Evaluation of a hand-held electrical conductivity meter for detection of subclinical mastitis in cattle

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Objective—To assess, under field conditions, whether a hand-held electrical conductivity (EC) meter could be used to detect subclinical mastitis caused by pathogens most commonly associated with mastitis in dairy cows.

Animals—425 lactating cows on 15 dairies in Costa Rica.

Procedure—Immediately prior to milking, milk samples from each quarter were tested, using a hand-held EC meter. A milk sample from the quarter with the highest score was submitted for bacteriologic culture. Results of bacteriologic culture were compared with highest absolute EC score for each cow and with differential EC score (ie, difference between the highest and lowest absolute EC scores for the 4 quarters of each cow).

Results—Absolute EC score for cows with subclinical mastitis was significantly higher than that for cows without subclinical mastitis, and absolute EC score was significantly associated with detection of subclinical mastitis. If absolute EC score ≥ 7 was considered indicative of subclinical mastitis, sensitivity was 0.43, specificity was 0.83, predictive value of a positive result was 0.39, and predictive value of a negative result was 0.85. Differential EC score for cows with mastitis was significantly higher than that for cows without subclinical mastitis. If differential EC score ≥ 2 was considered indicative of subclinical mastitis, sensitivity was 0.53, specificity was 0.77, predictive value of a positive result was 0.37, and predictive value of a negative result was 0.87.

Clinical Implications—A hand-held EC meter may be used to screen cows for subclinical mastitis. (*Am J Vet Res* 1998;59:1087–1091)

Subclinical mastitis is of major concern to dairy farmers because of its effect on milk production and because it represents a reservoir of infectious organisms.[1] Approximately 75% of the economic losses from mastitis can be attributed to lost milk production.[2] Thus, a rapid test for detection of subclinical mastitis would be useful.

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Use of electrical conductivity as an indicator of subclinical mastitis has been investigated, but results of using electrical conductivity to detect subclinical mastitis vary.[3-18] Sensitivity and specificity of electrical conductivity as a diagnostic test for subclinical mastitis have been reported to range from 6 to 100% and from 1 to 100%, respectively.[3] Disparity in conclusions on the usefulness of electrical conductivity are presumably because of application, use in the milk line compared with hand-held cow side, and the conditions under which the research was conducted. The purpose of the study reported here was to assess, under field conditions, whether a hand-held **electrical conductivity (EC)** meter could be used to detect subclinical mastitis caused by pathogens most commonly associated with mastitis in dairy cows.

Materials and Methods

Dairies and cows—Fifteen dairy farms that were members of the Centro de Analisis Para la Produccion de Leche and located in the canton of San Carlos, province of Alajuela, Costa Rica, participated in the study. All dairies milked cows twice daily.

Experimental procedure—Dairies were visited during a 2-week period in July 1994 and again during a 2-week period in February 1995. Cows that were lactating at the time of dairy visits were eligible for inclusion in the study. Cows with visibly abnormal milk (ie, bloody or containing clots), cows that had been treated with antimicrobials within the preceding 14 days, and cows that had calved within the preceding 10 days were excluded from the study.

For cows included in the study, the udder was prepared for milking as usual. One of the investigators then wiped the teats dry with a single-use paper towel, expressed 2 streams of milk from 1 of the quarters, then tested the next stream of milk for EC, using a hand-held EC meter.[a] If a reading was not obtained, additional milk was expressed into the meter until a reading was obtained. This procedure was repeated for the other 3 quarters, so that a separate EC reading was obtained for each quarter.

The EC meter was factory calibrated and gave a numerical reading from 0 to 9. In addition, it was self-purging so that flushing between samples was unnecessary.[b] To reduce cross contamination, the EC meter was flushed with water between cows. Absolute EC score was defined as the reading obtained directly from the EC meter. Differential EC score was defined as the difference between the highest and lowest absolute EC scores for the 4 quarters of each cow.

For each cow, a milk sample was collected from the quarter with the highest absolute EC score and submitted for bacteriologic culture. If more than 1 quarter shared the highest absolute EC score, the investigator chose the quarter from which the milk sample was to be collected on the basis of ease of collection. Milk samples were aseptically collected according to National Mastitis Council recommendations.[19]

Table 1—Highest absolute electrical conductivity (EC) scores for milk from cows with (n = 86) and without (339) subclinical mastitis, determined on the basis of bacteriologic culture of milk

Highest absolute EC score*	No. of cows without mastitis	No. of cows with mastitis
0	1	0
1	3	3
2	29	4
3	67	4
4	80	11
5	54	17
6	46	10
7	21	11
8	13	7
9	25	19

*For each cow, highest absolute EC score was the highest score for all 4 quarters.

