

Veterinary Technician Program

Milk Quality and Udder Health

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Abstract

When natural defense mechanisms fail to prevent invasion of the mammary gland by microbial pathogens, intramammary infection and mastitis may occur, producing illness, decreased milk production, and decreased milk quality. The presence of mastitis in a dairy cow may be detected by clinical signs such as abnormal milk and mammary swelling, or in the absence of clinical signs by increases in the cell population or conductivity of the milk. Decisions about whether to treat a particular case of mastitis and how to control and prevent mastitis in the dairy herd are more easily made when the pathogen or pathogens responsible for the infection are identified. Definitive identification of mastitis pathogens is made using microbial milk culture. Depending on the objectives of the practice and the level of motivation and training of the staff, there are various levels of investigation that may be undertaken, from only identifying the presence or absence of pathogen growth and whether pathogens are gram negative, to culturing for mycoplasmas and running further tests to identify certain bacterial genera or species. Whatever protocol is established, it is important to also establish practices for quality control to ensure accurate results. A milk microbiology laboratory in the veterinary clinic provides an excellent opportunity for expanded client services and professional development of the veterinary technician.

Résumé

Lorsque les mécanismes de défense naturelle n'empêchent pas l'invasion de la glande mammaire par des agents microbiens, l'infection intramammaire et la mammite peuvent en découler et entraîner la maladie, une perte de production de lait et une diminution de la qualité du lait. La présence de mammite chez la vache laitière peut se détecter par des signes cliniques comme l'enflure du pis ou du lait anormal. Sans signes cliniques, la mammite se détecte par un accroissement de la population cellulaire ou de la conductivité du lait. Les décisions qui concernent le traitement d'un cas particulier de mammite ou le contrôle et la prévention de la mammite dans un troupeau laitier se font plus facilement lorsque le ou les agents pathogènes responsables de l'infection

sont identifiés. L'identification définitive des agents pathogènes causant la mammite se fait par culture microbienne du lait. Selon les objectifs de la clinique et du niveau de motivation et de connaissance des employés, il y a plusieurs types de recherche qui peuvent être envisagés allant de la simple identification de la présence ou l'absence de croissance des agents pathogènes ou s'ils sont à Gram négatif, jusqu'à la culture des mycoplasmes et l'utilisation de tests plus avancés pour identifier certain genres ou espèces de bactéries. Peu importe le protocole utilisé, il est important de mettre en place un contrôle de qualité pour s'assurer d'obtenir des résultats conformes. Avoir un laboratoire microbiologique d'analyse du lait dans la clinique vétérinaire offre une belle opportunité d'élargir la gamme des services offerts aux clients et de développer les compétences professionnelles des techniciens vétérinaires.

Introduction: Mammary Anatomy and Immunity

The mammary gland of the dairy cow has several barriers that provide resistance to invasion of the gland by microbial pathogens. The teat sphincter is a muscular band around the teat opening that, when functioning normally, closes the teat opening between milkings and during the dry period. Teat closure is also facilitated by the formation of a keratin plug in the teat opening between milkings and during the dry period; the keratin plug contains antimicrobial proteins. In addition, the inner circumference of the teat opening contains the Rosette of Furstenburg, a site where immune cells are concentrated.

A healthy bovine udder contains about 50,000 to 100,000 cells per milliliter of milk. This cell population consists primarily of macrophages, along with neutrophils and epithelial cells. When a pathogen is introduced into the mammary gland, macrophages send out chemical signals which call neutrophils (also known as polymorphonuclear leukocytes or PMNs) from the bloodstream into the mammary gland to fight the infection by engulfing and destroying bacteria. The number of PMNs in the mammary gland during the acute phase of mastitis can increase to millions of cells per milliliter of milk and remain elevated for days to weeks.

Another change that takes place early in the inflammatory process caused by mammary infection

is increased permeability of the barrier between the blood and the milk, which alters the composition of the milk. Serum albumin, a blood protein, leaks into the mammary gland, various inflammation-mediating substances such as cytokines become present in the mammary gland, and the pH of the milk increases, although it remains more acidic than blood serum. Milk from cows with mastitis also contains less lactose and fat than milk from non-inflamed mammary glands. If the infection becomes chronic, particularly with certain bacterial species like *Staphylococcus aureus*, clumps of fibrin may occlude glandular ducts, and there may be areas of fibrosis in the gland.

