Electrical Conductivity of Milk: Measurement, Modifiers, and Meta Analysis of Mastitis Detection Performance

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ABSTRACT

The physics, physiology, and pathology of electrical conductivity of milk are described. Based on a meta analysis, the use of electrical conductivity as a mastitis detection tool is discussed. Most reports were based on subclinical mastitis data. The gold standards of the different reports are discussed. With an overall sensitivity of 66% and an overall specificity of 94%, the predictive value of a positive electrical conductivity test remains low in a low prevalence population. The use of on-line systems for clinical mastitis detection is discussed. Online systems that combine multiple data and perform multifactorial analyses will be of interest to the dairy industry.
(**Key words**: electrical conductivity, bovine mastitis, detection, meta analysis)

Abbreviation key: CMT = California mastitis test, EC = electrical conductivity, IQR = inter quarter ratio, WMT = Wisconsin mastitis test.

INTRODUCTION

The economic impact of both clinical and subclinical forms of mastitis is large in the current dairy industry (29, 52). Losses occur from decreased milk production, treatment, and labor costs, nondeliverable milk, veterinary fees, reduced milk quality, reduced milk price, increased risk of subsequent mastitis, and increased risk of culling or death of the cow.

On-line electrical conductivity (EC) measurements have recently been introduced commercially with the goal of aiding udder health monitoring on the farm. However, it is not well known how sensitive or specific these devices are for detection of either clinical or subclinical mastitis.

The objectives of this study were 1) to summarize the present knowledge on EC in relation to udder health, 2) to assess the use of EC as a mastitis detection tool, and 3) to discuss the potential application of EC in online automated mastitis detection systems.

CURRENT KNOWLEDGE

Definition of Electrical Conductivity

Electrical conductivity is a measure of the resistance of a particular material to an electric current. The electrical resistance of an electrolyte solution is defined as the resistance of a cube of the solution 1 cm^3 in volume. The conductivity is the reciprocal of the resistance.

Resistance (impedance) is measured in ohms and is calculated by dividing voltage by amperes. Conductivity is measured in Siemens and is calculated by dividing ampere by voltage.

Typical EC of normal milk appears to be between 4.0 and 5.5 mS/cm at 25°C (59), and the distribution of the measurements is log normal (27). The EC of milk has also been expressed as a concentration of NaCl with the same conductivity as the examined milk, in

Received July 10, 1991.
Accepted September 23, 1991.
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millimoles, which reflects the total ionic concentration of the milk in milliequivalents per liter (26, 32, 33, 47).

Measurements of Electrical Conductivity

Laboratory. In the laboratory, EC measurements are made using electrodes placed in glass cells with known volume. The cells are calibrated using a standard NaCl solution of a certain molarity. Corrections for temperature are made. The repeatability of the EC measurements on 25 milk samples measured 20 times was good; the standard error was only .5% of the mean of 20 measurements (34).

Cowside Tests. Cowside tests have been developed; most measure one quarter at a time (4, 6, 18, 20, 22, 42) or all four quarters separately at the same time (38, 40).

On-Line. The EC can be measured during milking by means of a measuring cell placed in the milking equipment (35, 50). Most on-line systems measure EC per quarter (14, 35, 43, 50, 51), except for one system that measures EC on composite milk per cow (5). Others are developing a system without electrodes being in contact with the milk, using two toroids and a milk loop in the system (43).

The on-line systems are further characterized by the automation of the measurements during milking and data storage on an electronic device such as a microcomputer. Data from several milkings can be stored and used to perform calculations.

Correlations between on-line EC measurements and EC measured in the laboratory were .83 for foremilk, .89 for peak flow milk, .85 for strippings, and .86 overall (*P* not given) (35).

Influence of Mastitis and Other Factors on Electrical Conductivity

In milk, EC is determined by the concentration of anions and cations. The most important ions in milk are Na^+, K^+, and Cl^- (25, 59). The secretory cells in the mammary gland have active transport systems for pumping Na^+ into the extracellular fluid and K^+ into the cells. The Na^+ and K^+ are transported passively from the secretory cells into the milk. The Na^+:K^+ ratio is 3:1 in extracellular fluid and blood and 1:3 both in intracellular fluid and the milk. The Cl^- concentration is higher in blood and extracellular fluid than in milk (25). The mammary ducts appear to be impermeable to ions and lactose (30).

