Mastitis diagnostics and performance monitoring: a practical approach

Lam TJGM [1,2], Olde Riekerink RGM[2], Sampimon OC[2] and Smith H[3]

[1] Dutch Udder Health Centre, PO Box 2030, 7420 AA Deventer, The Netherlands
[2] GD Animal Health Service, PO Box 9, 7400 AA Deventer, The Netherlands
[3] ASG-Wageningen UR, Dept. of Infectious Diseases, PO Box 65, 8200 AB Lelystad, The Netherlands

ABSTRACT

In this paper a review is given of frequently used mastitis diagnostic methods in modern dairy practice. Methods used at the quarter, cow, herd and regional or national level are discussed, including their usability for performance monitoring in udder health. Future developments, such as systems in which milk-derived parameters are combined with modern analytical techniques, are discussed. It is concluded that, although much knowledge is available and science is still developing and much knowledge is available, it is not always fully exploited in practice.

KEYWORDS: bacteriological culturing (BC); diagnosis; mastitis control; monitoring; somatic cell count (SCC)

CORRESPONDING AUTHOR:

Theo Lam
Dutch Udder Health Centre, PO Box 2030, 7420 AA Deventer, The Netherlands
E-mail: t.lam@gddeventer.com

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INTRODUCTION

Worldwide, much effort is put into mastitis research, knowledge-transfer programmes, herd health advisery programmes, and cow-based ambulatory work. Because healthy udders are economically profitable (Halasa *et al.* 2007), lead to a better quality product (Ma *et al.* 2000), and better cow welfare (Ekman and Sandgren 2006); all these programmes have the intention of ultimately improving udder health. To be able to judge whether these activities are successful, performance has to be monitored. Many possibilities exist to monitor udder health performance, which are not always fully exploited. In this review, mastitis diagnostics that are essential in performance monitoring are discussed.

Monitoring udder health performance is impossible without reliable and affordable diagnostic methods. Thus, there is a constant need to improve these methods, be it accuracy, cost price, or convenience. The most frequently used diagnostic methods are somatic cell counting (SCC) and bacteriological culturing (BC) of milk. Currently, methods such as measurement of N-acetyl-b-D-glucosaminidase (NAG-ase), lactate dehydrogenase (LDH), electrical conductivity (EC), and molecular methods such as polymerase chain reaction (PCR) technology are used less frequently. The latter has promising possibilities for future applications, especially in strain identification (Zadoks and Schukken 2006).

CLINICAL DIAGNOSIS

Mastitis diagnosis starts with visual observation. The role of the milker herein is undisputedly very important. It is generally known that large differences exist between farms in clinical mastitis diagnosis (Lam *et al.* 1993). This can have two causes – differences in farmers' definitions of clinical mastitis or differences in observing and identifying clinical cases. The latter is greatly improved by forestripping, which is also an important part of udder preparation (Reneau 2001). For a good clinical diagnosis, it is essential to be able to see abnormalities, for which you need sufficient light (at least 250 lux) underneath the udder, where teat cups are attached (Hulsen and Lam 2007). In the field of udder health, definitions agreed upon by the National Mastitis Council (1996) ought to be followed. If there is any detectable change in quarter and/

or any observable abnormality in the milk, the quarter is defined as having clinical mastitis. It is wise not to ignore clinical symptoms. Abnormal milk, even the smallest clot is a signal that something is wrong within that quarter. It should be noticed and action should be taken accordingly.

