Methods for Diagnosing Mastitis

Pamela R.F. Adkins, DVM, PhD*, John R. Middleton, DVM, PhD

KEYWORDS

• Bovine • Mastitis • Intramammary infection • Diagnosis

KEY POINTS

- The most common cause of mastitis is an intramammary infection.
- Considering cost and ease of data collection, somatic cell count is the most common diagnostic test used for the detection of subclinical mastitis.
- Bacteriologic culture and polymerase chain reaction are the primary methods currently in use to diagnose intramammary infection.
- There is no gold standard for the diagnosis of mastitis or intramammary infection.

INTRODUCTION

Mastitis is defined as inflammation of the mammary gland. The most common cause of mastitis is an intramammary infection (IMI). An IMI refers to the presence of an infectious organism in the mammary gland. Although these two often go hand in hand and the terms are frequently used interchangeably, no single diagnostic test is able to define both. A diagnosis of mastitis is generally based on measuring the inflammatory response, whereas diagnosis of an IMI is based on identification of the inciting infectious agent. Diagnosis of mastitis by measuring indicators of inflammation is often used as an indirect method to identify cows with an IMI.

DIAGNOSIS OF MASTITIS

Clinical Mastitis

Mastitis can be characterized as clinical or subclinical. Clinical mastitis is defined as visibly abnormal milk from a mammary quarter. With forestripping, that is, visual examination of a stream of milk collected immediately before routine milking, clinical mastitis can easily be detected (**Fig. 1**). Clinical mastitis can be defined based on severity as mild, moderate, or severe.[1] Severity scoring systems can be used to determine appropriate treatment and the risk of an undesirable outcome.[2]

Disclosure Statement: The authors have nothing to disclose.
Department of Veterinary Medicine and Surgery, University of Missouri, 900 East Campus Drive, Columbia, MO 65211, USA
* Corresponding author.
E-mail address: adkinsp@missouri.edu

Vet Clin Food Anim 34 (2018) 479–491
https://doi.org/10.1016/j.cvfa.2018.07.003
vetfood.theclinics.com

Fig. 1. Forestripping can help to identify cases of clinical mastitis in the parlor.

- Mild clinical mastitis: Abnormal milk only (usually manifest by clots, flakes, and/or changes in the color and consistency of the milk secretion).
- Moderate clinical mastitis: Abnormal milk and abnormal mammary gland (manifest by inflammatory changes in the tissue such as redness, heat, pain, and swelling).
- Severe clinical mastitis: Abnormal milk, abnormal mammary gland, and sick cow (manifest by changes in body temperature, rumination rate, appetite, hydration status, and demeanor).[2]

Subclinical Mastitis

Subclinical mastitis is defined as the presence of inflammation with a normal appearing mammary gland and visibly normal milk. Many tests have been evaluated for the diagnosis of subclinical mastitis. Some of the more common ones are listed here.

- Somatic cell count (SCC): Concentration of leukocytes (primarily) per milliliter of milk. Leukocytes comprise 80% of the somatic cells in uninfected quarters and 99% in infected quarters.[3] The most important factor that causes a rise in SCC is an IMI.
- Lactose: The percentage of lactose in mastitic milk is lower. This change occurs owing to tissue damage causing decreased synthetic ability of the enzyme systems in the secretory cells, resulting in reduced lactose biosynthesis.[4]
- Lactate dehydrogenase (LDH): An enzyme found in most tissues, including the cytoplasm of leukocytes. When cell damage occurs, to either mammary epithelial cells or leukoctyes, LDH is released into the milk.[4] Some commercially available mastitis detection tools incorporate measurement of LDH activity.

- *N*-acetyl-β-D-glucosaminidase (NAGase): A lysosomal enzyme that is released into the milk from damaged mammary epithelial cells and, to a lesser extent, from milk somatic cells.[5,6]
- Acute phase proteins: Haptoglobin and milk amyloid A have been found in milk owing to their migration from blood into milk across the blood–milk barrier because of increased capillary permeability and loss of tight junctions, or through local production by milk leukocytes or mammary epithelial cells.[7]
- Conductivity: Electrical conductivity (EC) of milk increases with mastitis owing to an increase in sodium and chloride concentrations and a decrease in the potassium concentration.[4] Several milking equipment manufacturers have used EC as an in-line method of detecting mastitis.

Somatic Cell Count

Taking cost and ease of data collection into consideration, SCC or the logarithmic transformation of SCC, the somatic cell score (SCS), is the most common diagnostic test used for the detection of subclinical mastitis. In a laboratory setting, SCC can be measured using microscopy, referred to as direct microscopic SCC or by using automated electronic cell counters. The direct microscopic SCC method is performed by spreading a specific volume of milk within a calibrated area of a microscopic slide. After the milk dries, the slide is stained, and visible cells are counted within the defined area. The method is labor intensive, requires a high-quality microscope, and necessitates thorough training of personnel to gain proficiency. Automated electronic cell counters, which commonly are based on flow cytometric methods, allow for rapid and easy determination of SCC. Creameries, Dairy Herd Information Association, and other dairy organizations use automated electronic cell counters, making these data highly accessible. Portable counters are also available and can be used to test SCC in the laboratory or on the farm.