Effect of Mastitis. Mastitis has a marked effect on milk production and composition (7). Milk concentrations of lactose and K^+ are decreased, and concentrations of Na^+ and Cl^- are increased (25, 37, 44). The ion concentrations in mastitic milk change because of increased blood capillary permeability, the destruction of tight junctions, and the destruction of the active ion-pumping systems (26). After the cell damage, Na^+ and Cl^-, which have high concentrations in extracellular fluid, pour into the lumen of the alveolus. In order to maintain osmotic pressure, K^+ and lactose concentrations decrease in the milk (25, 44). The decrease of lactose in the milk could be caused by leakage of lactose into the extracellular fluid and blood after the destruction of the normal lactose barrier (37), or by the reduced biosynthesis of lactose caused by destruction of secretory cells (1, 25), or by both mechanisms.

The altered concentration of Na^+, K^+, and Cl^- in mastitic milk cause the EC of mastitic milk to be increased (25) with no change in osmotic pressure (37, 44). A higher variability in on-line EC measurements of infected quarters, compared with uninfected quarters, has also been reported (61).

Other Factors. In addition to mastitis, other circumstances that cause the ionic content of milk to change will have an influence on the EC of milk.

Reported factors that were probably mastitis-related include variation among cows (42, 51), variation among days within cows (14, 31, 33, 34, 51), stage of lactation (3, 38, 51, 55), and parity (50, 51, 55). Herd differences were also reported (6, 24, 54, 56).

In the laboratory, EC of milk samples increased with sample temperature. Between 15 and 40°C, the relationship between temperature and EC was approximately linear with an increase of .113 mS/°C (45). Temperature correction coefficients have been proposed (45, 49).

Some reported factors were associated with milk composition or milking times. With increased fat percentage of the milk, EC decreased (10, 48). The fat fraction of milk is virtually a nonconductor because of the reduction of volume of conducting medium and the physical obstacle fat globules form to migrating ions (48). An increased milking interval caused the EC of milk to be higher (10, 51). This could be caused by the increase in Na^+ and Cl^- and the decrease of K^+ found after incomplete milkings and after an extended milking interval (57). A diurnal pattern of EC was described (23) as well as a difference between morning and evening milking, the evening EC being lower (10, 35). The difference between morning and evening was most marked in infected quarters (50). During milking, EC varied between subsequent fractions of the milk (16, 23, 32, 35, 37, 61); an increase during milking was also found (14). The EC in foremilk was higher before milk ejection than after milk ejection (16).

Other factors that have been reported to influence EC include estrus (24, 32), genetic group (3), cows being turned out on pasture in the spring (24, 31), milk fever (31), feed poisoning with bad quality silage (24), and intramammary antibiotic treatment of uninfected quarters (32). Oxytocin appeared to change milk composition in a way similar to mastitis (1).

MASTITIS DETECTION

Calculations to Detect Abnormal Milk

To distinguish between normal and mastitic milk using EC, several methods have been investigated.

Absolute Thresholds. Several researchers have reported a higher mean EC in diseased quarters or abnormal milk than in normal quarters or normal milk, respectively (3, 6, 9, 10, 11, 12, 14, 16, 20, 26). Thresholds above which the milk was considered abnormal were used, either in composite foremilk samples (all four quarters) (3), in quarter foremilk samples (4, 6, 10, 15, 18, 40, 54, 56), or in quarter strippings (9, 10). For on-line systems, absolute thresholds based on means or running averages were used (50).

Inter Quarter Ratio. Inter quarter ratio (IQR) was defined as the ratio between the quarter with the lowest conductivity and the other quarters of the same cow (11, 14, 17, 32). Because the nonmastitic influences on EC seemed to affect all quarters of a cow equally, the difference in EC between quarters of a cow might indicate mastitis in a particular quarter (31, 33). One assumption was that a cow would almost never have mastitis in all four quarters at the same time. Thresholds that have been suggested for the IQR were between >1.00 and 1.40 (10, 11, 14, 17, 32, 51). Several variations in the IQR were proposed, including IQR based on means of quarters from repeated measurements and two by two comparison of quarters (33, 46, 47). For on-line detection, IQR was used, based on means within milking or running averages over milkings (51). To improve statistical analysis of the IQR, a redefinition of the IQR was proposed. The parameter should first be normalized by logarithmic transformation and then corrected for the mean, thus creating a normally distributed parameter with mean zero (27).

Differences Between Quarters. In addition to the IQR, thresholds for the difference between the highest and the lowest quarter have been used (40, 41, 42, 44, 50, 56); different thresholds were used for main milk and strippings (24), for foremilk and strippings (11), and for morning and evening milkings (10). When compared with the IQR, the difference measure was superior in detecting abnormal milk (50).