SOMATIC CELL COUNT

SCCs have been known to be an important indicator of intramammary infections (IMI) for years (Schukken *et al.* 2003). An easy, cheap, and quick cow-side test to estimate SCC on farm is the California Mastitis Test (CMT). It is a semi-quantitative SCC measure by forming a stringy mass from the reagent (3% sodium lauryl sulphate) and DNA out of disrupted cells. Although the CMT test is not very complicated, it is not always executed correctly in practice. As a consequence, its usability is sometimes questioned. For practical guidelines the reader should refer to Hulsen and Lam (2007). Although the CMT has been proven to be valuable for over 40 years (Luedecke *et al.* 1967), results obtained from fresh cows are difficult to interpret and CMT should not be used to detect pathogens in milk from cows earlier than four days postcalving (Sargeant *et al.* 2001; Dingwell *et al.* 2003). The CMT can be valuable, for instance, to assess therapy success based on SCC estimation after treatment in clinically recovered cows, or to identify the affected quarter within a cow with elevated SCC. Direct SCC measurement, of course, is much more accurate, but more expensive and not always available cow-side. If SCC has to be measured in large numbers of samples, they are generally sent to a laboratory using high capacity cell counters based on the flow cytometry principle, such as the Fossomatic cell counter. This is a reliable way to measure SCC (Miller *et al.* 1986). Nowadays, tests such as Porta SCC and the DeLaval Cell Counter are available, which seem to be accurate on-farm methods of estimating SCC (Barratt *et al.* 2003). Interpretation of SCC is sometimes difficult, because it is a variable parameter that is influenced by many factors, such as diurnal variation (Olde Riekerink *et al.* 2007), stage of lactation, parity, and fraction of the milk sampled (Sarikaya and Bruckmaier 2006). The most important factor influencing SCC, however, is bacterial IMI (Dohoo and Meek 1982). Milk samples for SCC measurement should be taken immediately before milking, after removing three squirts of milk. Because of the variability of SCC, for monitoring infection dynamics longitudinal data are necessary, which should be based on multiple test results (Schukken *et al.* 2003). In literature many different thresholds are used to differentiate 'healthy' from 'unhealthy' quarters or udders. The SCC in truly uninfected quarters does not exceed approximately 70,000 cells/ml, slightly increasing with age and lactation days (Schepers *et al.* 1997). Optimal sensitivity and specificity of SCC at a threshold of 200,000 cells/ml as indicator of presence of IMI are estimated at 73% and 86%, respectively (Dohoo and Leslie 1991). Thus, an operational threshold of practical value with minimal diagnostic error, 200,000 cells/ml, was proposed by Schukken *et al.* (2003).

BACTERIOLOGICAL CULTURING

Bacteriological culturing can be executed at herd, as well as cow and quarter level, each with its own specific goal. Bacteriological culturing is most often used as a diagnostic tool to solve mastitis problems. Knowledge on the infectious status of mammary glands, however, can also be very helpful to prevent transmission of pathogens by diagnosing a reservoir at an early stage. Additionally, historical BC results give herd-based information that can be helpful in optimising the treatment of future mastitis cases.

Bulk milk BC, used for diagnostic purposes has been reviewed by Jayarao *et al.* (2004). They described that environmental mastitis pathogens found in bulk milk may also be derived from non-specific contamination from cow skin, bedding, manure, or water. Contagious pathogens, such as *Staphylococcus aureus* and *Streptococcus agalactiae* generally do come from IMI, but due to dilution, latent infections, and intermittent shedding, sensitivity of a single bulk milk BC is low. Successive samples over a period of time, however, are considered as a good indicator for these infections in the herd (Jayarao *et al.* 2004). Sensitivity of BC of a single bulk milk sample for *Mycoplasma* spp. likewise, is not a perfect test (Biddle *et al.* 2003). In regions where the prevalence of Mycoplasma is high, however, routine testing for *Mycoplasma* spp. is recommended (Kirk *et al.* 1997).

To judge IMI at the cow level, composite milk samples have been used for a long time (Williams 1937). This relatively cheap method is still used frequently and can be an important source of information. Test characteristics of composite samples for detection of *Strep. agalactiae* (Dinsmore *et al.* 1991) and *Mycoplasma* spp. (Biddle *et al.* 2003) are good. Approximately 40% of quarters infected with *Staph. aureus*, however, will not be cultured from composite milk samples (Lam *et al.* 1996). In situations where one is interested in culture results of staphylococci or environmental streptococci, a selection of quarters for culturing, based on SCC or CMT, is probably more cost-effective and reliable than the use of composite samples. At the quarter level, approximately 10-40% of milk samples from cases of clinical mastitis yield no growth. This may be due to several reasons (i.e., too few or no bacteria present or pathogens that require special culture techniques such as *Mycoplasma* spp.). In subclinical mastitis, latent infections or shedding cycles may also play a role (Sears *et al.* 1990). Through the years several methods, such as preculture incubation, preculture freezing, and increased plate inoculation volumes were tested trying to increase the recovery rate of pathogens. Increasing the inoculation volume to 0.1 ml significantly increased sensitivity (Lam *et al.* 1996), as did preincubation for four hours (Dinsmore *et al.* 1992). Schukken *et al.* (1989) found that freezing milk before culturing increased only the number of coagulase negative staphylococci found, whereas Dinsmore *et al.* (1992) showed a significantly higher positive overall culture rate. In general, these methods do increase the number of positive cultures, but it has to be realised that the growth

