Microbiology, MALDI-TOF, Microbiome and more: How to understand and use diagnostic tests to improve udder health

Pamela L. Ruegg, DVM, MPVM

College of Veterinary Medicine, Michigan State University, East Lansing MI 48824

Abstract

Determining an appropriate intervention after intramammary infection depends on an accurate diagnosis, but there are a bewildering variety of tests that can be used to detect mastitis and determine etiology. Traditional microbiological tests rely on phenotypic and/or biochemical methods to identify bacteria but more accurate identification is achieved using newer methods such as Maldi-Tof which often results in a confusing array of unfamiliar bacterial names. Non-culture-based methods such as PCR are increasingly affordable but have both strengths and limitations that need to be acknowledged when making management decisions. Other technologies, such as determination of the milk microbiome have unfamiliar terminology and methods that require explanation before used to make decisions about handling mastitis cases. In this presentation the methods and clinical implications of using traditional microbiology and farm based or laboratory based molecular methods to diagnose mastitis will be discussed with emphasis on practical decision making.

Key words: mastitis, dairy, udder health, milk quality

Introduction

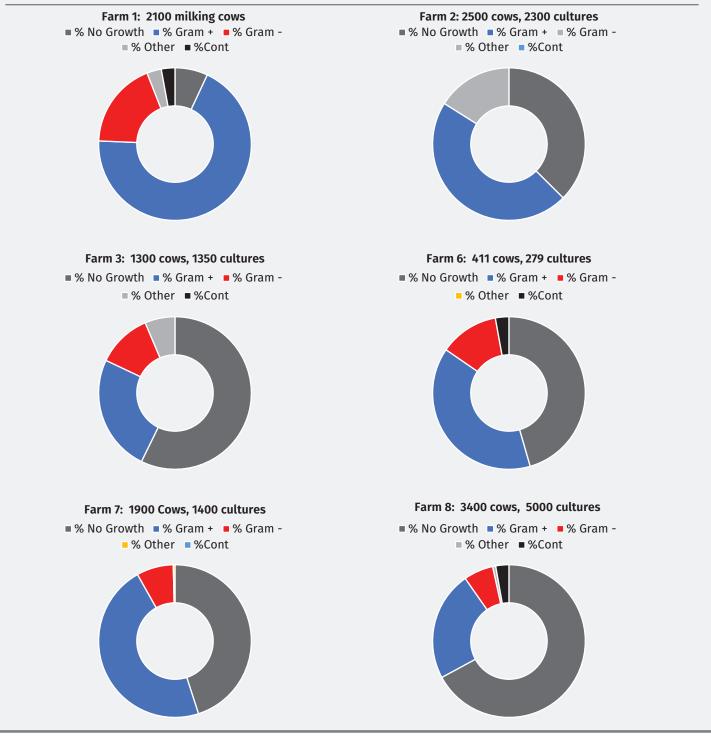
In 2002, we published a paper in The Bovine Practitioner "Milk Quality and Mastitis Tests". 1 We began that article by stating that the prevalence of subclinical mastitis ranged from about 26-50% of lactating cows and that "mastitis was the most costly disease of dairy cattle". Twenty years later, we have made tremendous progress in controlling subclinical mastitis. Since 2002, the average bulk tank somatic cell count (SCC) for U.S. dairy herds has fallen from 320,000 cells/mL to 179,000 cells/mL² and the prevalence of subclinical mastitis has decreased about 50%. Despite this progress, mastitis continues to be the most common and costly disease of mature dairy cows and requires constant vigilance by dairy producers and veterinarians to minimize its impact. Mastitis is caused by a variety of pathogens and has both subclinical and clinical presentations; thus, detection and control programs are dependent on use of diagnostic testing. Diagnostic tests for mastitis are used to determine many of the strategies that are effective for managing mastitis. A variety of older (i.e., California Mastitis Test [CMT], examination of foremilk) and newer diagnostic technologies (i.e., Maldi-Tof and molecular testing) are used in mastitis control programs. Results of tests are used to diagnose subclinical mastitis, identify newly infected cows, determine likely areas where animals are becoming infected, determine etiology to direct treatment, determine if animals are infected with contagious organisms and pose a risk of transmission to other cows, identify chronic cows, and make culling decisions. Helping producers understand how to link diagnostic test results to management decisions is an important service that veterinarians can provide to dairy clients and the objective of this paper is to provide an update on practical uses of some diagnostic tests used to control mastitis.