Detection of Mastitis

Mastitis in dairy animals can cause illness, pain, and death. In addition, inflammation decreases mammary gland function and mastitis may decrease milk production for a limited period, for the rest of the lactation, or indefinitely. Mastitis also decreases milk quality; milk from cows with mastitis may spoil more quickly, is more likely to have “off flavor”, is less useful for making cheese, and may reduce quality premiums paid to the farmer.

Mastitis, which is almost always due to intramammary infection, may or may not be evident upon examination of the cow and milk. Subclinical mastitis occurs when infection is present in the mammary gland but cannot be detected without some type of testing beyond examination of the cow and the milk. Clinical mastitis, when mild, may be manifested only as changes in the milk; the nature of these changes ranges from the presence of small “flakes” or “clots” of organic material in the milk to bigger clumps or discoloration of the milk. Clinical mastitis is generally considered moderate in severity when visibly abnormal milk is accompanied by swelling of the affected gland or glands, and severe when the cow is systemically ill with signs such as fever or depression.

Clinical mastitis is commonly diagnosed in the milking parlor by milking out a small amount of milk prior to attaching the milking unit (called “forestripping”) and examining it for abnormalities such as clots, and by observing cows for signs of illness and/or swelling of the udder. This process will not detect subclinical mastitis because there are not clinical signs. On farms that do not forestrip or when there is mastitis in the absence of clinical signs, other methods must be used to diagnose mastitis.

The most common method of diagnosing subclinical mastitis is by counting or estimating the number of cells in the milk. As described earlier, milk from cows without mastitis typically contains less than 100,000 cells per milliliter; the presence of 200,000 cells per milliliter or more is considered an indication of intramammary infection.

An inexpensive and simple way to estimate the number of cells in milk (usually referred to as the somatic cell count or SCC) is to use the California Mastitis Test (CMT). It may be performed on-farm in less than a minute by mixing a small amount of milk with the CMT reagent. As cell numbers increase, the mixture of the two liquids progresses from liquid to slimy to gelatinous. CMT results are generally scored and recorded by evaluating the degree of reaction, but an estimate of cell numbers is not a part of routine test evaluation. Advantages of the CMT are low price and ease of use, while disadvantages include variability in how users conduct and interpret the test, and that the test is not quantitative. As seen in Figure 1, the CMT provides only a rough estimate of the number of cells in the milk.

A more accurate estimate of somatic cell count may be performed on the farm or in a clinic laboratory using the PortaSCC[®] (PortaCheck, Inc., Moorestown, NJ) test. The PortaSCC[®] test has a lower threshold of detection than the California Mastitis Test, so it can detect less severely elevated cell counts than the CMT. In addition, the test provides a numerical estimate of the number of cells as part of reading the test. The PortaSCC[®] is not a cow-side test like the CMT, but it can be conducted relatively quickly and easily after a limited amount of training. The test uses the enzyme linked immunosorbent assay (ELISA), and the technique to run the test is similar to other commercial applications of the ELISA, such as heartworm antigen tests in dogs or tests for retroviruses in cats. Advantages of the PortaSCC[®] are that it is relatively fast and easy to run, and the cost is likely to be affordable for most veterinary clinics or dairies. Disadvantages are that it is more expensive than CMT and that it requires that a sample of milk be analyzed within eight hours of collection because chemically preserved or frozen milk samples cannot be used.

The most precise and sensitive method of counting cells in milk is using an automated cell counter, such as the Fossomatic[™] models commonly used at Dairy Herd Improvement Association (DHIA) laboratories. These sophisticated machines are not affordable for clinics or farms, but university, milk co-operative, and DHIA laboratories provide valuable services to many dairy farms using these machines for routine herd monitoring, usually at a reasonable cost to the farmer. Milk samples to be analyzed using cell counters require preservatives, but do not require refrigeration. Regular laboratory testing is probably most useful as a tool to guide management decision making, while the speed and simplicity of other SCC tests makes them useful for guiding decisions about individual cows.

Electrical conductivity may also be used to detect mastitis. Among the changes associated with breakdown of the blood-milk barrier that occur in mastitis are increased levels of sodium and chloride in the milk.

The increased presence of these ions increases the milk's ability to carry an electrical charge, or electrical conductivity. Meters that measure electrical conductivity of milk can be added to milking systems to monitor changes in milk of individual cows. Unlike SCC, there is no standard level of conductivity that definitively indicates that mastitis is present. Instead, cows are monitored for sudden or large increases in milk conductivity that are suggestive of mastitis and will trigger further investigation.