promotion also affects possible contaminants in the milk. For clinical mastitis diagnosis, it is important that BC results are available as soon as possible to optimise treatment results, to save costs and to prevent the ineffective use of antibiotics. For that reason, several commercial on-farm culturing systems such as the Minnesota Easy Culture System II and the Petrifilm system became available during recent years. Both are user-friendly systems, applying milk with a sterile cotton swab or a plastic pipette, and have test characteristics (compared to traditional culture methods) that are good enough to make it a useful management tool. Reading results from the Petrifilm system, however, needs some more experience and ability than reading the Minnesota system (Godden *et al.* 2007).

SELECTING COWS FOR BACTERIOLOGICAL CULTURING

To effectively use bacteriological culturing as a diagnostic tool, milk samples have to be collected from the correct cows and quarters at the correct point in time. As discussed above, most information is gathered if samples are collected at the quarter level. The most practical approach to select cows is to use cow-level SCC information. To select quarters, either CMT or quarter-level SCC can be used. SCC patterns are, to some extent, related to the type of mastitis causing pathogen (de Haas *et al.* 2004). Some IMI are of short duration, and thus of limited importance to the farmer. On the other hand, subclinical infections can have shedding patterns and thus may not always be diagnosed in a single BC sample (Sears *et al.* 1990). Ideally, subsequent quarter samples are collected to increase diagnostic properties. However, that approach is time consuming and expensive, and therefore not very practical. Comparing bacteriological data from single samples to the actual situation of those quarters based on subsequent samples (gold standard) in five dairy herds showed that predictive values of a single test increased when SCC information was used to select cows (Lam and Schukken 2005). The probability that a *Staph. aureus* infected cow will still be infected a month after initial sampling is twice as high in a cow that has had a SCC > 200,000 cells/ml repeatedly, compared with all cows with SCC > 200,000 cells/ml (**Table 1**). Thus, for approaching mastitis problems at the herd level, it seems wise to select cows with at least two consecutive samples with elevated SCC, measured on a monthly basis.
In addition to SCC information, measuring NAG-ase can be informative in selecting samples for BC. In a study of 10 commercial dairy herds, Berning and Shook (1992) showed that, after selection of infected quarters based on SCC, the log NAG-ase was more effective in identifying major from minor pathogen infections. Thus, information on NAG-ase may be helpful in selecting IMI with major pathogens.

Table 1: Validity of single quarter samples in diagnosing intramammary *Staph. aureus* infections (95% confidence interval between brackets)

	All cows	Cows with SCC >200,000 cells/ml	Cows with SCC repeatedly > 200,000 cells/ml
Sensitivity	0.92 (0.90; 0.95)	0.91 (0.88; 0.95)	0.90 (0.86; 0.95)
Specificity	0.98 (0.98; 0.98)	0.87 (0.86; 0.89)	0.97 (0.97; 0.98)
Pred. Value Pos.	0.54 (0.51; 0.58)	0.41 (0.38; 0.46)	0.82 (0.78; 0.88)
Pred. Value Neg.	0.99 (0.99; 1.00)	0.99 (0.99; 0.99)	0.98 (0.98; 0.99)

CLINICAL MASTITIS IN RELATION TO INTRAMAMMARY INFECTIONS

Clinical mastitis is the most costly form of mastitis, due to discarded milk, treatment, and other costs (Halasa *et al.* 2007). Subclinical mastitis leads to production losses, can be a source of new infections (Lam *et al.* 1996a), and is also related to the occurrence of clinical mastitis cases. In a study in seven low bulk milk SCC herds, IMI status of all quarters, as well as clinical mastitis cases were monitored in detail during a period of 20 months (Lam 1996). In these herds, approximately 40% of IMI never showed clinical signs (**Table 2**). Conversely, approximately 50% of clinical mastitis cases in these herds were part of chronic IMI, indicating that, even in herds with low SCC, clinical mastitis is to some extent associated with subclinical IMI. It is likely that this relation is even stronger in herds with high SCC.

