, sampling of milk, or storage of the samples until cultured, there is a great likelihood that contamination or misinterpretation of results will occur. Garbage in...garbage out! Thus, veterinarians and their staff should provide user-friendly methods of assuring compliance for teat-end asepsis techniques (e.g. alcohol-soaked gauze pads or wipes) and also train farm personnel in the proper collection and storage of milk samples. This is the most critical step in assuring useful outcomes in milk bacteriology.

It is beyond the scope of this paper to give an exhaustive review of the epidemiology of mastitis pathogens, their control, therapy, and the bacteriologic methods needed to isolate them in the laboratory. Excellent reviews are available.[11-13] In particular, the establishment of a milk culture laboratory takes dedica-

tion on the part of an individual either on the farm or in the veterinary clinic to "take charge" that protocols are established and that consistent quality control is maintained. A user-friendly guide to help beginners understand proper sample collection, handling, and basic bacteriology is available through the National Mastitis Council.[11] A more detailed reference that should be kept in any veterinary clinic that provides either milk bacteriology or support for on-farm culture is also available.[10] A list of suppliers that provide items necessary for milk bacteriology basics is listed in the appendix.

Depending on the labor, time, and monetary resources of a veterinary clinic or farm to apply towards milk bacteriology, the "diagnostic depth" of the techniques can vary from simple mastitis surveillance to detailed epidemiological and therapeutic support. The reality is that for many clinics, and especially on-farm, culture of milk samples is done to ensure that basic mastitis pathogens are identified. Ninety-five percent of pathogens isolated from milk samples on most dairies are streptococci, staphylococci, and coliforms. When milk culture reveals repeated isolation of pathogens other than the above-mentioned pathogen groups, care should be exercised in therapy and an attempt made to identify potential reservoirs of infection. Thus, a good starting place for basic milk bacteriology is to be able to identify streptococci, staphylococci, and coliforms, perhaps also with the ability to identify coagulase-postive staphylococci (presumptively *Staph aureus*) as well. A simple flow chart to outline the tests needed is provided in Figure 1.