Mastitis-Causing Pathogens

Once it is established that the cow has mastitis, via clinical signs or some method of detecting increased numbers of cells in milk, other questions naturally arise, such as: 1) is the causative agent contagious to other cows; 2) is antibiotic drug treatment indicated; 3) if drug treatment is indicated, what drug is the best choice; and 4) how can this type of infection be prevented in the future?

All of these questions may be addressed using results of microbial milk culture. By knowing which microbe is causing the infection, or if microbial culture fails to detect the presence of a microbial pathogen, valuable information is gained. For example, some pathogens are generally responsive to treatment, while others are unlikely to respond to even the most aggressive drug therapy. When no pathogen is detected, treatment is not initiated, and unnecessary expense is avoided. In addition, many pathogens have typical reservoirs, either in the cow or in the cow's environment, which provide a source of pathogens. Knowing this information greatly enhances the efficacy of efforts to prevent future cases of mastitis in other cows in the herd.

A few mastitis pathogens are considered primarily contagious in nature; they are spread from cow to cow at milking time by the milking cluster, milkers' hands, or other fomites. The reservoir of these pathogens is in the udder of infected cows. Perhaps the most common contagious mastitis pathogen is *Staphylococcus aureus*. Mastitis due to *S. aureus* may respond to appropriate drug therapy if discovered early in the course of the disease. When it becomes chronic, the formation of micro-abscesses in the udder make the infection very difficult to eliminate. *Mycoplasma* species, most commonly *M. bovis*, may be spread from cow to cow at milking time, or may occasionally be spread from one body system to another; mycoplasmas are commonly present in the respiratory and/or urogenital systems of cattle. Mammary infection with *Mycoplasma* seldom resolves, with or without treatment. Unlike *S. aureus* and *Mycoplasma* spp, *Streptococcus agalactiae* is a contagious mastitis pathogen that responds well to antibiotic treatment. In addition to these three contagious pathogens, some pathogens contracted from the environment may also have a contagious component to their epidemiology. Contagious pathogens are usually addressed using some combination of hygienic milking procedures, testing of cows for the disease, and treatment or culling.

There are many organisms in a cow's environment that may cause infection in the mammary gland. One of the most common is *Escherichia coli*, which is found in feces and may cause mastitis that varies in severity from mild and short-lived to severe and life-threatening. *Klebsiella* spp, which are sometimes associated with wood-based bedding such as sawdust or shavings, may also cause life-threatening mastitis, and even when the infection is less severe, it is often very difficult to treat

CMT score	Description	Est. SCC (cells/mL)	Mastitis diagnosis
0 (Negative)	Mixture remains liquid with no evidence of thickening or formation of a precipitate.	< 200,000	No mastitis
T (Trace)	Slight thickening that tends to disappear with continued movement of the paddle.	150,000 to 500,000/mL	Suspicious
1 (Weak)	Distinct thickening, but no tendency toward gel formation. Thickening may disappear after prolonged rotation of the paddle.	400,000 to 1,500,000/mL	Suspicious
2 (Distinct)	Mixture thickens immediately. With continued rotation of paddle, liquid moves towards the center, leaving the bottom of the outer edge of the cup exposed.	800,000 to 5,000,000/mL	Mastitis
3 (Strong)	A distinct gel forms which tends to adhere to the bottom of the paddle and during swirling a distinct mass forms.	Over 5,000,000/mL	Mastitis

Source: ImmuCell CMT (California Mastitis Test) Product Insert

Figure 1. Interpretation of results when using the California Mastitis Text (CMT).

successfully. Streptococci other than *Streptococcus agalactiae* and staphylococci other than *Staphylococcus aureus* are also environmental pathogens which usually cause mild or subclinical diseases. The list of environmental pathogens is quite long, including such pathogens as *Arcanobacterium pyogenes*, *Bacillus* spp, *Corynebacterium* spp, enterococci, *Pasteurella* spp, *Proteus* spp, *Serratia marcescens*, yeasts, molds, and others. Each mastitis pathogen has its own epidemiological profile, including the typical environmental reservoir for the pathogen, preferred strategies for prevention and treatment, and prognosis.

Much information about individual mastitis pathogens can be found through universities and extension services and on the internet. The National Mastitis Council has an excellent website that includes profiles of common pathogens at www.nmconline.org.

Fortunately, basic microbial culture of milk can be performed relatively easily in a veterinary clinic, enabling the rapid generation of valuable information to guide management of the herd and care of individual cows.