MOLECULAR METHODS FOR DIAGNOSTIC PURPOSES

The use of molecular methods in pathogen detection has increased over the last years. Often, these methods use polymerase chain reaction (PCR) technology. Testing the presence of a specific bacterial species, a part of the DNA of that pathogen is amplified and subsequently visualised. For a number of mastitis pathogens, PCR-based techniques have been described (Lee *et al.* 1998; Baird *et al.* 1999; Hassan *et al.* 2001; Daly *et al.* 2002). These methods are currently very labour-intensive and it is expensive to do a separate PCR test for every possible mastitis pathogen. For that reason, multiplex PCR tests are of interest, in which several pathogens can be tested at the same time (Phuektes *et al.* 2003; Bottero *et al.* 2004).
Additionally, real-time PCR assays are being developed (Lightcycler, Taqman, Luminex, Biacore) for detection and quantifying mastitis pathogens in milk.
Molecular methods can also be used to differentiate bacterial strains within one bacterial species. These differences may be of importance, because they may be associated with differences in virulence, epidemiology, and cure rates. For these purposes, phenotypic characteristics,

Table 2: Number of intramammary infections (IMI) with clinical signs in seven dairy herds during a 20 month period

	Number of IMI	Number of IMI with clinical signs
E. coli	105	99 (94%)
Staph. aureus	171	62 (36%)
Strep. dysgalactiae	80	52 (65%)
Strep. uberis	79	49 (62%)

such as phage types, serotypes, and antibiotic sensitivity patterns can be used. Another possibility is to test for differences in strains by genotyping (fingerprinting) their genome. In epidemiological research, these methods are frequently used (Zadoks and Schukken 2006). Several molecular methods, such as PFGE (pulse field gel electrophoresis), ribotyping, RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), and MLST (multi locus sequence typing) are used for genotyping. Generally, these methods use DNA that became available after digestion with restriction enzymes, after amplification with PCR techniques, DNA sequence analyses, or combinations of these techniques. For a review of these techniques, see Zadoks and Schukken (2006). In most countries PCR technology is not available for routine use in mastitis diagnostics. Recently more information became available on a commercial multiplex PCR test performed directly on milk, which is available for routine use (Koskinen *et al.* 2008).

FUTURE DEVELOPMENTS

Numerous different techniques to diagnose mastitis have been developed over the years. Electrical conductivity of milk is not a new method, but so far, using the test by itself, its characteristics, especially specificity, are not that good (Nielen *et al.* 1992), and its use on farm has been limited. New developments, such as the use of fuzzy logic (de Mol and Woldt 2001), and an approach through Bayesian Networks (Steeneveld *et al.* 2008) enhance test characteristics. To improve these, information from other sources is necessary to distinguish healthy cows, cows with subclinical, and cows with clinical mastitis (Norberg *et al.* 2004). Combining different types of milk-derived data, like yield, temperature and EC of milk, can be used for automated cow status monitoring. This can overcome problems like the occurrence of many false-positive alerts, when EC is used as a single parameter (Nielen *et al.* 1992). The inclusion of additional information like LDH, but also herd and udder characteristics, leads to early diagnosis of clinical mastitis cases (Chagunda *et al.* 2006). This methodology has been developed and is currently being tested in a number of commercial dairy farms in Denmark (Blom and Nielsen 2008). If it actually proves to be able to predict the occurrence of clinical mastitis a few days ahead (Friggens *et al.* 2007), it may be a step forward in mastitis diagnosis.

PERFORMANCE MONITORING

The diagnostic methods discussed above, can be used to monitor udder health performance at the cow and the herd level, as well as the regional or national level.