At the herd level, SCC data are generally available on every shipment of milk that leaves the farm and these data provide an estimation of overall udder health among cows contributing to the bulk tank milk. At the cow level, herds that use a testing laboratory such as the Dairy Herd Information Association, generally have monthly data reflecting the udder health of each cow, and these data can be used in parallel to predict which cows have healthy mammary glands versus those with acute, resolved, or chronic cases of subclinical mastitis (**Fig. 2**).

Although cow-level composite SCC samples are useful for separating infected from uninfected cows, these data are imperfect. The sensitivity of composite SCC as an indicator for IMI in at least one-quarter ranges from 30% to 89%, whereas the specificity ranges from 60% to 90%.[8–10] The sensitivity and specificity using a threshold of 200,000 cells/mL for a single composite SCC obtained closest to the time of culture were 44% and 87%, respectively, for cows infected with any pathogen and 65% and 73%, respectively, for cows infected with major pathogens.[10]

The most accurate relationship between IMI and SCC exists at the quarter level. Data suggest that uninfected quarters have a mean SCC of approximately 70,000 cells/mL[11,12] and an SCC of 200,000 cells/mL or greater or an SCS of 4 or higher is often used as a threshold to define infected quarters.[11] That said, diagnostic sensitivity of quarter-level SCC for subclinical mastitis can also be imperfect, and somewhat depends on the pathogen inciting the mastitis. Middleton and colleagues[13] reported that sensitivity of quarter-level SCC using a threshold of 100,000 cells/mL (SCS = 3) was 0.60 for all bacterial IMI, 0.53 for coagulase negative staphylococcal IMI, 0.96 for coagulase positive staphylococcal IMI, and 0.71 for IMI with non-*agalactiae Streptococcus*-like organisms.

Other limitations have been identified that impact the use of SCC as a diagnostic tool. Milk SCC can remain elevated for some time after an organism has been eliminated, resulting in a false-positive test for IMI. Also, although IMI is the predominant factor associated with variation in the SCC, other factors can affect the SCC including herd, cow, breed, quarter, month of sampling, season, stage of lactation, age of the cow, parity, frequency of milking, and stressors.[14–17]

Estimating the Somatic Cell Count at the Cow Side

A number of cow-side methods have been developed and studied for counting or approximating milk SCC.

- California mastitis test (CMT): A qualitative measurement of SCC. The reagent causes lysis of cell membranes and precipitation of the cell DNA and proteins resulting in change in viscosity of the reagent when added to milk.
- Wisconsin mastitis test (WMT): A modification of the CMT developed to increase the objectivity of measuring the viscosity. A modification of the WMT has been adapted for on-farm use[18] that can be performed in a few minutes and results in a semiquantitative measurement of the SCC; however, the test requires a refrigerated sample collected within 5 hours of testing.
- Esterase activity test: A qualitative test that converts the results of an enzymatic reaction into an estimated SCC. Requires 5 to 45 minutes of incubation, depending on the test type.

With regards to cow-side methods, again, not all methods have been researched appropriately. A modified WMT test was evaluated in the laboratory and found to have similar results to electronic somatic cell counting with a high degree of agreement when a threshold of 205,000 cells/mL was used to define an IMI.[18] However,

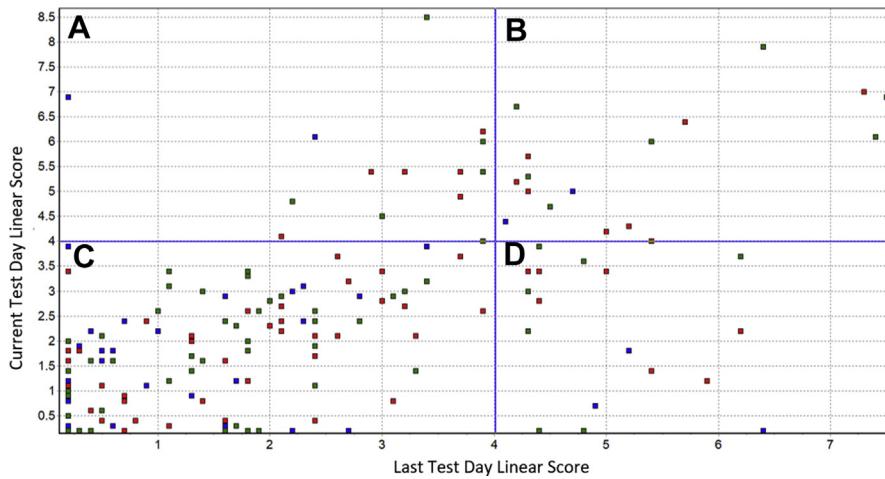

Fig. 2. Computer software can be used to plot somatic cell score from current (y-axis) and previous (x-axis) Dairy Herd Information Association test days to help determine mastitis status. (*A*) Cows with new cases of mastitis (low previous test day somatic cell count [SCC], high current test day SCC). (*B*) Cows with chronic cases of mastitis (high previous test day SCC, high current test day SCC). (*C*) Healthy cows (low previous test day SCC, low current test day SCC). (*D*) Cows with cured cases of mastitis (high previous test day SCC, low current test day SCC). (*Courtesy of* Scott E. Poock, University of Missouri, Columbia, MO.)

when using this same test in a cow-side manner, it markedly underestimated the SCC,[19] making it impractical for on-farm use.