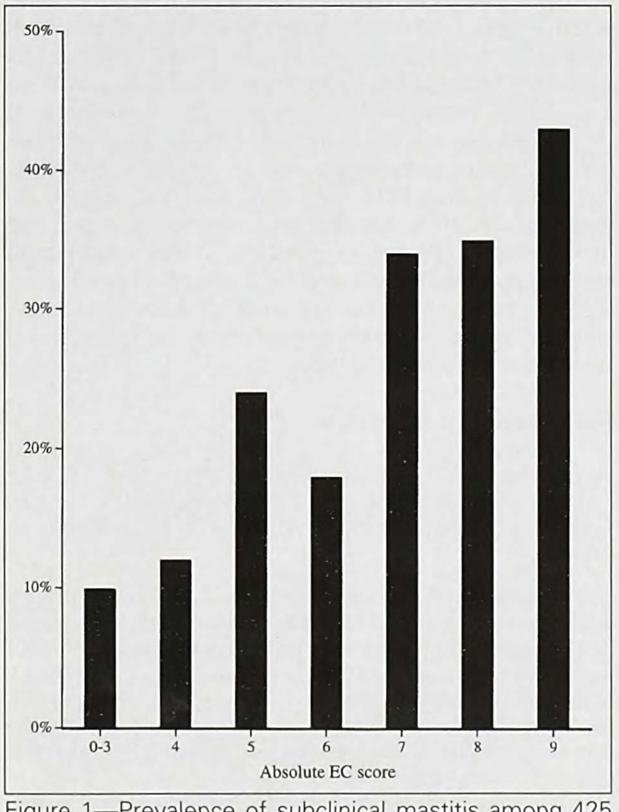

Figure 1—Prevalence of subclinical mastitis among 425 cows classified according to absolute electrical conductivity (EC) score for milk. Subclinical mastitis was diagnosed on the basis of bacteriologic culture of milk from the quarter with the highest absolute score.

Samples were immediately stored in a portable cooler with cold packs and frozen within 12 hours after collection.

Bacteriologic culture procedures—Milk samples (approx 10 µl) were streaked onto sheep's blood, MacConkey, and mannitol salt agar plates. Bacterial colonies were Gram stained, and gram-positive cocci were catalase tested. Catalase-negative cocci were identified with a commercial streptococcus identification system.[c] Catalase-positive cocci were subjected to coagulase and Voges-Proskauer tests. Catalase-positive cocci for which results of both tests were positive were recorded as *Staphylococcus aureus*. Catalase-positive cocci for which results of 1 or both tests were negative were identified with a commercial identification system.[d] Pleomorphic gram-positive cocci were identified with a commercial identification system for corynebacteria.[e] Gram-negative rods were identified on the basis of IMViC reactions and by use of commercial identification systems for enterobacteriaceae[f] and nonfermenters.[g]

Data analysis—Cows from which *S aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Escherichia coli, Pseudomonas* spp, *Klebsiella* spp, or *Enterobacter* spp was isolated were considered to have subclinical mastitis. Cows from which bacteria were not isolated or from which only bacteria not commonly associated with mastitis (eg, *Agrobacterium radiobacter, Bacillus* spp, *Brevibacterium* spp, *Lactobacillus* spp) were isolated were considered to not have subclinical mastitis. Cows from which Corynebacterium spp; *Staphylococcus* spp other than *S aureus; Streptococcus* spp other than *S agalactiae, S dysgalactiae*, or *S uberis*; or mixed pathogens or contaminants were isolated were excluded from analyses, because these results were considered ambiguous. Farm prevalence of subclinical mastitis was obtained by dividing number of cows with subclinical mastitis by number of cows with subclinical mastitis plus number of cows without subclinical mastitis.

Mantel-Haenszel χ^2 analysis for increasing trends[h] was used to evaluate the association between EC scores and subclinical mastitis (yes vs no). Differences in absolute and differential EC scores between cows with subclinical mastitis and cows without were evaluated by use of the Wilcoxon's test[i] for nonparametric data. Sensitivity, specificity, and predictive values of positive and negative test results were calculated, using results of bacteriologic culture as the criterion standard and various EC scores as the cutoff between positive and negative test results.

Results

Milk samples were collected from 536 dairy cows. However, 7 cows were excluded from the analyses because results of bacteriologic culture were not available (eg, the sample was lost or not submitted for bacteriologic culture) and 104 were excluded because results of bacteriologic culture were ambiguous (*Corynebacterium* spp were isolated from 33; *Staphylococcus* spp other than *S aureus* were isolated from 56; *Streptococcus* spp other than *S agalactiae, S dysgalactiae*, or *S uberis* were isolated from 4; mixed pathogens were isolated from 4; 7 were contaminated). Three hundred thirty-nine cows were classified as not having subclinical mastitis because bacteria were not isolated (n = 328) or only nonspecific pathogens were isolated (11). The remaining 86 cows were classified as having subclinical mastitis (*S aureus*, 37; *S agalactiae*, 29; *S dysgalactiae*, 3; *S uberis*, 4; *E coli*, 1; *Pseudomonas* spp, 5; *Klebsiella* spp, 2; *Enterobacter* spp, 5). Overall prevalence of subclinical mastitis was 20.2% (86/425). Prevalences of subclinical mastitis caused by *S aureus* and *S agalactiae* were 8.7 and 6.8%, respectively.