Definitions and test interpretation

Although several non-bacterial agents are known, bovine mastitis is usually the result of an intramammary infection (IMI) of the mammary gland by a bacterial pathogen. The risk factors, pathogenesis and control programs vary depending on etiology, so diagnostic testing is a fundamental aspect of mastitis control. Mastitis is usually defined based on the extent of the inflammatory response that occurs after an IMI is established. When milk appears visually normal but contains an excessive number of white blood cells (WBC) the quarter (or cow) is considered to have subclinical mastitis (SCM). Subclinical mastitis can only be detected using an indirect test and is most commonly diagnosed when the somatic cell count (SCC) exceeds 200,000 cells/mL.³ Clinical mastitis is diagnosed when the inflammatory response after IMI is large enough to cause visible changes in milk or the udder. While about 15% of clinical cases present with systemic involvement, a large majority of cases of clinical mastitis are non-severe with about 50% detectable only by observation of abnormalities in foremilk and an additional 35% presenting with swollen udders.^{4,5} Mastitis is detected based on non-specific signs of inflammation and can be caused by dozens of bacterial pathogens, which vary based on location of exposure, expected pathogenesis, and prognosis (with or without antimicrobial therapy). Inflammation often persists longer than IMI and detection of mastitis (inflammation) does not always indicate that sufficient bacteria are present in milk from the affected quarter to result in a positive bacterial diagnosis. The distribution of etiologies varies among farms (Figure 1) and effective prevention and treatment must be targeted at the prevalent pathogens. Determination of etiology is important for guiding treatment programs, determining risk factors for exposure and or making individual cow decisions. Several methods are available for determining etiology, including identification of live bacteria at genus or species level using conventional or enhanced microbiological techniques or use of molecular techniques that identify the presence of DNA fragments from both live and dead bacteria.

Important characteristics of diagnostic tests include the sensitivity (Se) and specificity (Sp) which are determined by comparing test performance against a presumed gold standard or by use of latent class analysis. Sensitivity is the proportion of truly diseased samples that are test-positive and specificity is the proportion of truly healthy samples that are test-negative. While sensitivity and specificity are functional characteristics of tests, from a practical standpoint, the predictive values (probability that test-positive results are truly diseased or test-negative results are truly healthy) are highly relevant and are greatly influenced by the underlying prevalence of the condition. The impact of prevalence on diagnostic test results must be understood to interpret and use results. When the prevalence is low (for example, endemic subclinical IMI caused by *Mycoplasma bovis*) the predictive value of a positive test is low

Figure 1: Annual distribution of culture results entered in DairyComp305 for 6 farms.



but the predictive value of a negative test is high. When the prevalence is high, the opposite is true. As no test is perfect, integration of clinical findings with test results will improve diagnostic performance. For example, in a herd that is known to have cows subclinically infected with *Staphylococcus aureus*, a culture-negative milk sample from a cow that has a long history of chronically high SCC, should be viewed as a potential false negative and appropriate management practices implemented (such as segregation or culling). Similarly, unexpected positive results of diagnostic tests should be confirmed with further testing. For example, it may be prudent to repeat testing (or

culture milk) if cow with a low SCC, and no history of clinical mastitis tests positive for *M. bovis* using a PCR test.

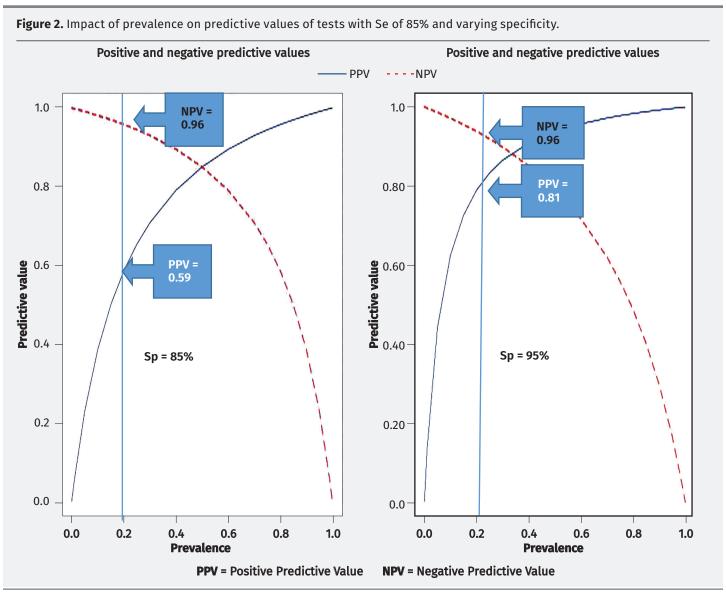
A broad range of sensitivities and specificities have been reported for on-farm culture (OFC) systems, but Se typically exceeds 80% and Sp is often greater than 90%.⁷ If the sensitivity and specificity were both 85%, when the true prevalence of IMI in cows is 20% (for example, quarter milk samples at dry off) about 60% of culture-positive and 96% of culture-negative samples each would be from truly infected and truly healthy cows, respectively (Figure 2). If the culture media was changed to an agar with greater specificity (Sp increased to 95%), the predictive value of a

positive test would increase to 81% while the predictive value of a negative test would not change appreciably. When Se increases, Sp is decreased, and the number of false positives also increases. For example, a positive diagnosis of Gram-positive IMI using an OFC system may be defined to require visible growth of > 3 CFU of identical colonies. Sensitivity of detection of IMI can be improved by dropping the CFU requirement to growth of 1 CFU but that will result in more animals treated, including some that are falsely positive (reduced specificity). In other words, we need to know something about the test and the purpose that we are trying to achieve (i.e., reduce antibiotic usage or treat as many cows as possible). In general, highly sensitive tests are desired when it is important to ensure that all affected animals are found (i.e., test and culling for highly contagious organisms) and highly specific tests are desired when the test will be used to make highly critical decisions (culling).