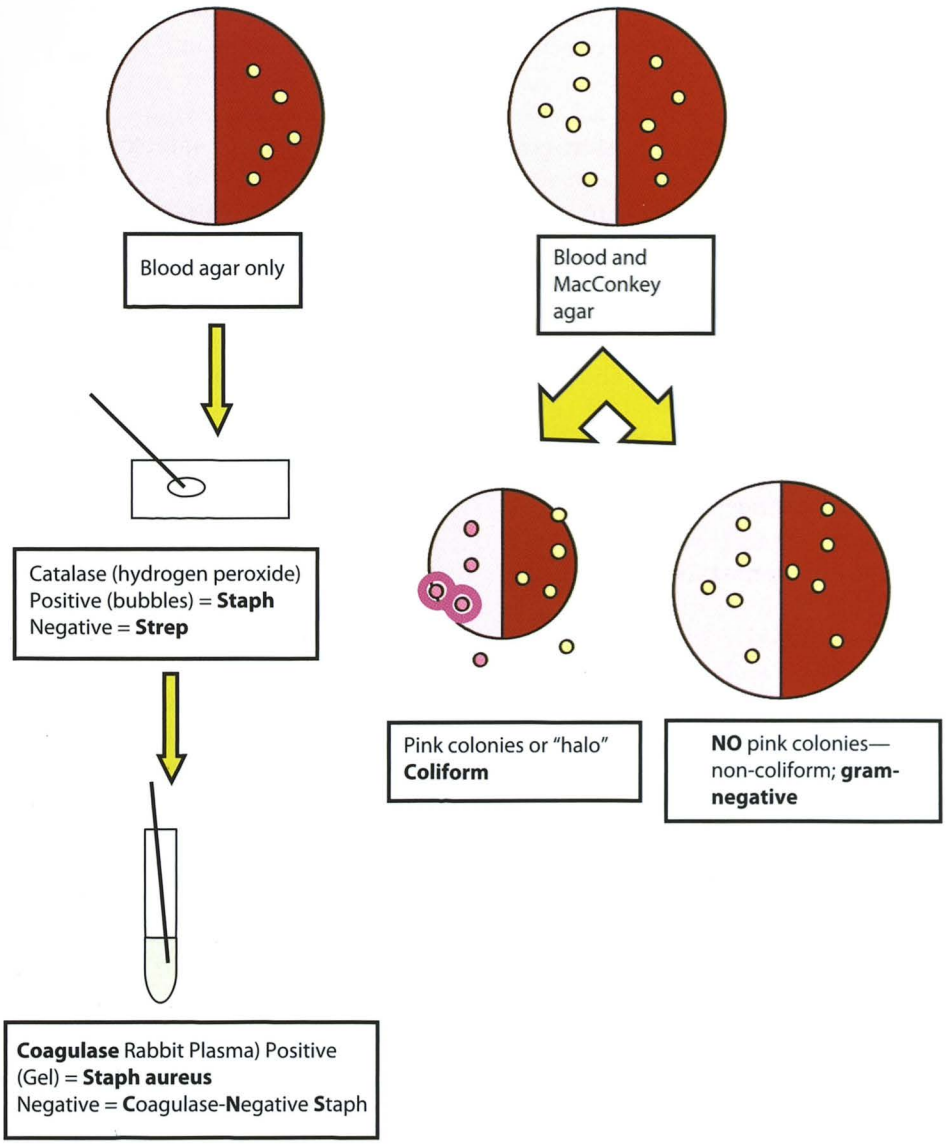

Figure 1. Milk bacteriology flow chart for on-farm culture.

On-Farm Culture

On-farm culture of milk is a practical tool to identify causative agents, and the foundation to better target therapeutic regimens for mastitis. A previous review described basic material and bacteriologic tests that may be useful for this purpose.[2] In a large Michigan dairy, cows with mild to moderate clinical mastitis were sampled for on-farm bacteriologic culture of milk samples before therapy was initiated. Cows that were infected with gram-positive cocci were treated, but cows with no organism isolated or gram-negative organisms were excluded from therapy. This reduced the number of treated cows by 80%.[5] A recent multi-state study further demonstrated that culture-based therapy of gram-positive and mixed isolates only, as compared to empirical therapy of all mild mastitis cases, reduced antimicrobial use by 50%.[7] Furthermore, no differences were found between mastitis cases that were assigned to the empirical therapy or culture-based treatment program in terms of risk for recurrence of clinical mastitis (35% vs 43%), linear somatic cell count (4.2 vs 4.4), daily milk production (66 lb vs 67.5 lb; 30.0 kg vs 30.7 kg), and risk for culling or death (28% d vs 32%) for the rest of the lactation after the clinical mastitis case.[8]

Veterinary oversight of on-farm culture should be extensive, with regular review of techniques, interpretation of culture isolates, and application of culture results into farm management decisions. It is also an opportunity for veterinarians and technicians to restock and ensure proper storage of supplies. Care should be particularly exercised to help farm personnel avoid using on-farm culture solely to rapidly diagnosis and treat mastitis cases. The use of on-farm culture to diagnose more specific pathogens (such as *Mycoplasma bovis*) will likely require extensive training and is best performed by veterinary clinic staff or a diagnostic laboratory.

Limitatons of Antimicrobial Susceptibility Testing

Antimicrobial susceptibility test results (disc assay) should only be done by strictly following Clinical and Laboratory Standards Institute (CLSI; http://www.clsi.org/) guidelines, which include testing of standard pathogens (ATCC) with known disc assay results against the clinic results, establishment of proper concentrations of bacteria for susceptibility testing, and use of Mueller-Hinton agar. This is often beyond the scope of most clinic or farm laboratories. Failure to follow these rigorous guidelines will result in misinformation regarding antimicrobial susceptibility and thus, therapeutic outcome.