Microbial Culture of Milk

Perhaps the simplest method of identifying the pathogen causing an intramammary infection is to obtain a milk sample and submit it to a local independent or university-affiliated diagnostic laboratory for microbial culture. The disadvantages of this approach are the expense and the delay in receipt of results, particularly if the samples have to be shipped to the laboratory by mail. The advantage is the high likelihood of accurate identification of the causative pathogen, even when the pathogen is not a common one.

Fortunately, a quicker and less expensive basic microbial culturing program is relatively easy to establish at a veterinary clinic. A few key considerations must be taken into account before an in-clinic milk culture service is initiated, including:

- **Profitability.** It is best to start small while the service is being developed, but eventually there must be enough volume of submissions to generate income.
- **Time and labor.** There must be an interested person available who will have adequate resources to operate the laboratory, including time every day for the conduct of microbiological procedures, access to sufficient initial and ongoing training, and support from practice management for the task.
- **Communication.** Systems must be established for recording culture results and reporting them in a timely manner to farms that submit milk samples.
- **Integration/additional services.** Thought should be given to what will be done with microbial

culture results. The venture is more likely to succeed if dairies know how to make the most use of the information generated. Assisting dairies in interpreting and applying the findings of microbiological testing can help them understand and utilize the service, and can provide another source of income for the practice. In addition, many dairies have difficulty obtaining appropriate samples in a timely manner, therefore offering the services of a skilled veterinary technician to identify cows for culture, collection of milk samples, or both, can also contribute to producer utilization of milk microbiology services.

- **Support.** A microbiological laboratory should be identified where samples can be sent for definitive identification. Definitive identification may be requested for cows that have not responded to treatment based on simple Gram-negative/not Gram-negative culturing schemes, for colonies that look unusual, and/or samples may be submitted at regular intervals to validate the in-clinic laboratory. Developing a relationship with the microbiologists at a diagnostic laboratory will help tremendously in the development of skills, knowledge, and confidence.

Setting up the Laboratory

Setting up and operating a basic milk microbiology laboratory is relatively simple.

The laboratory staff and management must decide which approach will best suit their goals and limitations, in order to determine the type of agar gel plates to be used for basic cultures.

A simple determination of whether a microbe is Gram-negative or not may be made using two types of agar gel; a non-selective blood agar gel (typically 5% sheep's blood agar) that supports growth of most microbes, and MacConkeys' agar gel, which is selective and supports the growth of Gram-negative bacterial strains only. It is important to recognize that blood agar gel also supports the growth of non-bacterial microbes such as yeasts and molds. Individual blood agar and MacConkey's agar gel plates may be purchased, or combination "biplates" that split a typical-sized agar gel plate in two, with half containing blood agar gel and half containing MacConkey's agar gel are available from many sources. More discrimination among microbial species is provided by "triplates", which are agar gel plates split into three compartments containing Factor media, which supports the growth of Gram-positive bacteria and yeast, MacConkey's agar gel, and modified thallium sulfate-crystal violet-B toxin (MTKT) agar gel, which is selective for *Streptococcus* spp.

Biplate or triplate systems are useful when a laboratory system is just being started because the

plates are relatively simple to use and results are not difficult to interpret. At the same time, it is critical to recognize the limitations of a simple biplate or triplate system. If the laboratory can only identify pathogens as gram-negative, gram-positive, or streptococci, results may only be reported using these categories; further testing would be necessary to identify pathogens more specifically. If the laboratory is not performing culture for mycoplasmas, it is important to inform participating farms of this fact and its implications.

A relatively easy test to perform in a clinic laboratory is the coagulase test for an enzyme produced by *Staphylococcus aureus* that causes fibrin in blood plasma to clot. Biplates and triplates described above do not support the growth of *Mycoplasma* spp, but mycoplasmas can also be grown in a clinic laboratory using specialized media and an oxygen-poor environment, which may be created using a candle and a closed jar. In addition, another simple test is the catalase test, which can be performed to differentiate *Streptococci* spp from *Staphylococci* spp.

Although the use of commercially available biplates and triplates is not difficult, training is essential if protocols are to be followed accurately, results interpreted correctly, and meaningful results produced. For example, many laboratories do not consider growth of less than three organisms on any media significant, so growth of one or two microbial colonies is reported as “no growth” or “no significant growth”. However, this breakpoint may not be appropriate for all species, as growth of even one colony of *Staphylococcus aureus* may be considered significant.