The predictive values of tests can also be improved by combining results of multiple tests. For example, parallel testing is used in some algorithms for determining selective dry cow therapy. Parallel testing means that an animal that tests positive to either of 2 tests (for example, had a clinical case of mastitis in that lactation or had a last test SCC > 200,000 cells/mL) is considered positive. Parallel test interpretation increases sensitivity and potentially

detects more cows which would benefit from dry cow antibiotic therapy. However, parallel testing in this manner would result in reduced specificity, thus more truly negative cows (cows that may not benefit from DCT) would be treated. Serial testing (deciding based on sequential test results) can be used to increase specificity. For example, to identify cows that are infected with *S. aureus*, cows may be first screened for SCC, and then milk samples cultured only from high SCC quarters. Repeated culture of milk samples from quarters suspected to have IMI is another example of a sequential testing strategy. Using traditional microbiological techniques, the Se of detection of *S. aureus* can be increased from 64% (a quarter milk sampled and cultured 1 time) to 83% (culture of milk samples from 3 consecutive milkings). Tools to calculate predictive values are at https://epitools.ausvet.com.au/predictivevalues.

With the advent of automated sensors that can measure a variety of biomarkers at each milking, the ideal frequency of applying tests is often questioned. In principle, the value of any diagnostic test (pregnancy checking, determination of dry matter intake, immunoglobulin testing of colostrum, etc.) is based on the value of the decision that is made based on the test result. For example, performing OFC of cases of clinical mastitis is not valuable, unless the results are used to make a treatment



or culling decision. Likewise, monthly SCC testing is only useful when results are used to make management decisions (segregation, culling, selective dry cow therapy etc.). Before investing resources in testing, a plan should be developed to identify the management actions that will occur based on the test outcome. If no decisions will or can be made, it may be more cost effective to spend resources in other ways.

Diagnostic tests commonly used in udder health programs

Conventional microbiological techniques

Culturing of aseptically collected milk samples is the only practical method for determining if a live (actively dividing) pathogen is present in a mastitic gland and is the most accurate method of guiding selective treatment decisions for clinical mastitis. Microbiological procedures for conventional culturing of milk samples have been well defined. To ensure that culturing techniques are aligned with consensus standards veterinarians should refer to the most up to date NMC Laboratory Handbook. 10 Proper aseptic collection and handling of an appropriate milk sample (usually a quarter sample) is the most critical aspect of the entire process and training of people responsible for collection of milk samples should be emphasized and re-emphasized. To maximize sensitivity and minimize risks of contamination, in most instances, quarter milk samples (rather than composite) should be collected. When cows are milked in linear or rotary parlors, if possible, quarter milk samples should be collected after the teats have been prepared for milking (application of pre-milking disinfectant and drying, followed by sanitation using 70% ethanol) but before the units are attached. Collection of quarter milk samples from cows milked in automatic milking systems (AMS) can be challenging and usually requires restraint of individual cows in an area that allows workers to safely access and sanitize teats. Improper collection of milk samples is a common reason for misleading results and videos and other resources are available for training (see resources section). After collection, milk samples should be immediately cooled and cultured as soon as possible. If samples cannot be processed within 24 hours, they should be frozen. Freezing and thawing milk reduces the number of colonies but for most pathogens, freezing for < 2 weeks has minimal effects on recovery. However, bacteria that lack protective mechanisms (such as Mycoplasma spp. which lack a cell wall) are more fragile and are more likely to be damaged by freezing.

A wide range of methods and laboratories are used for culturing milk samples and results need to be interpreted based on an understanding of the capabilities of the methods that have been used. A full-service, accredited veterinary diagnostic laboratory typically cultures 10µL of milk on blood agar and then identifies the etiology based on phenotypic and growth characteristics as well as using additional biochemical or other tests. More recently, many specialized laboratories are using mass spectroscopy (Maldi-Tof) to compare bacterial protein profiles to existing databases. 11,12 This technique requires initial growth on traditional agars and allows for separation of bacteria that have similar phenotypic and biochemical profiles. Gram-positive cocci that had been previously grouped as "environmental Streptococci spp." are now more correctly identified as Enterococcus, Lactococcus or "strep-like organisms." Traditional laboratories may identify bacteria based on algorithms that use phenotypic characteristics, Gram-staining and simple tests such as catalase and coagulase testing. On-farm culturing

systems that base diagnosis on phenotypic characteristics of bacteria plated on selective agars were initially described about 20 years ago and are often used to direct mastitis therapy. 13,14 A large variety of OFC systems have been assessed, 15 and they vary in diagnostic capabilities, price and test characteristics. Some selective media are designed to determine if a milk sample is culture negative, or contain Gram-positive or Gramnegative bacteria, while others can diagnose specific bacterial species such as S. aureus. Sensitivity and specificity of OFC systems has varied depending on the "gold-standard" used to assess the tests, the experience and training of the users and the level of discrimination needed, but most systems can be effectively used in selective treatment programs. While many farms successfully use OFC to guide therapy, 16 there are numerous opportunities for mistakes, and engagement of veterinary practitioners (or technicians) to supervise and provide quality control is an important service. Online resources describing use, interpretation and troubleshooting of OFC programs are available (see resources section).