The CMT, when used at a cut point of trace or higher, had a much higher test sensitivity and specificity than the cow-side version of the WMT test. When comparing the CMT, cow-side WMT, and 3 esterase tests, the CMT provided the most accurate, practical, and least cost on-farm screening test to predict subclinical mastitis at dry-off.[20] The CMT also provided a faster and more accurate cow-side screening test to predict subclinical mastitis defined as an SCC of greater than 200,000 cells/mL at dry-off and freshening.[19]

The sensitivity and specificity of the CMT has been evaluated in multiple studies. When evaluating the tests ability to detect an IMI with a major mastitis pathogen (*Staphylococcus aureus*, *Streptococcus spp*, and gram-negative organisms) in early lactation, the sensitivity was 82.4% and specificity was 80.6% on day 4 of lactation.[21] When assessed to determine the ability of the CMT to identity IMI with any pathogen, including minor pathogens, the sensitivity was much lower at 61%, but specificity was the same at 80%.[13] When assessing the CMT to identify IMI at dry-off at the cow level for all pathogens, the sensitivity was 70% and specificity was 48%.[22] Overall, although the CMT lacks diagnostic sensitivity for detecting any IMI, when IMI are caused my major pathogens sensitivity is reasonable, suggesting that CMT is still a useful screening tool for the more inflammatory mastitis pathogens.[23]

Other Measures of Mammary Gland Inflammation

Among the other tests available to detect subclinical mastitis discussed at the beginning of this section, few have been validated against reference methods, for example, SCC measurement or detection of IMI, making it challenging to determine which of these is the best detection method. Of those methods that have been evaluated, it has been found that the SCC provides superior diagnostic performance in detecting IMI-negative and IMI-positive cows than LDH and NAGase.[23,24] The milk amyloid A enzyme-linked immunosorbent assay has been shown to be as accurate as the SCC.[25] Other investigators have shown that haptoglobin performs better than milk amyloid A, because a constant increase in the haptoglobin concentration was found in the milk along with increasing quantities of bacterial DNA.[7] Although acute phase proteins may be useful, currently they are not an economically feasible option for diagnosing subclinical mastitis. Like with SCC, cow factors can also affect other measurements used to diagnosis subclinical mastitis, such as LDH and NAGase, and in some cases to a greater extent than SCC.[23]

Although EC is commonly used as an in-line indicator of mastitis, for example, in automated milking systems, its usefulness in detecting cases of mastitis is impacted by multiple factors, including whether the case is clinical or subclinical and changes in milk composition. At the cow-level interquarter comparisons of EC improve test sensitivity and specificity.[26] Hand-held EC meters for cow-side use tend to perform poorly for detecting IMI and seem to be inferior to SCC measurement or CMT. Milk lactose concentration can likewise be measured in-line and has been used to predict IMI.[27] A recent study suggested that, when using attribute weighting analysis of data collected longitudinally during milking (milk volume, protein concentration, lactose concentration, milking time, peak flow, and EC) and comparing these data to 3 SCC thresholds for the detection of subclinical mastitis ($\geq$250,000, $\geq$200,000, or $\geq$150,000 cells/mL), in the absence of SCC, lactose concentration followed by EC were strong indicators of subclinical mastitis.[28]

None of the diagnostic tests used to define mastitis can specify the pathogen causing the infection and, therefore, excludes the information necessary to make a

treatment decision. Thus, it is recommended to follow up a diagnosis of mastitis with a diagnostic test to determine the cause of the IMI.

DIAGNOSIS OF INTRAMAMMARY INFECTION

In general, the goals of determining the cause of an IMI are to either select a treatment protocol or determine where control measures need to be implemented or improved on the farm to reduce disease incidence and improve udder health and milk quality. As with SCC, data can be collected at the herd (bulk tank) or pen (in-line sampling), cow, or mammary quarter levels. Bacteriologic culture and polymerase chain reaction (PCR) are the primary methods currently in-use to diagnose IMI. Most PCR assays use real-time multiplex PCR to identify an array of common mastitis pathogens. Regardless of diagnostic method used, there is no true gold standard available to diagnose an IMI.