Combination of IQR and Absolute Values. A combination of absolute and IQR thresholds has been used (10, 11, 17, 20, 32, 46). Using logistic regression, the combination of both the absolute value and the IQR, without using thresholds, seemed to provide the best model fit (9).

Meta Analysis of Reports on Electrical Conductivity

Material and Methods. The methodology and results of several studies have been reanalyzed using test evaluation procedures (36). Using 77 observations from 17 studies presenting results, it was possible to calculate sensitivity, specificity, prevalence, and predictive values for meta analysis.

Most studies yielded more than one observation, i.e., 15 (17), 14 (11), 8 (10), 6 (47), 5 (33, 51), 4 (18), 3 (6, 12, 40, 54), 2 (14, 60), and 1 (3, 4, 44, 50). Reasons were use of different gold standards, more than one con-

TABLE 1. Sensitivity and specificity of electrical conductivity as a mastitis detection tool.[1]

	Median	Minimum	Maximum
Sensitivity	66	6	100
Specificity	94	0	100

[1]n = 77.

ductivity measurement, or use of several data sets.

Data on correlation coefficients between EC and SCC, California mastitis test (CMT), or Wisconsin mastitis test (WMT) were taken from 16 different reports (2, 3, 6, 9, 12, 14, 15, 20, 26, 34, 38, 41, 42, 47, 56, 60).

Results. Overall, test results showed higher specificity than sensitivity (Table 1). The median sensitivity was 66%, and the median specificity was 94%. Different gold standards led to different sensitivities and specificities (Table 2). The highest sensitivity and specificity were observed when bacteriological culturing was used as the gold standard. Comparing different conductivity calculations, sensitivity was lowest when absolute conductivity thresholds were used and highest when a combination of absolute threshold and a difference between quarters was used (Table 3). Because of insufficient numbers, it was not possible to make a comparison between single and repeated measurements of EC.

The positive and negative predictive values varied greatly between reports, which was likely due to differences in prevalence, as is to be expected from test evaluation theory (Figures 1 and 2). Correlations between EC of milk and SCC, CMT, or WMT varied greatly, but they were clearly >0. The reported correlations were not dependent on mean SCC per report or on prevalence of mastitis (Table 4).

Discussion. In general, reports on the usefulness of EC for detecting mastitis offer conflicting results.

A major difference between EC studies pertained to the definition of the diseased and healthy animals (gold standard). Gold standards that have been used in EC research include SCC, CMT, or WMT (17, 20, 23, 24, 26, 34, 35, 38, 40, 44); bacteriological culturing (3, 9, 10, 11, 12, 20, 32, 33, 42, 47, 50, 54, 55, 56); a combination of bacteriological culturing with SCC, CMT, or WMT (4, 6, 14, 15, 18, 20, 31, 41, 51); clinical mastitis signs (31, 40); a combination of clinical signs and bacteriological culturing (31); another EC measurement (38); or ion and lactose concentration in the milk (44). In some studies, the gold standard was not specifically defined (2, 5, 16, 37, 61).

In the meta analysis, the sensitivity with bacteriological culturing as the gold standard was remarkably higher than the sensitivity with SCC, CMT, or WMT as the gold standard

TABLE 2. Sensitivity and specificity of electrical conductivity for mastitis detection using different gold standards.

	Median	Minimum	Maximum
WBC[1] (n = 23)			
Sensitivity	57	22	100
Specificity	94	0	99
BC[2] (n = 41)			
Sensitivity	75	16	100
Specificity	95	67	100
WBC and BC[3] (n = 12)			
Sensitivity	60	6	100
Specificity	91	1	100

[1]WBC = White blood cell measurement (SCC, CMT = California mastitis test, or WMT = Wisconsin mastitis test).

[2]BC = Bacteriological culturing.

[3]WBC and BC = Combination of both gold standards.

TABLE 3. Sensitivity and specificity of electrical conductivity for mastitis detection using different calculations to define abnormality.

	Median	Minimum	Maximum
Absolute threshold (n = 26)			
Sensitivity	57	16	100
Specificity	91	1	99
Difference[1] (n = 30)			
Sensitivity	68	6	100
Specificity	96	32	100
Both[2] (n = 21)			
Sensitivity	79	27	100
Specificity	96	0	100

[1]Difference = A measurement of difference in electrical conductivity between quarters of the same cow.

[2]Both = Combination of absolute threshold and a measure of difference between quarters of the same cow.