The proportion of no-growth results is an important quality control indicator for OFC programs. The amount of milk that is plated (inoculum volume) is an important determinant of Se and determines the lower limit of detection. For example, one colony observed on a plate inoculated with 0.01-mL (10-µL) is equivalent to approximately 100 CFU/mL of milk while one colony observed using a 0.1-mL (100-µL) inoculum is equivalent to approximately 10 CFU/mL. The inoculum volume for swabs used to inoculate plates will vary but is likely at least 100µL. Use of larger inoculum volumes increases sensitivity but also increases the possibility of contamination. Culture-negative milk samples can occur for different reasons. In milk obtained from high SCC quarters, culture-negative milk samples may result from truly infected glands because a robust immune response has suppressed bacterial growth to less than the detection limit.¹⁷ In contrast, for many cases of clinical mastitis, culture-negative milk samples are an indication that a successful inflammatory response has eliminated the pathogen before visible signs of inflammation have waned. 18 While there are some differences among farms, when samples are properly collected and processed, about 25-30% of quarter samples collected from cases of clinical mastitis should be culture negative (Table 1). If the proportion of culture negative samples is less, the process of collecting, processing and interpreting results should be evaluated.

Interpretation of culture results is not always straight forward. Expectations for the distribution of results vary depending on presentation of the case (Table 1). For example, results of quarter milk samples collected from cases of clinical mastitis are often distributed as about 30% culture-negative, 35% Gram-negative and 35% Gram-positive. In contrast, culture-positive milk samples obtained from quarters of late-lactation cows typically have very few Gram-negative bacteria (Table 1). 19 Composite milk samples usually contain a greater proportion of non-aureus Staphylococci (NAS), presumably due to the large number of these organisms that colonize the teat canal. ²⁰

Antimicrobial sensitivity testing is another conventional microbiological method that is often considered but is rarely cost effective when applied to mastitis control programs. In North America, the spectrum of activity of antibiotics used for treatment of mastitis is quite similar among the limited number of approved products, and determination of etiology is usually more predictive of treatment success as compared to results of susceptibility tests. ^{21,22} In rare instances when treatment of *S. aureus* is considered, identification of strains that lack

Table 1: Comparison of results of quarter milk samples cultured from cows with clinical cases of mastitis or normal milk collected from late lactation cows.

		Oliveira et al., 2013	Rowe et al., 2019
Herds (n)		50	80
Quarter milk samples (n)		741	10,448
Type of cases		Clinical mastitis	Late lactation cows: non-clinical
Gram negative N (%)		264 (36%)	88 (<1%)
%	E. coli	23%	
	Klebsiella sp.	7%	
	Others	6%	
Gram positive N (%)		204 (38%)	2,110 (20%)
%	Strep-like organisms	13%	6%
	NASa	6%	11%
	S. aureus	3%	<1%
	Others	6%	4%
Other organism N (%)		10%	<1%
No growth N (%)		27%	79%
a non-aureus staphylococci spp.			

 β -lactamases is highly predictive of the potential for treatment success and may be considered. ²³

Molecular methods

Polymerase chain reaction (PCR). PCR is commonly used to diagnose mastitis and is based on the recovery of DNA with unique nucleotide sequences from the nucleus of bacterial cells. When PCR is used, bacterial DNA is extracted from milk samples and mixed with "primers" which are templates of nucleotide sequences from known bacterial species. The primers copy complementary sequences found in the milk until enough copies are made so that they can be matched to a bacterial species in the library. When PCR is used, only bacteria that match the primers included in the PCR kit can be identified. Some kits include primers that can identify bacterial DNA from just a few organisms (M. bovis, S. aureus and Streptococcus agalactiae) while other kits include primers for up to 16 organisms. Bacteria vary among farms and evolve; thus, it is possible that some mastitis pathogens may not contain the nucleotide sequences that are used in the primers. Intramammary infection is not the only source of bacterial DNA found in milk samples, as teat skin, the streak canal and sampling methods can contaminate milk with bacterial DNA. To ensure a useful result, milk samples used for PCR testing must be collected using aseptic technique and errors can occur when non-aseptically collected samples are used. Even when aseptically collected milk samples are used, false-positive results of PCR testing occur. 24,25 In one study, the use of this test resulted in identification of bacterial DNA of potential mastitis pathogens in 43% of culture-negative milk samples, but 31% of the samples contained DNA from > 2 types of organisms and major mastitis pathogens were cultured from some PCR negative sampes. 24 PCR testing is a fast and accurate method to detect contagious organisms such as M. bovis and S. aureus, but because it gives no indication of viability, it is not useful to guide selective treatment programs.