Even if performed properly, in most cases, susceptibility cut-points for zone diameters (for the disk diffusion test) are based on antibiotic concentrations in serum or interstitial fluid of people after oral or intravenous (IV) dosing; these are not equivalent to concentrations achieved in milk or mammary tissue after intramammary or systemic dosing.[4] Several studies have determined that susceptibility test results (susceptible vs. resistant) had no impact on the outcome (clinical cure, bacteriologic cure) of clinical mastitis episodes.[1,3,6]

Common Pitfalls of On-Farm and Veterinary Clinic Bacteriology

Like any diagnostic support system, milk culture results are most useful when they are put into context of the individual animal being sampled or the herd history (e.g. housing, bedding, season). Thus, for both on-farm and veterinary clinic culture, records should be maintained on individual cow history and timely reviews of herd management. The cause for the sample to be cultured, e.g., fresh cow surveillance, clinical case, high somatic cell count, or bulk-tank sample, should also be recorded.

Contamination is a common problem with many milk samples, and reflects the cleanliness of the cows as well as the sampling technique of the personnel collecting the milk. It is imperative that samples be collected aseptically from clean, dry teats. One of the biggest frustrations with milk bacteriology is the proportion of samples that result in "no organism isolated", especially when collected from a cow with clinical mastitis or a high somatic cell count. This typically occurs in 30 to 40% of the samples when using standard methods (24 hr incubation and 10µL inoculums). This may occur from intermittent shedding of pathogens, the resolution of the infection by the time the milk sample is collected, prior antibiotic therapy, or the reluctance of some pathogens to vigorously grow under standard techniques. Also, the use of selective media on bi- and tri-plates is common for many clinical and farm laboratories. While a useful tool to help identify pathogen classes, these media can reduce the culture yield of even targeted bacteria and care should be taken not to use these media solely to "weed out" background contamination from poor sampling technique. Depending on the pathogen, preincubation of milk in enrichment broths, larger size of inoculums, longer incubation periods, or in the case of *Staph aureus*, freezing of samples before culture, may be helpful. If a disproportionate amount of cultures fail to yield pathogens, diagnostic laboratory support is advised.

Conclusions

Milk bacteriology is a valuable tool in the veterinary clinic's toolbox to help establish quality milk programs on dairy farms. Meaningful results can be

obtained with a minimal amount of investment and time on the part of the veterinary clinic or farm to sample and culture milk samples. However, care must be taken to apply quality control standards regardless of the level of bacteriology used and understanding the limitations of interpretation of results.

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Appendix – List of suppliers for milk bacteriology laboratories

Culture Plates/ Media and Supplies

University of Minnesota- Laboratory for Udder Health - (excellent support and service)
http://www.vdl.umn.edu/ourservices/udderhealth/

Quality Milk Production Services - Cornell University- (provides supplies and support for University of Minnesota Easy Culture II System in the northeast)
http://ahdc.vet.cornell.edu/sects/QMPS/Services/minnesota_plates.cfm

Udder Health Systems, Inc (Dr. Allan Britten- excellent support)
http://www.udderhealth.com/products.htm

Weber Scientific (good source and assortment of "Capitol" snap vials
http://weberscientific.com/app/catalog/index.php?_wsAction=view-product&id=690

Econo-Lab 10 uL loops L200-2 Pack (25)
http://www.econo-lab.com/Inoc

Fisher culture vials 14-956-1J Case (500)
www.fisherhealthcare.com

Incubators

Nasco Farm and Ranch
http://www.enasco.com/Search?q=egg+incubator&page=1

Animart
*www.**animart**.com/*
(entire kit available)

The Incubator Warehouse
http://incubatorwarehouse.com/index.php/?gclid=CMn7vYnypbcCFYYWMgodJkgAkA

Fisher Scientific
http://www.fishersci.com/ecomm/servlet/Search?keyWord=Standard+Incubators&store=&nav=4294878704&offSet=0&storeId=10652&langId=-1&fromSearchPage=1&searchType=PROD&typeAheadCat=prodCat