To judge treatment outcome, the level of judgement is the quarter or the cow. In research, generally BC is used for this purpose, sometimes supplemented with SCC. Although they are of great importance, cure-rates are hardly evaluated quantitatively in daily practice. Because information concerning cure rates gives the possibility to really judge treatment success in a herd, the use of these data by farmers and practitioners should be advocated. Ideally, all clinical mastitis cases are sampled and results are used to optimise treatment of individual cows. If that is not practised, it is still recommended to collect samples of all cases of clinical mastitis, and store them (correctly identified) in a freezer. They can, if required, be cultured later. A general recommendation is to make up the bacteriological udder health status of a herd at least once a year, or more frequent if needed. For such a bacteriological udder health status, a selection of at least 10 high SCC cows and 10 clinical mastitis cows should be cultured using quarter milk samples (Lam *et al.* 2008). Based on the bacteriological information, combined with the evaluation of recent treatments, a standard treatment protocol for the forthcoming period can then be developed.

At the herd level, udder health performance can be judged based on information on clinical mastitis and SCC, preferably supplemented with BC. This information is even more important than data on cure rates, because it is highly correlated with general udder health management. Based on the results of the previous year, realistic goals for SCC can be set for the next year, followed by an action plan, including a timeline. Estimates of the annual economic impact of mastitis for the herd can be used to motivate the farmer and prioritize actions.

Performance of knowledge-transfer projects can also be monitored. The easiest approach is to measure bulk milk SCC, but clinical mastitis incidence at herd level and the effect on the use of antibiotics can also become available (Green *et al.* 2007).

Finally, udder health performance can be measured on a regional or a national basis. Again, bulk milk SCC is the easiest parameter, which is monitored in many countries. Clinical mastitis incidence, antibiotic use, BC, and antibiotic sensitivity patterns of cultured bacteria, however, are much more informative than SCC data. In Scandinavian countries especially, valuable data on this subject are available and used, trying to further improve udder health in these countries (Østerås *et al.* 2007).

CONCLUSIONS

Much knowledge in the field of mastitis diagnostics is available and science is still developing. Several diagnostic methods are available to monitor the effect of interventions in udder health, but these are not always fully exploited. At the cow level, cure rate after therapy is measurable, but hardly quantified. Clinical diagnosis, together with SCC measurement and BC are the most frequently used parameters for this aim. At the herd level, goal setting based on available retrospective data could be practised more often. The same parameters can be used, supplemented with SCC and bulk milk BC. Future developments, such as systems in which several milk-obtained parameters such as EC and LDH are combined, may lead to changes in this field in the near future.

At the regional or national level, only limited data are presented in scientific publications, whereas these data are often available and informative on the effect of certain

approaches chosen in a certain area.

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REFERENCES

Baird SC, Carman J , Dinsmore RP et al. (1999) Detection and identification of Mycoplasma from bovine mastitis infections using a nested polymerase chain reaction. Journal of Veterinary Diagnostic Investigation 11, 432-435.

Barratt K, Leslie K and Bashiri A (2003) An evaluation of the Porta SCCTM for determining udder health status in dairy cows at drying off. Proceedings NMC 42nd annual meeting 280-281.

Berning LM, Shook GE (1992) Prediction of mastitis using milk somatic cell count, N-acetyl-ß-D-glucosaminidase, and lactose. Journal of Dairy Science 75, 1840-1848.

Biddle MK, Fox LK and Hancock DD (2003) Patterns of Mycoplasma shedding in the milk of dairy cows with intramammary Mycoplasma infection. Journal of the American Veterinary Medical Association 223, 1163-1166.

Blom JY, Nielsen LAH (2008) A novel in-line system to manage mastitis, ketosis, reproduction and feeding in dairy herds. Proceedings Mastitis Control 2008, The Hague, The Netherlands 297.

Bottero MT, Dalmasso A, Soglia D et al. (2004) Development of a multiplex PCR assay for the identification of pathogenic genes of *Escherichia coli* in milk and milk products. Molecular and Cellular Probes 18, 283-288.