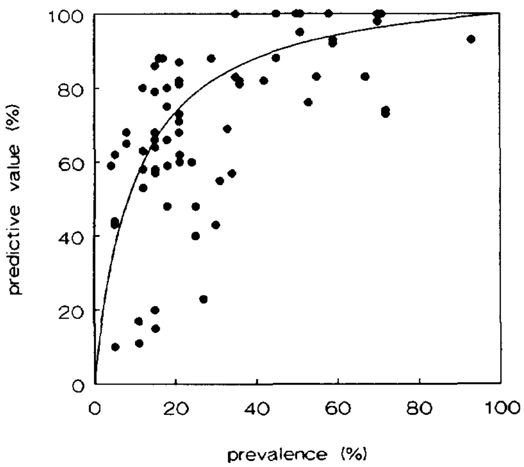

Figure 1. Predictive value of a positive electrical conductivity test (PV+) versus prevalence (PR). ● = Predictive value for each observation (n = 77); — = expected predictive value based on a sensitivity of 66% and a specificity of 94%:

expected PV+ =

$$100 \times \frac{.66 \times PR}{(.66 \times PR) + [.06\ (100 - PR)]}.$$

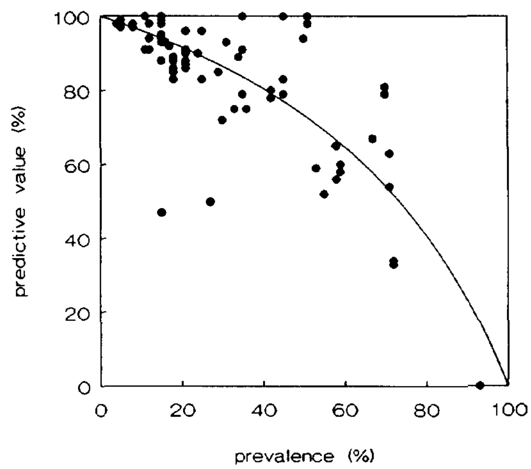

Figure 2. Predictive value of a negative electrical conductivity test (PV–) versus prevalence (PR). ● = Predictive value for each observation (n = 77); — = expected predictive value based on a sensitivity of 66% and a specificity of 94%:

expected PV– =

$$100 \times \frac{.94 \times (100 - PR)}{(.34 \times PR) + [.94 \times (100 - PR)]}.$$

(75 and 57%, respectively). However, specificity, remained high regardless of the gold standard. In subclinical mastitis, SCC may have more to do with the defense status of the cow than with the destruction of cells and tight junctions, which is measured by EC. Wide and overlapping ranges of EC were found in various SCC groups (34). The variability of correlations between cell counts and EC of –.36 to .82 also supports the idea that different processes are measured (Table 4). Milk production losses in quarters with a low SCC were described (13).

The low sensitivity of EC could be caused by the use of an "incorrect" gold standard, rather than deficiency of EC being not very good at detecting mastitis positives. If a new test disagrees with the gold standard, sensitivity, specificity, or both will be lower than 100%. A disagreement with the gold standard will often be interpreted as the test being worse. However, if the test were better than the gold standard, the test would also disagree and therefore have sensitivity or specificity below 100%. The EC could be better at detecting negatives than the current gold standard. Hence, the false negatives may be true negatives, and the true sensitivity may be higher than the currently observed sensitivity.

In mastitis research, the definition of the gold standard for subclinical mastitis does not represent a perfect gold standard, because repeated measurements and combinations of tests are recommended (8). In most cases of EC research, the definition of the gold standard

TABLE 4. Summary of reported correlation coefficients between electrical conductivity and white blood cell measures.

	n	Median	Minimum	Maximum
WBC[1]	22	.37	–.36	.82
Log WBC[2]	16	.47	.20	.63
Log EC/log WBC[3]	12	.40	.10	.68

[1]WBC = Correlation between white blood cell measure (SCC, CMT = California mastitis test, or WMT = Wisconsin mastitis test) and electrical conductivity.

[2]Log WBC = Correlation between the logarithm of white blood cell measure and electrical conductivity.

[3]Log WBC/log EC = Correlation between the logarithm of white blood cell measure and the logarithm of electrical conductivity.

was based on single measurements, even though mostly subclinical mastitis cases were studied. Perhaps, the gold standard for subclinical mastitis should also include decreased milk production (7). Because EC is related to epithelial damage, this might provide for a more accurate definition of subclinical mastitis. Milk production loss has been found to be correlated with EC. In a review, correlations of .49, .24, and .58 between quarter EC differences and quarter production losses were reported (53). Using a mean quarter difference value based on repeated measurements, a milk production loss per quarter was approximately 3% for every .1-mS/cm increase in EC (46). An approximate quarter milk production loss of 2% per .1-mS/cm difference between two quarters was also reported (53). Compensatory milk production of quarters has been described (21) and might have had an influence on the results.