Culture

Bacterial culture techniques are generally inexpensive and simple to perform, but need to be performed using standardized repeatable methods.[29] Although many mastitis pathogens are readily grown under aerobic conditions on a blood-based agar medium, some pathogens require specific growth media and growth conditions, for example, *Mycoplasma* spp. After culture results are obtained, definitions need to be established to standardize diagnoses.

Standardized methods are described for characterizing bacteria in bulk tank milk.[29] In general, the goals of bulk tank cultures are to (1) monitor raw milk quality and (2) gain herd-level information about the presence of mastitis pathogens, particularly contagious mastitis pathogens such as *S aureus*, *Streptococcus agalactiae*, and *Mycoplasma* spp. The presence of other potential mastitis pathogens in bulk milk may or may not be associated with IMI because many of the other bacteria could come from nonmammary sources, for example, contaminated teat skin and soiled or poorly sanitized milking equipment. The standard plate count (SPC) gives an estimate of the total bacterial load in the bulk tank. The laboratory pasteurized count gives an estimate of thermoduric bacteria (those that survive pasteurization), and the preliminary incubation count estimates the number of psychotropic bacteria (those that grow at cold temperatures). Recommended thresholds for SPC, laboratory pasteurized count, and preliminary incubation count are less than 5000 CFU/mL, less than 100 CFU/mL, and less than 10,000 CFU/mL, respectively.[29] Increases in the SPC, laboratory pasteurized count, and preliminary incubation count can be associated with poor udder cleanliness and/or poor milking system sanitation, but increases in the SPC alone could indicate cases of IMI.

At the cow or mammary quarter level, factors involved in diagnosing an IMI include the number of colonies of the organism isolated from the milk sample, whether the organism is isolated in pure or mixed culture, and if a measure of inflammation is included in the definition. When a quarter milk sample results in the growth of 3 or more colony types, the sample is most likely contaminated.[29] However, it is important to remember that all organisms isolated from a milk sample could be the result of contamination, including known mastitis pathogens such as *S aureus*, *S agalactiae*, and *Mycoplasma* species. Single, duplicate, and triplicate quarter milk samples used in series or in parallel have been used to determined IMI status.

All culture procedures have limited sensitivity and requiring anything other than isolation of 1 colony forming unit (CFU) of an organism from 0.01 mL of milk (100 CFU/ml) further limits the sensitivity.[30] In general, the current recommendation for considering a single quarter sample positive for an IMI is to use 100 CFU/mL,

except for non-*aureus Staphylococcus*, where the recommendation is 200 CFU/mL.[30] The use of the results of duplicate and triplicate samples gives high test specificity with a decrease in test sensitivity or results in little gain compared with a single sample.[31] This does not mean the recommended definition stated is always appropriate, because it can depend on the organism isolated, the goals of the farm, and the control program that is planned based on the definition.

Composite milk samples, a sample containing milk from all four quarters of 1 cow, are often used for diagnosis of IMI in cows with subclinical mastitis. In general, composite samples have a low sensitivity, but a high specificity for most organisms.[32] The low sensitivity is caused by the dilution of bacterial numbers by milk from uninfected quarters in the composite sample, similar to the dilution seen with composite SCC. Quarter-level samples are therefore recommended as the first line in mastitis diagnosis, whereas composite samples are useful in surveillance when considering their limitations.[32]

Secondary (Confirmatory) Tests

After primary isolation of a bacterial colony or colonies, additional tests must be applied to determine the identity of the organism. Most laboratories and on-farm culture systems rely on an initial assessment of phenotypic characteristics to help distinguish mastitis pathogens into broad groups. Some common phenotypic tests used to crudely differentiate organisms isolated from milk include visual evaluation of colony morphology, examination of the culture medium for hemolysis, and Gram staining or KOH gelation testing. For gram-positive bacterial isolates, frequently used tests include the catalase test, coagulase test, and CAMP/esculin test to aid in the differentiation of contagious gram-positive bacteria, for example, *S aureus* or *S agalactiae*, from noncontagious gram-positive bacteria, for example, non-*aureus* staphylococci, non-*agalactiae* streptococci, or streptococcal-like organisms. For gram-negative bacterial isolates, growth on selective medium, for example, lactose fermentation on MacConkey agar, as well as triple sugar iron reaction, growth on Simmons citrate agar, oxidase test, and motility testing may be used to differentiate the various environmental gram-negative pathogens. Although these methods are useful for broadly grouping pathogens based on their putative source and also for making preliminary decisions about treatment, they are not entirely accurate.

Historically, further speciation was conducted using proprietary biochemical test panels that, based on colorimetric analysis, yielded a likely bacterial genus and species identity for a given isolate. Available data now suggest that, for some genus and species of bacteria isolated from cases of bovine mastitis, these methods are inaccurate. Hence, other methods to identify organisms to the species level have been explored. Until recently, the most commonly used alternative to biochemical testing was partial sequence analysis of bacterial housekeeping genes, with 16S rRNA being the most universal target.[33] The usefulness of 16S rRNA gene sequencing is limited when applied to certain staphylococcal species owing to the high degree of gene similarity.[34] Therefore, several other housekeeping genes have been used to differentiate staphylococcal species, including, *rpoB*,[35] *tuf*,[36] *sodA*,[37] *gap*,[38] *dnaJ*,[39] and *hsp60*.[40] Although these methods are generally considered accurate, they can be time consuming and costly to perform, and are not always readily available to mastitis diagnostic laboratories.