The use of PCR testing of bulk tank milk has also been investigated.²⁶ As expected, PCR testing is useful to detect the presence of obligate udder pathogens (such as S. agalactiae) in bulk milk, especially when there is a low prevalence of infected cows within the herd.²⁶ However, interpretation of PCR results for other bacteria found in bulk milk is difficult. PCR tests are interpreted relative to the cycling threshold values (Ct) which indicates the number of PCR cycles that are required to make enough copies to reach the signaling threshold. The lower the Ct value the greater the amount of the specified DNA in the sample and high Ct values can result in identification of sparse numbers of contaminants. When using PCR on bulk milk samples, there is limited understanding of how to interpret Ct values for bacteria that can originate from either the environment or IMI and validated guidelines for interpretation at the herd level are not available.

Milk microbiome

The microbiome refers to the collective genomes of microbial communities found in a defined system and is determined using PCR of bacterial DNA extracted from milk and tested using universal primers that amplify DNA from any bacteria. Results are characterized as Operational Taxonomic Units (OTU) that range from kingdom to species. Mucosal organs such as the gut have rich and well-characterized culturable and non-culturable microbial communities that results in many OTU using relative few PCR cycles. Milk from the healthy bovine mammary gland usually contains few or no viable bacteria, and often require many PCR cycles resulting in methodological challenges, a high probability of contamination and a lack of consistency in studies of the milk microbiome. While viable and non-viable microbial communities are well recognized in the teat canal, at this point, there is no consensus about the origin, existence or function of a potential milk microbiome and there are no current practical applications for this tool in managing udder health.

Detection of Mycoplasma bovis

While several Mycoplasma species are associated with bovine disease, M. bovis is the most isolated species that is associated with bovine mastitis. Several methods including culture, PCR and detection of antibodies are used to diagnose Mycoplasma infections.²⁷ Differences in targets for the tests are important to understand. Culture will identify live bacteria when they are shed in sufficient quantities but M. bovis is well known to shed intermittently, ²⁸ so false-negative tests are possible. When culture is used to identify herds or cows with IMI caused by M. bovis, it requires use of media containing specific nutrients and incubation in a CO2 enhanced environment for up to 10 d. M. bovis can infect multiple organ systems but identification of this organism in milk samples is strongly predictive of IMI and PCR tests are often used to identify infected animals. More recently researchers have evaluated use of ELISA to detect M. bovis antibodies in samples of bulk tank and individual cow milk. Detection of antibodies to M. bovis can be performed using ELI-SA and represent animals that have been exposed and mounted an immune response to this organism, however an ELISA positive animal may not have an active infection and antibodies persist for an indeterminant amount of time.²⁹ While M. bovis ELISA tests may be useful for screening herd additions, many ELISA-positive animals are not infectious nor exhibiting clinical signs of disease, thus use of this test for test-and-culling programs is not advised.

Measures of inflammation

Somatic Cell Count (SCC)

Milk obtained from quarters of cows experiencing SCM appears visually normal (even when millions of somatic cells are present) but contains an excessive number of somatic cells (with or without the detectable presence of pathogenic organisms). Somatic cells in milk consist of neutrophils, macrophages, lymphocytes and a smaller percentage of epithelial cells. The SCC of healthy quarters is usually well below 100,000 cells/mL and is composed primarily of macrophages. After establishment of IMI, inflammatory mediators attract large numbers of phagocytes to migrate from the bloodstream to the udder, dramatically increasing the SCC in milk and shifting the distribution of cells from macrophages and lymphocytes to primarily neutrophils. Detection of SCM is based on measurement

of SCC in milk collected from individual quarters or composite milk samples that are a mixture of milk from all functional glands of an individual cow. When using SCC to diagnose SCM, a threshold of approximately 200,000 cells/mL has been considered optimal to reduce diagnostic error. ^{3,30,31} It is important to remember that composite milk samples that are greater than this value have at least 1 quarter with SCC > 800,000 cells/mL.