Chagunda MGC, Friggens NC, Rasmussen MD et al. (2006) A model for detection of individual cow mastitis based on an indicator measured in milk. Journal of Dairy Science 89, 2980-2998.

Daly P, Collier T and Doyle S (2002) PCR-ELISA detection of *Escherichia coli* in milk. Letters in Applied Microbiology 34,222-226.

Dingwell RT, Leslie KE, Schukken YH et al. (2003) Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation. Canadian Veterinary Journal 44, 413-415.

Dinsmore RP, English PB, Gonzalez RN et al. (1991) Evaluation of methods for the diagnosis of *Streptococcus agalactiae* intramammary infections in dairy cattle. Journal of Dairy Science 74, 1521-1526.

Dinsmore RP, English PB, Gonzalez RN et al. (1992) Use of augmented cultural techniques in the diagnosis of the bacterial cause of clinical bovine mastitis. Journal of Dairy Science 75, 2706-2712.

Dohoo IR, Meek AH (1982) Somatic Cell Counts in Bovine milk. Canadian Veterinary Journal 23, 119-125.

Dohoo IR, Leslie KE (1991) Evaluation of changes in somatic cell counts as indicators of new intramammary infections. Preventive Veterinary Medicine 10, 225-237.

Ekman T, Sandgren CH (2006) An overview of animal welfare and mastitis. Proceedings NMC 45th annual meeting 122-129.

Friggens NC, Chagunda MGG, Bjerring M et al. (2007) Estimating degree of mastitis from time-series measurements in milk: a test of a model based on lactate dehydrogenase measurements. Journal of Dairy Science 90, 5415-5427.

Godden S, Lago A, Bey R et al. (2007) Use of on-farm culture systems in mastitis control programmes. Proceedings NMC regional meeting 1-9.

Green MJ, Leach KA, Breen JE et al. (2007) National intervention study of mastitis control in dairy herds in England and Wales. Veterinary Record 160, 287-293.

de Haas Y, Veerkamp RF, Barkema HW et al. (2004) Associations between pathogen-specific cases of clinical mastitis and somatic cell count patterns. Journal of Dairy Science 87, 95-105.

Halasa T, Huijps K, Østerås O et al. (2007) Economic effects of bovine mastitis and mastitis management: a review. Veterinary Quarterly 29,18-31.

Hassan AA, Khan IU, Abdulmawjood A et al. (2001) Evaluation of PCR methods for rapid identification and differentiation of *Streptococcus uberis* and *Streptococcus parauberis*. Journal of Clinical Microbiology 39, 1618-1621.

Hulsen JHJL, Lam TJGM (2007) Udder health: a practical guide to first-rate udder health. ISBN 9789087400149.

Jayarao BM, Pillai SR, Sawant AA et al. (2004) Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. Journal of Dairy Science 87, 3561-3573.

Kirk JH, Glenn K, Ruiz L et al. (1997) Epidemiologic analysis of *Mycoplasma* spp. isolated from bulk tank milk samples obtained from dairy herds that were members of a milk cooperative. Journal of the American Veterinary Medical Association 211, 1036-1038.

Koskinen MT, Holopainen J, Salmikivi L et al. (2008) Analytical detection limit of the Pathoproof mastitis PCR assay using two different experimental approaches. Proceedings Mastitis Control 2008, The Hague, The Netherlands 183-189.

Lam TJGM, Schukken YH, Grommers FJ et al. (1993) Within-herd and between-herd variation in diagnosis of clinical mastitis in cattle. Journal of the American Veterinary Medical Association 202, 938-942.

Lam TJGM (1996) Dynamics of bovine mastitis. Ph.D. thesis, Utrecht University.

Lam TJGM, van Wuijckhuise LA, Franken P et al. (1996) Use of composite milk samples for diagnosis of *Staphylococcus aureus* mastitis in dairy cattle. Journal of the American Veterinary Medical Association 208, 1705–1708.

Lam TJGM, de Jong MCM, Schukken YH et al. (1996a) Mathematical modeling to estimate efficacy of postmilking teat disinfection in split-udder trials of dairy cows. Journal of Dairy Science 79, 62-70.