In the last few years, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry has been evaluated for the genus and species identification of mastitis pathogens. This technology is becoming widely adopted in many diagnostic and research laboratories. MALDI-TOF is a high-throughput technology that uses a protein fingerprint and a database of reference spectra to determine a bacterial species. This test has been validated as an accurate secondary test for some mastitis

pathogens. Overall, MALDI-TOF has been found to be accurate in diagnosis of *Staphylococcus* spp.[41,42] and *Corynebacterium* spp.[43] Although this method has been determined to be a rapid technique for speciation of bacteria, the initial equipment set up is costly and, for the most part, it requires the organism be cultured first. One recent study has determined that MALDI-TOF can be used in a culture-independent fashion to identify bacterial species in experimentally inoculated milk samples. However, the required colony forming unit per milliliter to make an accurate diagnosis was very high, generally much higher than expected in naturally occurring cases of IMI in the field; therefore, direct from milk MALDI-TOF is not currently recommended owing to the high likelihood of false negative results in cows infected with mastitis pathogens.[44]

On-Farm Culture Systems

Although milk cultures can be performed by veterinary practices and diagnostic laboratories, there can be a benefit to having cultures performed on the farm giving producers ready access to timely data for making targeted treatment decisions. On-farm systems can include traditional plating methods using nonselective media such as blood agar or using a combination of selective media such as MacConkey agar for gram-negative pathogens, TKT agar for streptococci, and Baird-Parker for staphylococci.[45] Other simplified options include the use of biplates or triplates that use a combination of these media on a single segregated Petri dish or use of commercially available selective culture films.

Biplate and triplate systems are commercially available. The biplate system has 2 agar types, one for selective growth of gram-negative organisms and one for the selective growth of gram-positive organisms. The triplate has 3 agar types, which in addition to differentiating gram-positive from gram-negative organisms, also helps differentiate staphylococcal species from streptococcal species.[46] Microbial growth films are commercially available for aerobic counts, coliform counts, and staphylococcal counts. One limitation of using the aerobic count bacterial growth film is that it does not allow for species identification, making it impossible to differentiate contamination from an IMI.[47]

With the use of selective medias, it is expected that on-farm culture systems will not detect all mastitis pathogens.[46] These systems are often most successful when interpretation is simplified, such as to differentiate growth from no growth, gram-positive from gram-negative, or for a triplate system, staphylococci from streptococci.[45,46] On-farm culture systems are generally aimed at broadly categorizing mastitis pathogens to select treatment and are not designed to make species-level pathogen diagnoses.[48]

Real-Time Multiplex Polymerase Chain Reaction

Although culture-based methods are still the mainstay in many diagnostic laboratories and veterinary practices for diagnosing IMI, culture-independent methods for identifying bacterial pathogens in milk have become more common over the last decade. The first PCR for the identification of pathogens associated with IMI was made commercially available in 2008. When compared with culture-based methods, PCR is faster, because the results can be provided to the producer within 4 hours, and it has been found to be more sensitive when compared with traditional culture.[49] PCR has been shown to provide a diagnosis for 43% to 47% of mastitic milk samples that were negative based on conventional culture.[50,51] The results of the PCR assay are expressed as a cycle threshold value (Ct); the lower the Ct value, the greater the amount of DNA of the specific pathogen being detected is in the sample and thus the greater the likelihood of a true positive diagnosis. Generally, the cutoff for a positive result is a Ct value of 37.0.[52]

Commercial PCR assays are available for detecting mastitis-causing pathogen DNA in mammary quarter milk samples, cow-level composite milk samples, and bulk tank milk samples. Bulk tank PCR assays can be used in the same way as bulk tank cultures as an indicator of udder health, milking time hygiene, and storage conditions on the farm. Additionally, application of PCR to bulk tank samples can be used to monitor bacteria with low prevalence, such as *S agalactiae*.[53] With that in mind, when using comingled milk (eg, bulk tank or pen) samples, it is recommended to only test for contagious pathogens (such as *S aureus*, *S agalactiae*, and *Mycoplasma* spp.) because there is a high probability that these bacteria originated from the mammary gland.[54]

Commercial PCR tests have been used on cow-level samples collected using an in-line sampling device (such as those used by Dairy Herd Information Association to collect monthly SCC samples). However, it must be remembered that these are not aseptically collected samples and are prone to risk of false-positive results because of teat skin contaminants, contaminated teat orifices, contaminated equipment, and carryover of contaminated milk from other cows.[55,56] Carryover can occur owing to residual milk in the unit, meter, or sampler. Carryover has been found to affect the PCR results for *S aureus* and *S agalactiae* diagnosis. Based on these data, modified cut points have been recommended for *S aureus* diagnosis when using in-line composite samples, with a Ct value of less than 32 being very likely to be infected, a Ct value of greater than 37 very likely to be IMI negative, and a Ct value of 32 to 37 being of undetermined status.[55]