Monitoring monthly incidence and prevalence trends from monthly individual cow SCC testing is essential for investigating herd problems and SCC patterns by parity and stage of lactation (Figure 3) often give a strong indication of the type of pathogens and mode of transmission. At the herd-level, evaluation of the pattern of newly and chronically increased SCC can be highly diagnostic for troubleshooting high BTSCC. For example, when many cows have increased SCC in early lactation, exposure to environmental mastitis pathogens during the dry and transition periods should be evaluated (Figure 3A). In these herds, transition and dry cow management should be evaluated with special emphasis on the condition of pastures (when used) and density of cow pens. In contrast, when contagious mastitis is a problem, the proportion of cows with increased SCC usually increases as lactation progresses and as cows age (because of the longer a cow milks, the greater the opportunities for exposure to infected milk) (Figure 3B). In these herds, emphasis should be placed on detecting inadequate teat dipping or the presence of fomites that can transfer infected milk among cows (such as towels used to clean or dry teats on more than one cow). When a large proportion of cows have chronically increased SCC (more than 2 consecutive monthly tests with increased SCC) it indicates that cows are infected with host adapted pathogens that are usually transmitted in a contagious manner. In these instances, it is useful to review a list of individual cows sorted by SCC to identify cows that may require specific interventions. The use of a rapid cow-side quarter-level SCC test, can help farmers make important management decisions such as whether to segregate, treat, culture, withhold high SCC quarters or cull the cow.

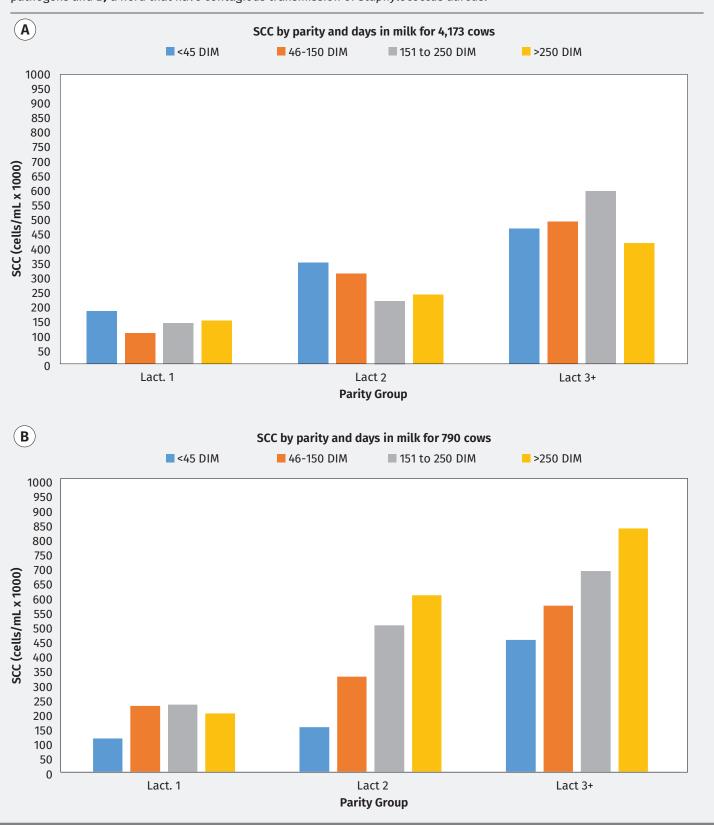
After effective treatment or spontaneous cure of an IMI, the SCC will gradually return to < 200,000 cells/mL, but the time required to for the SCC to diminish varies among etiologies and CMT values should not be used to determine when treatment has been effective. ³² Even in large herds with relatively low BTSCC, the prevalence of SCM is strongly predictive of bulk tank SCC and access to individual SCC is essential for managing udder health (Figure 4).

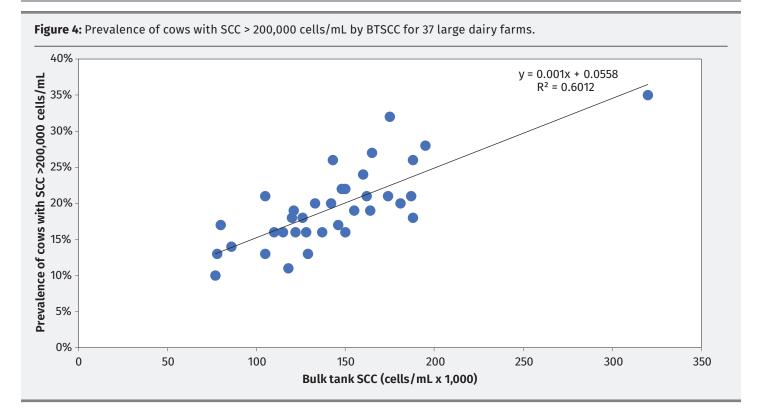
Table 2: Usefulness of diagnostic tests for making udder health decisions. +++ indicates very useful; ++ indicates somewhat useful; + indicates occasionally useful

	Culture-based techniques to detect viable organisms	PCR test for presence of bacterial DNA	Detection of inflammation (SCC, CMT or others)
Guide selective treatment decisions for clincial mastitis	+++	+	++ ^a
Identify cows/herds infected with M. bovis, S. aureus and Prototheca bovis	++	+++	+++b
Guide treatment decision in selective DCT algorithms	+	+	+++
Identify cows for segregation pens	++	++	+++
Herd level investigation of transmission and risk factors	++	++	+++

^a SCC history should be reviewed before deciding that antibiotic therapy is indicated (chronically infected cows are often poor candidates for antimicrobial therapy); ^bSCC history is useful to identify chronic cows for culture or PCR testing.