“No growth” results are important in any milk culturing program, and they typically constitute one-quarter to one-third of all milk cultures. Not treating cows that have no growth on microbial milk culture saves money for the dairy farm using a culturing program, and these savings may be more than adequate to recover the cost of culturing. At the other extreme are cultures of contaminated milk samples that will grow many different types of organisms (three or more colony types is usually used as a breakpoint indicating contamination). Many people just learning to perform milk microbiology have difficulty determining whether colonies are of the same or different types.

There is no substitute for working with an experienced trainer to learn basic microbiological skills. Any clinic starting up a milk microbiology laboratory will benefit tremendously by sending at least one staff member for training. Training courses are offered through organizations such as the Quality Milk Production Service at Cornell University, or less structured training may be arranged by contacting a nearby veterinary diagnostic laboratory, a commercial microbiology laboratory, or a clinic that has an established milk microbiology labora-

tory. After initial training, there are many websites that offer photographs and guidelines that may be helpful for evaluating microbial growth on biplates or triplates, such as this one from Michigan State University: <http://user.cvm.msu.edu/~sears/Mastitis%20Isolation.pdf> If the technician is unsure how to interpret microbial growth, the agar gel plate may be taped shut and submitted to another laboratory for definitive identification.

After labor and training, the biggest start-up expense is an incubator. If the laboratory is to be located in an area where the temperature is well-controlled, a Styrofoam egg incubator may be used to incubate samples while the volume of samples being tested in the clinic is small. Many functional used incubators may also be found at reasonable prices through internet resale websites. A thermometer should be placed inside the incubator to monitor accuracy of temperature settings. Other important items to have include 10 microliter loops for inoculating plates (either metal with a source of flame for sterilization or disposable plastic), glass slides, a microscope, a system for staining slides, designated laboratory space, bleach for disinfection of the working space, a freezer to hold milk samples, and a refrigerator for unused agar gel plates.

Quality Control

The importance of quality control to a microbiological laboratory, even a small one, cannot be overstated. It is critical that the laboratory not overstate microbial identification. A laboratory that performs cultures using only biplates may report results using descriptors such as

- Pure growth, contaminated, no significant growth, no growth
- Few colonies, moderate growth, heavy growth, number of colonies
- Gram-negative, not Gram-negative
- Hemolysis on agar, colony colors, colony description

This information can be quite useful, especially when combined with a consultation between the dairy and a veterinarian who is familiar with mastitis microbiology. Pathogens such as *E. coli* and *Klebsiella* have typical colony appearances on biplates, therefore, the veterinarian in consultation may suggest that the isolated colonies have this typical appearance, but must be circumspect about making definitive identification. Results may be used to guide decisions about whether to treat and how to treat cows affected with mastitis, which can improve treatment successes and save money for the farmer by avoidance of treating cows with no microbial growth on milk culture. A laboratory that performs culturing using only biplates cannot definitively identify microbial species without further testing, and should avoid doing so. If the dairy requests further identification of the

microbe, this can be done in the clinic laboratory or by sending the frozen milk sample or the agar gel plate to a diagnostic laboratory or specialized milk quality laboratory.

Laboratories that use triplates or conduct tests such as the coagulase test and catalase test are able to provide more specific information about isolated pathogens, but must still be careful to provide only the information that is known.

All laboratories should participate in some type of regular monitoring/quality control of their procedures and outcomes. This may be done informally, by sending a certain number or percentage of samples to a specialty or diagnostic laboratory for confirmation of organism identification, or it may be done more formally. For example, the Quality Milk Production Service has a program that regularly sends known samples for identification to enrolled laboratories so that they may test the accuracy of their work. In any case, some program must be in place to ensure that laboratory results remain accurate and therefore useful.

Conclusion

In summary, a properly operated in-clinic milk microbiology lab can provide tremendous benefit to dairies, veterinary technicians, and veterinary practices. Dairies obtain valuable information to improve mastitis prevention and control, and may realize savings even after the cost of milk culture by not treating cows with no microbial growth on culture. Veterinary technicians can develop new skills, and are provided with an autonomous specialty within the practice as trained leaders of milk microbiology services. Veterinary practices, in addition to enhancing professional development of their veterinary technicians, provide a valuable service to their dairy clients and can develop other profitable services in conjunction with laboratory services, such as reviews of records and facilities, identification of cows to be cultured, milk sample collection, and reporting of milk culture results. With the right people and procedures in place, a milk microbiology laboratory can be a valuable addition to dairy practice.