The pros and cons of PCR compared with culture must be acknowledged (**Table 1**). Some concerns with PCR assays include the fact that they only detect the target species that are included in the PCR, which is based on the primer sets included with that

Table 1
Comparison of conventional bacterial culture and PCR-based approaches to diagnosing intramammary infection

	Bacteriologic Culture	PCR
Detects	Bacterial colonies	Bacterial DNA
Diagnostic threshold	CFU/mL	Ct
Live organism	Yes	Not necessarily
Virulence factor detection	Limited (eg, hemolysins)	If PCR primers are included
Factors influencing Se	Growth media and conditions; incubation time; CFU/mL detection threshold/inoculum volume; interpreter	Included primers, primer specificity; Ct threshold
Factors influencing Sp	Contaminated sample; CFU/mL threshold/inoculum volume; interpreter	Contaminated sample; Ct threshold; detection of DNA from nonviable bacteria, primer specificity; carryover when using in-line sampler
Time to result	24 h – 10 d	4 h
Cost	Low	Currently, 4–5 × conventional culture

Abbreviations: CFU, colony forming units; Ct, cycle threshold; PCR, polymerase chain reaction; Se, sensitivity; SP, specificity.

From Middleton JR, Fox LK, Pighetti G, et al. The laboratory handbook on bovine mastitis. Reprinted with permission from the National Mastitis Council Inc., New Prague, Minnesota, USA, 2017. NMC is a not-for-profit organization that provides a forum for the global exchange of information on mastitis control and milk quality. Available at: www.nmconline.org.

specific multiplex kit. There are no guidelines for how to report multispecies results. Additionally, PCR can detect DNA from dead bacteria. In an experimental challenge trail, PCR detected *Staphylococcus* spp. DNA for several days after the bacteria was no longer detected with conventional culture.[57] It is unknown if these were truly dead cells, if the milk contained growth inhibitors preventing bacterial growth on agar, or the bacterial load had dropped below the detection limit of conventional culture, that is, less than 100 CFU/mL.[57] These data are important to consider if PCR is being used as a follow-up test to assess response to treatment. Based on results of Hiitio and co-workers,[57] it is recommend waiting at least 2 to 3 weeks after the onset of mastitis or treatment or until the quarter milk SCC returns to normal levels before using PCR to assess response to treatment.

SUMMARY

The diagnosis of mastitis is generally based on clinical observations or direct or indirect measures of the inflammatory response to infection, whereas the diagnosis of an IMI is based on identification of the infectious agent. Mastitis can be characterized as clinical or subclinical, with subclinical being more common and more challenging to diagnose. SCC or SCS are the most common diagnostic tests used for the detection of subclinical mastitis. Both culture and PCR can be useful in the diagnosis of an IMI; however, both have their advantages and disadvantages. Diagnosing the bacterial agent causing the IMI can help to determine treatment and prevention strategies on the farm, which in turn can help to decrease the incidence and prevalence of mastitis.

REFERENCES

1. Ruegg PL. Managing mastitis and producing quality milk. In: Risco CA, Melendez PR, editors. Dairy production medicine. West Sussex (Shire): John Wiley & Sons; 2011. p. 207–32.
2. Wenz JR, Barrington GM, Garry FB, et al. Use of systemic disease signs to assess disease severity in dairy cows with acute coliform mastitis. J Am Vet Med Assoc 2001;218(4):567–72.
3. Sordillo LM, Shafer-Weaver K, DeRosa D. Immunobiology of the mammary gland. J Dairy Sci 1997;80(8):1851–65.
4. Kitchen BJ. Review of the progress of dairy science: bovine mastitis: milk compositional changes and related diagnostic tests. J Dairy Res 1981;48(1):167–88.
5. Fox LK, Hancock DD, McDonald JS, et al. N-acetyl-beta-D-glucosaminidase activity in whole milk and milk fractions. J Dairy Sci 1988;71(11):2915–22.
6. Kitchen BJ, Middleton G, Salmon M. Bovine milk N-acetyl-beta-D-glucosaminidase and its significance in the detection of abnormal udder secretions. J Dairy Res 1978;45(1):15–20.
7. Kalmus P, Simojoki H, Pyorala S, et al. Milk haptoglobin, milk amyloid A, and N-acetyl-beta-D-glucosaminidase activity in bovines with naturally occurring clinical mastitis diagnosed with a quantitative PCR test. J Dairy Sci 2013;96(6):3662–70.
8. McDermott MP, Erb HN, Natzke RP. Predictability by somatic cell counts related to prevalence of intrammary infection within herds. J Dairy Sci 1982;65(8):1535–9.
9. Reksen O, Solverod L, Osteras O. Relationships between milk culture results and composite milk somatic cell counts in Norwegian dairy cattle. J Dairy Sci 2008; 91(8):3102–13.
10. Jashari R, Piepers S, De Vliegher S. Evaluation of the composite milk somatic cell count as a predictor of intramammary infection in dairy cattle. J Dairy Sci 2016; 99(11):9271–86.