Lam TJGM, Schukken YH (2005) Testing for *Staphylococcus aureus* in herds with a low bulk milk somatic cell count. Proceedings 4th IDF Mastitis Conference, Maastricht, The Netherlands 422-426.

Lam TJGM, Ruegg PL and McDougall S (2008) Good Veterinary Practice to improve udder health: do's, don'ts, and opportunities. Proceedings WBC 2008, Budapest, Hungary 70-74.

Lee SU, Quesnell M, Fox LK et al. (1998) Characterisation of staphylococcal bovine mastitis isolates using the polymerase chain reaction. Journal of Food Protection 61, 1384-1386.

Luedecke LO, Forster TL and Ashworth US (1967) Relationship between California Mastitis Test reaction and leucocyte count, catalase activity, and A-esterase activity of milk from opposite quarters. Journal of Dairy Science 50, 1592-1596.

Ma Y, Ryan C, Barbano DM et al. (2000) Effects of somatic cell count on quality and shelf-life of pasteurised fluid milk. Journal of Dairy Science 83, 264-274.

Miller RH, Paape MJ and Acton JC (1986) Comparison of milk somatic cell counts by coulter and fossomatic counters.

Journal of Dairy Science 69, 1942-1946.
de Mol RM, Woldt WE (2001) Application of fuzzy logic in automated cow status monitoring. Journal of Dairy Science 84, 400-410.
National Mastitis Council (1996) Current Concepts of Bovine mastitis. Fourth Edition.
Nielen M, Deluyker H, Schukken YH et al. (1992) Electrical conductivity of milk: measurement, modifiers, and meta analysis of mastitis detection performance. Journal of Dairy Science 75, 606-614.
Norberg E, Hogeveen H, Korsgaard IR et al. (2004) Electrical conductivity of milk: ability to predict mastitis status. Journal of Dairy Science 87, 1099-1107.
Olde Riekerink RGM, Barkema HW, Veenstra W et al. (2007) Somatic cell count during and between milkings. Journal of Dairy Science 90, 3733-3741.
Østerås O, Solbu H and Refsdal AO (2007) Results and evaluation of thirty years of health recordings in the Norwegian dairy cattle population. Journal of Dairy Science 90, 4483-4497.
Phuektes P, Browning GF, Anderson G et al. (2003) Multiplex polymerase chain reaction as a mastitis screening test for *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis* in bulk milk samples. Journal of Dairy Research 70, 149-155.
Reneau JK (2001) Prepping cows: who needs it? Proceedings NMC 40th annual meeting 33-42.
Sargeant JM, Leslie KE, Shirley JE et al. (2001) Sensitivity and specificity of somatic cell count and California Mastitis Test for identifying intramammary infection in early lactation. Journal of Dairy Science 84, 2018-2024.
Sarikaya H, Bruckmaier RM (2006) Importance of the sampled milk fraction for the prediction of total quarter somatic cell count. Journal of Dairy Science 89, 4246-4250.
Schepers AJ, Lam TJGM, Schukken YH et al. (1997) Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. Journal of Dairy Science 80, 1833-1840.
Schukken YH, Smit JAH, Grommers FJ et al. (1989) Effect of freezing on bacteriologic culturing of mastitis milk samples. Journal of Dairy Science 72, 1900-1906.
Schukken YH, Wilson DJ, Welcome F et al. (2003) Monitoring udder health and milking quality using somatic cell counts. Veterinary Research 34, 579-596.
Sears PM, Smith BS, English PB, Herer PS et al. (1990) Shedding pattern of *Staphylococcus aureus* from bovine intramammary infections. Journal of Dairy Science 73, 2785-2789.
Steeneveld W, van der Gaag LC, Barkema HW et al. (2008) Using cow-specific risks to support the detection of clinical mastitis on farms with an automatic milking system. Proceedings Mastitis Control 2008, The Hague, The Netherlands 370.
Williams WL (1937) The detection of shedders of the streptococcus of mastitis in composite control milk samples. Journal of Dairy Science 20, 711-717.
Zadoks RN, Schukken YH (2006) Use of molecular epidemiology in veterinary practice. Veterinary Clinics of North America Food Animal Practice 2, 229-261.