Other differences between reports on EC were measurement frequency of EC and EC calculations used to detect abnormal milk. When comparing different EC calculations, absolute thresholds gave the lowest sensitivity. Most likely this was the result of all the nonmastitic cow factors that influence EC (3, 4, 6, 17, 24, 34, 54). Comparisons within cow gave better sensitivity, but the combination of both an absolute threshold and a comparison within cow appeared to give the highest results for sensitivity and specificity.

The use of EC as a screening or diagnostic test will depend not only on sensitivity and specificity but also on the prevalence of mastitis. Based on the performed meta analysis, EC seems to have some use as a screening test for subclinical mastitis. A typical screening test has a very high negative predictive value, based on high sensitivity and a somewhat lower specificity. The predictive value of a positive EC test will be low (Figure 1), and the predictive value of a negative test will be high (Figure 2) when prevalence of subclinical mastitis is low, for instance, around or below 20%. Therefore, EC could be used as a screening test even when the sensitivity is not very high. If EC is to be used as a decision criterion to treat or to cull animals, a low predictive value of a positive test is not acceptable, considering the economics of treatment, culling, and disease. However, in case of a herd with a high prevalence of subclinical mastitis, for instance over 60%, a positive EC test would have a higher predictive value and could be used to dictate actions (Figure 1).

APPLICATIONS IN ON-LINE SYSTEMS

Use of Electrical Conductivity in Mastitis Detection

Subclinical Mastitis. An advantage of on-line systems is that data are collected at each milking, and screening for the onset or presence of subclinical mastitis is possible after each milking. Whether, with the current gold standards, on-line systems could reach a high sensitivity for subclinical mastitis remains to be seen and is of great importance for the routine use of EC as a screening or diagnostic tool.

Clinical Mastitis. Based on the meta analysis, not much can be concluded about use of on-line systems for clinical mastitis detection. The use of on-line systems in early clinical mastitis detection will depend on the sequence of events in mastitis. When conductivity rises one or more milkings before clinical signs of mastitis appear, on-line systems may be useful for early treatment. If the pattern of EC during milking might be an indication for clinical mastitis, only on-line systems would be able to detect this.

Experimental infection with *Staphylococcus aureus* and *Streptococcus uberis* showed an increase in EC at the first milking after the challenge before clots in the milk appeared at the fourth milking after challenge (22). The EC was found to be closely associated with an experimental intramammary infection with *Staph. aureus* (58). After experimental *Staph. aureus* infection, the EC pattern of the infected quarters changed (61). Using doses of 10 and 100 μg of *Escherichia coli* endotoxin to induce mastitis, an increase of EC preceded the increase in leucocytes in the milk at 10 and 16 h after endotoxin infusion, respectively (19). Another study in goats, using *E. coli* endotoxin, found the peak of changes in milk composition, including Na^+ and K^+, between 5 to 7 h after endotoxin infusion (28). Therefore, it might be possible that *E. coli* infections cause mastitis so soon after invasion of the quarter that detection with an on-line system based on twice daily milking would be difficult.

Treatment Decision. The practical use of EC for early subclinical or clinical mastitis detection largely depends on the predictive value of a positive test and the economics of treatment and mastitis losses in a particular cow population. In addition, not much is known on the expected benefits of early treatment of subclinically or not yet clinically mastitic cows.

Gold Standard for On-Line Data

Because of the high frequency of measurements of an on-line system, the definition of the gold standard for especially subclinical mastitis remains a problem. Specifically, there is a need to measure the gold standard frequently to match the frequency of the probably very dynamic data from the on-line system.

Multifactorial Analyses and Sequential Testing

Multifactorial Approach. Future analysis of on-line EC data may be combined with other on-line data, such as milk production, cow temperature, cow activity, or concentrate consumption (39). Microcomputers used with on-line systems provide the opportunity to calculate and report results based on multifactorial models.

Sequential Approach. Sequential testing offers the opportunity to influence the prevalence of disease in a particular population. When EC is used as a first screening test, other data from the on-line system can subsequently be used to find the abnormal cows in the EC positive population, e.g., those cows with a high milk temperature. Another system could start with finding cows with reduced milk production and then proceed to test EC or other data.

ACKNOWLEDGMENTS

This research has been funded by the Dutch Foundation for Knowledge-Based Systems.

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