11. Schukken YH, Wilson DJ, Welcome F, et al. Monitoring udder health and milk quality using somatic cell counts. Vet Res 2003;34(5):579–96.
12. Djabri B, Bareille N, Beaudeau F, et al. Quarter milk somatic cell count in infected dairy cows: a meta-analysis. Vet Res 2002;33(4):335–57.
13. Middleton JR, Hardin D, Steevens B, et al. Use of somatic cell counts and California mastitis test results from individual quarter milk samples to detect subclinical intramammary infection in dairy cattle from a herd with a high bulk tank somatic cell count. J Am Vet Med Assoc 2004;224(3):419–23.
14. Schepers AJ, Lam TJ, Schukken YH, et al. Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. J Dairy Sci 1997;80(8):1833–40.
15. Brolund L. Cell counts in bovine milk. Causes of variation and applicability for diagnosis of subclinical mastitis. Acta Vet Scand Suppl 1985;80:1–123.
16. Harmon RJ. Physiology of mastitis and factors affecting somatic cell counts. J Dairy Sci 1994;77(7):2103–12.
17. Walsh S, Buckley F, Berry DP, et al. Effects of breed, feeding system, and parity on udder health and milking characteristics. J Dairy Sci 2007;90(12):5767–79.
18. Rodrigues AC, Cassoli LD, Machado PF, et al. Short communication: evaluation of an on-farm test to estimate somatic cell count. J Dairy Sci 2009;92(3):990–5.
19. Kandeel SA, Megahed AA, Arnaout FK, et al. Evaluation and comparison of 2 on-farm tests for estimating somatic cell count in quarter milk samples from lactating dairy cattle. J Vet Intern Med 2018;32(1):506–15.
20. Kandeel SA, Megahed AA, Constable PD. Comparison of six on-farm tests to estimate somatic cell count at dry-off in dairy cattle. Paper presented at: American College of Veterinary Internal Medicine Forum 2017. National Harbor, MD, June 8-9, 2017. p.1358.
21. Dingwell RT, Leslie KE, Schukken YH, et al. Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. Can Vet J 2003;44(5):413–5.
22. Sanford CJ, Keefe GP, Sanchez J, et al. Test characteristics from latent-class models of the California Mastitis Test. Prev Vet Med 2006;77(1–2):96–108.
23. Nyman AK, Persson Waller K, Bennedsgaard TW, et al. Associations of udder-health indicators with cow factors and with intramammary infection in dairy cows. J Dairy Sci 2014;97(9):5459–73.
24. 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(2):1440–8.
25. Jaeger S, Virchow F, Torgerson PR, et al. Test characteristics of milk amyloid A ELISA, somatic cell count, and bacteriological culture for detection of intramammary pathogens that cause subclinical mastitis. J Dairy Sci 2017;100(9):7419–26.
26. Jensen NE, Knudsen K. Interquarter comparison of markers of subclinical mastitis: somatic cell count, electrical conductivity, N-acetyl-beta-glucosaminidase and antitrypsin. J Dairy Res 1991;58(4):389–99.
27. Pyorala S. Indicators of inflammation in the diagnosis of mastitis. Vet Res 2003; 34(5):565–78.
28. Ebrahimie E, Ebrahimi F, Ebrahimi M, et al. A large-scale study of indicators of sub-clinical mastitis in dairy cattle by attribute weighting analysis of milk composition features: highlighting the predictive power of lactose and electrical conductivity. J Dairy Res 2018;85(2):193–200.
29. Middleton JR, Fox LK, Pighetti G, et al. Laboratory handbook on bovine mastitis. New Prague (MN): National Mastitis Council; 2017.