Figure 3: Characteristic SCC patterns for a herd experiencing **A)** early lactation mastitis problems with environmental pathogens and **B)** a herd that have contagious transmission of *Staphylococcus aureus*.





Differential SCC

The distribution of WBC in milk from healthy glands is predominantly lymphocytes and macrophages, but when IMI is established the SCC is dominated by neutrophils.³³ The totality of WBC plus some mammary epithelial cells are included in measurements of the SCC, but recent technology has allowed the ability to differentiate WBC. The differential SCC (DSCC) can be determined in the laboratory (Foss Flow Cytometry [F-DSCC]) and on-farm (Q-Scout Milk Leukocyte Differential [MLD]). Both of these technologies appear to be able to accurately perform a differential count of WBC, but their output varies. The F-DSCC calculates the proportion of PMN, and lymphocytes as compared to the sum of PMN, lymphocytes and macrophages, whereas the MLD calculates the absolute values and proportion of each cell type. Theoretically, this information could help identify the stage of an inflammatory response which may be useful to predict outcomes. However, while the F-DSCC and MLD are reasonably correlated with SCC, research has not yet demonstrated that either of these tests can differentiate among pathogens nor predict outcomes and additional research is needed to demonstrate value beyond that provided by SCC.³⁴

A note on other indicators of inflammation

Several other biomarkers for mammary gland inflammation have been identified including changes in conductivity, Nagase, lactate dehydrogenase (LDH), and others. ^{6,35,36} All these biomarkers are activated at various stages of the inflammatory response, and most are highly correlated with SCC. Several of these are used in automatic milking systems to detect mastitis but a review of these biomarkers is beyond the scope of this presentation.

Summary

The adage that "If you don't monitor it, you can't manage it" is highly applicable to managing mastitis at both the cow and herd level. Mastitis can be caused by a variety of pathogens but is detected based on non-specific inflammatory responses that define the subclinical and clinical presentations. Depending on the interventions that are being considered, a various diagnostic tests are available for practitioners to use as they direct therapy, identify infected cows and solve herd problems (Table 2). A thorough understanding of the capabilities of these tests is useful for determining effective interventions.

Additional resources

Using on-farm culturing to improve mastitis treatments. Twelve videos available at https://www.youtube.com/playlist?list=PLrOXYxghhxVYtK6TrtVtj9r_Q960Mj-8k

 $\label{lem:complex} \begin{tabular}{ll} Collecting an aseptic milk sample: https://www.youtube.com/watch?v=Zt6APgWWQGo. \end{tabular}$

Numerous udder health resources and links: https://topmilk. msu.edu/ $\,$

Funding statement

Dr. Ruegg's work is supported in part by the David J. Ellis Endowment

References

- 1. Ruegg PL, Reinemann DJ. Milk quality and mastitis tests. *Bov Pract.* 2002;36:41-54.
- 2. Norman HD, Guinan JH, Megonigal JH, et al. Milk somatic cell count from Dairy Herd Improvement herds during 2021. SCC23-22 ed, 2022.

- 3. Dohoo IR, Leslie KE. Evaluation of Changes in Somatic-Cell Counts as Indicators of New Intramammary Infections. *Prev Vet Med* 1991;10:225-237.
- 4. Oliveira L, Hulland C, Ruegg PL. Characterization of clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin. *J Dairy Sci* 2013;96:7538-7549.
- 5. Pinzon-Sanchez C, Hulland C, Ruegg PL. Post treatment outcomes of clinical mastitis on commercial dairy farms. *J Dairy Sci* 2010;93:79-79.
- 6. Nyman AK, Emanuelson U, Waller KP. Diagnostic test performance of somatic cell count, lactate dehydrogenase, and N-acetyl-beta-D-glucosaminidase for detecting dairy cows with intramammary infection. *J Dairy Sci* 2016;99:1440-1448.
- 7. Malcata FB, Pepler PT, O'Reilly EL, et al. Point-of-care tests for bovine clinical mastitis: what do we have and what do we need? *J Dairy Res* 2020;87:60-66.
- 8. Nyman AK, Persson Waller K, Emanuelson U, et al. Sensitivity and specificity of PCR analysis and bacteriological culture of milk samples for identification of intramammary infections in dairy cows using latent class analysis. *Prev Vet Med* 2016;135:123-131.
- 9. Hogeveen H, Klaas IC, Dalen G, et al. Novel ways to use sensor data to improve mastitis management. *J Dairy Sci* 2021;104:11317-11332.
- 10. NMC. *Laboratory Handbook on Bovine Mastitis*. 3rd ed. New Prague, MN, USA: NMC, 2017.
- 11. Tomazi T, Goncalves JL, Barreiro JR, et al. Identification of coagulase-negative staphylococci from bovine intramammary infection by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2014;52:1658-1663.
- 12. Barreiro JR, Ferreira CR, Sanvido GB, et al. Short communication: Identification of subclinical cow mastitis pathogens in milk by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J Dairy Sci* 2010;93:5661-5667.
- 13. Sears PM, McCarthy KK. Diagnosis of mastitis for therapy decisions. *Vet Clin North Am Food Anim Pract* 2003;19:93-108.
- 14. Lago A, Godden S, Bey R, et al. Preliminary validation of an on-farm culture system. *J Anim Sci* 2006;84:199-199.
- 15. Malcata FB, Pepler PT, Zadoks RN, et al. Laboratory-based evaluation of a simplified point-of-care test intended to support treatment decisions in non-severe bovine clinical mastitis. *J Dairy Res* 2021;88:170-175.
- 16. Lago A, Godden SM. Use of Rapid Culture Systems to Guide Clinical Mastitis Treatment Decisions. *Vet Clin North Am Food Anim Pract* 2018;34:389-412.
- 17. Pantoja JCF, Hulland C, Ruegg PL. Dynamics of somatic cell counts and intramammary infections across the dry period. *Prev Vet Med* 2009;90:43-54.
- 18. Fuenzalida MJ, Ruegg PL. Negatively controlled, randomized clinical trial to evaluate use of intramammary ceftiofur for treatment of nonsevere culture-negative clinical mastitis. *J Dairy Sci* 2019;102:3321-3338.
- 19. Rowe SM, Godden SM, Royster E, et al. Cross-sectional study of the relationships among bedding materials, bedding bacteria counts, and intramammary infection in late-lactation dairy cows. *J Dairy Sci.* 2019;102:11384-11400.