30. Dohoo IR, Smith J, Andersen S, et al. Diagnosing intramammary infections: evaluation of definitions based on a single milk sample. J Dairy Sci 2011;94(1):250–61.
31. Dohoo I, Andersen S, Dingwell R, et al. Diagnosing intramammary infections: comparison of multiple versus single quarter milk samples for the identification of intramammary infections in lactating dairy cows. J Dairy Sci 2011;94(11): 5515–22.
32. Reyher KK, Dohoo IR. Diagnosing intramammary infections: evaluation of composite milk samples to detect intramammary infections. J Dairy Sci 2011;94(7):3387–96.
33. Patel JB. 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. Mol Diagn 2001;6(4):313–21.
34. Becker K, Harmsen D, Mellmann A, et al. Development and evaluation of a quality-controlled ribosomal sequence database for 16S ribosomal DNA-based identification of *Staphylococcus* species. J Clin Microbiol 2004;42(11):4988–95.
35. Drancourt M, Raoult D. *rpoB* gene sequence-based identification of *Staphylococcus* species. J Clin Microbiol 2002;40(4):1333–8.
36. Hwang SM, Kim MS, Park KU, et al. Tuf gene sequence analysis has greater discriminatory power than 16S rRNA sequence analysis in identification of clinical isolates of coagulase-negative staphylococci. J Clin Microbiol 2011;49(12): 4142–9.
37. Poyart C, Quesne G, Boumaila C, et al. Rapid and accurate species-level identification of coagulase-negative staphylococci by using the *sodA* gene as a target. J Clin Microbiol 2001;39(12):4296–301.
38. Ghebremedhin B, Layer F, Konig W, et al. Genetic classification and distinguishing of *Staphylococcus* species based on different partial gap, 16S rRNA, *hsp60, rpoB, sodA*, and *tuf* gene sequences. J Clin Microbiol 2008;46(3):1019–25.
39. Shah MM, Iihara H, Noda M, et al. *dnaJ* gene sequence-based assay for species identification and phylogenetic grouping in the genus *Staphylococcus*. Int J Syst Evol Microbiol 2007;57(Pt 1):25–30.
40. Kwok AY, Su SC, Reynolds RP, et al. Species identification and phylogenetic relationships based on partial HSP60 gene sequences within the genus *Staphylococcus*. Int J Syst Bacteriol 1999;49(Pt 3):1181–92.
41. Cameron M, Barkema HW, De Buck J, et al. Identification of bovine-associated coagulase-negative staphylococci by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a direct transfer protocol. J Dairy Sci 2017;100(3):2137–47.
42. Cameron M, Perry J, Middleton JR, et al. Short communication: evaluation of MALDI-TOF mass spectrometry and a custom reference spectra expanded database for the identification of bovine-associated coagulase-negative staphylococci. J Dairy Sci 2018;101(1):590–5.
43. Goncalves JL, Tomazi T, Barreiro JR, et al. Identification of *Corynebacterium* spp. isolated from bovine intramammary infections by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Vet Microbiol 2014;173(1–2):147–51.
44. Barreiro JR, Goncalves JL, Braga PAC, et al. Non-culture-based identification of mastitis-causing bacteria by MALDI-TOF mass spectrometry. J Dairy Sci 2017; 100(4):2928–34.
45. Ruegg PL. On-farm diagnosis of mastitis infections and organisms. Paper presented at: National Mastitis Council Regional Meeting 2005. Burlington, VT, July 12-13, 2005. p. 24-30.
46. Godden S, Lago A, Bey R, et al. Dingwell. Use of on-farm culture systems in mastitis control programs. Paper presented at: National Mastitis Council Regional Meeting 2007. Visalia, CA, May 22-23, 2007. p. 1–9.

47. Cameron M, Keefe GP, Roy JP, et al. Evaluation of a 3M Petrifilm on-farm culture system for the detection of intramammary infection at the end of lactation. Prev Vet Med 2013;111(1–2):1–9.
48. Royster E, Godden S, Goulart D, et al. Evaluation of the Minnesota Easy Culture System II Bi-Plate and Tri-Plate for identification of common mastitis pathogens in milk. J Dairy Sci 2014;97(6):3648–59.
49. 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(12):5707–15.
50. Taponen S, Salmikivi L, Simojoki H, et al. Real-time polymerase chain reaction-based identification of bacteria in milk samples from bovine clinical mastitis with no growth in conventional culturing. J Dairy Sci 2009;92(6):2610–7.
51. Bexiga R, Koskinen MT, Holopainen J, et al. Diagnosis of intramammary infection in samples yielding negative results or minor pathogens in conventional bacterial culturing. J Dairy Res 2011;78(1):49–55.
52. Koskinen MT, Holopainen J, Pyorala S, et al. Analytical specificity and sensitivity of a real-time polymerase chain reaction assay for identification of bovine mastitis pathogens. J Dairy Sci 2009;92(3):952–9.
53. 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(10):5702–8.
54. Kelton DF, Godkin MA. Frequently asked questions about the Pathoproof mastitis 3 PCR assay. Paper presented at: NMC 53rd Annual Meeting 2014. Fort Worth, TX, January 26-28, 2014. p. 127–32.
55. Mahmmod YS, Klaas IC, Enevoldsen C. DNA carryover in milk samples from routine milk recording used for PCR-based diagnosis of bovine *Staphylococcus aureus* mastitis. J Dairy Sci 2017;100(7):5709–16.
56. Mahmmod YS, Mweu MM, Nielsen SS, et al. Effect of carryover and presampling procedures on the results of real-time PCR used for diagnosis of bovine intramammary infections with *Streptococcus agalactiae* at routine milk recordings. Prev Vet Med 2014;113(4):512–21.
57. Hiitio H, Simojoki H, Kalmus P, et al. The effect of sampling technique on PCR-based bacteriological results of bovine milk samples. J Dairy Sci 2016;99(8):6532–41.