- 20. Braem G, De Vliegher S, Verbist B, et al. Unraveling the microbiota of teat apices of clinically healthy lactating dairy cows, with special emphasis on coagulase-negative staphylococci. *J Dairy Sci* 2013;96:1499-1510.
- 21. Hoe FG, Ruegg PL. Relationship between antimicrobial susceptibility of clinical mastitis pathogens and treatment outcome in cows. *J Am Vet Med Assoc* 2005;227:1461-1468.
- 22. Apparao D, Oliveira L, Ruegg PL. Relationship between results of in vitro susceptibility tests and outcomes following treatment with pirlimycin hydrochloride in cows with subclinical mastitis associated with gram-positive pathogens. *J Am Vet Med Assoc* 2009;234:1437-1446.
- 23. Barkema HW, Schukken YH, Zadoks RN. Invited review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. *J Dairy Sci* 2006;89:1877-1895.
- 24. Koskinen MT, Wellenberg GJ, Sampimon OC, et al. Field comparison of real-time polymerase chain reaction and bacterial culture for identification of bovine mastitis bacteria. *J Dairy Sci* 2010;93:5707-5715.
- 25. Mahmmod YS, Klaas IC, Nielsen SS, et al. Effect of presampling procedures on real-time PCR used for diagnosis of intramammary infections with *Staphylococcus aureus* in dairy cows at routine milk recordings. *J Dairy Sci* 2013;96:2226-2233.
- 26. Katholm J, Bennedsgaard TW, Koskinen MT, et al. Quality of bulk tank milk samples from Danish dairy herds based on real-time polymerase chain reaction identification of mastitis pathogens. *J Dairy Sci* 2012;95:5702-5708.
- 27. Parker AM, Sheehy PA, Hazelton MS, et al. A review of mycoplasma diagnostics in cattle. *J Vet Intern Med* 2018;32:1241-1252.
- 28. 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* 2003;223:1163-1166.
- 29. Parker AM, House JK, Hazelton MS, et al. Bulk tank milk antibody ELISA as a biosecurity tool for detecting dairy herds with past exposure to *Mycoplasma bovis*. *J Dairy Sci* 2017;100:8296-8309.
- 30. Schepers AJ, Lam TJGM, Schukken YH, et al. Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. *J Dairy Sci* 1997;80:1833-1840.
- 31. Djabri B, Bareille N, Beaudeau F, et al. Quarter milk somatic cell count in infected dairy cows: a meta-analysis. *Vet Res* 2002;33:335-357.
- 32. Ruegg PL. Mastitis, detection, diagnosis and managment. *J Dairy Science* 2017; in press.
- 33. Halasa T, Kirkeby C. Differential Somatic Cell Count: Value for Udder Health Management. *Front Vet Sci* 2020;7:609055.
- 34. Godden SM, Royster E, Timmerman J, et al. Evaluation of an automated milk leukocyte differential test and the California Mastitis Test for detecting intramammary infection in early- and late-lactation quarters and cows. *J Dairy Sci* 2017;100:6527-6544.
- 35. Pyorala S. Indicators of inflammation to evaluate the recovery from acute bovine mastitis. *Res Vet Sci* 1988;45:166-169.
- 36. Pyorala S. Indicators of inflammation in the diagnosis of mastitis. *Vet Res* 2003;34:565-578.