Highest absolute EC score ranged from 1 to 9 (median, 6; **Table 1**) for cows with subclinical mastitis and from 0 to 9 (median, 4) for cows without. However, absolute score for cows with subclinical mastitis was significantly ($P \leq 0.001$) higher than that for cows without subclinical mastitis, and absolute EC score was significantly ($P < 0.01$) associated with detection of subclinical mastitis (**Fig 1**). Differential EC score ranged from 0 to 5 (median, 2; **Table 2**) for cows with subclinical mastitis and from 0 to 7 for cows without (median, 1). However, differential EC score for cows with mastitis was significantly ($P < 0.001$) higher than that for cows without subclinical mastitis.

Characteristics of using highest absolute EC score as a test for subclinical mastitis were calculated, using absolute EC scores from 4 through 8 as cutoffs be-

Table 2—Differential EC scores for milk from cows with (n = 86) and without (339) subclinical mastitis, determined on the basis of bacteriologic culture of milk

Differential EC score*	No. of cows without mastitis	No. of cows with mastitis
0	88	16
1	174	24
2	44	20
3	19	14
4	10	6
5	2	6
6	1	0
7	1	0

*Defined as the difference between the highest and lowest absolute EC scores for the 4 quarters of each cow.

Table 3—Characteristics of highest absolute EC score as a test for subclinical mastitis in cows

Cutoff*	Sensitivity	Specificity	Predictive value: Positive test result	Predictive value: Negative test result
4	0.87	0.30	0.24	0.90
5	0.74	0.53	0.29	0.89
6	0.55	0.69	0.32	0.86
7	0.43	0.83	0.39	0.85
8	0.30	0.89	0.41	0.83

*Cows with an absolute EC score greater than or equal to the cutoff score were considered to have subclinical mastitis.

Table 4—Characteristics of differential EC score as a test for subclinical mastitis in cows

Cutoff*	Sensitivity	Specificity	Predictive value: Positive test result	Predictive value: Negative test result
1	0.81	0.26	0.22	0.85
2	0.53	0.77	0.37	0.87
3	0.30	0.90	0.44	0.84

*Cows with a differential EC score greater than or equal to the cutoff score were considered to have subclinical mastitis.

tween positive and a negative test results (**Table 3**). Similarly, characteristics of using differential EC score as a test for subclinical mastitis were calculated, using differential EC scores from 1 through 3 as cutoffs between positive and negative test results (**Table 4**).

Discussion

Previous studies[3-18] differ as to the validity of using EC of milk for detection of subclinical mastitis. Disparate results among studies may be attributable to numerous factors, including differences in criterion standards used to determine whether cows actually had subclinical mastitis, differences in threshold values used as cutoffs between positive and negative test results, variations in sampling time in relation to milking (eg, milk obtained at the beginning of milking vs milk obtained during the middle of milking vs milk obtained at the end of milking vs composite milk samples), differences in type of sample (eg, fresh vs refrigerated vs frozen), and breed of cows.

In this study, the criterion standard used to determine whether cows had subclinical mastitis was bacteriologic culture of a milk sample from the quarter with the highest absolute EC score. Unfortunately, there are several limitations associated with this method. For instance, milk samples were not collected from the 3 other quarters even if scores for those quarters were equal to the highest absolute EC score, and it is possible that some cows classified as not having subclinical mastitis would have been found to have been infected if samples from all 4 quarters had been submitted for bacteriologic culture. However, in a previous study[20] in Costa Rica, 46% of dairy cows with subclinical mastitis had 2 or more infected quarters, and it seems likely that if this result held constant for our study, the possibility of not culturing bacteria from an infected cow would be low. In addition, bacteriologic culture of single milk samples is not a 100% accurate method of detecting intramammary infection,[21,22] particularly because cows with chronic subclinical mastitis may shed bacteria only intermittently. Thus, use of milk samples from only 1 quarter and use of single milk samples may help explain the high rate of false-positive results (ie, percentage of cows with high absolute EC scores but for which results of bacteriologic culture were negative) in the present study. For instance, using absolute EC score ≥ 7 and differential EC score ≥ 2 as indicative of subclinical mastitis, false-positive rates were 17% (59/339) and 23% (77/339), respectively. However, these high false-positive rates may also be a reflection of the limitations of the test itself.

To reduce the rate of false-negative results (ie, percentage of cows with low absolute EC scores but for which results of bacteriologic culture were positive) in the present study, only cows from which pathogens most commonly associated with mastitis were classified as having subclinical mastitis. *Staphylococcus aureus* and *S agalactiae* are common causes of mastitis in cows and are commonly isolated from the mammary gland or udder and teat skin[23]; *S dysgalactiae, S uberis, E coli, Pseudomonas* spp, *Klebsiella* spp, and *Enterobacter* spp are also considered to be common causes of mastitis. Bacteria other than these pathogens could have been contaminants from the environment and, therefore, were not considered indicative of subclinical mastitis.[24] This procedure may have excluded from analysis cows that did have subclinical mastitis caused by other pathogens, possibly reducing the number of cows included in the analyses and lowering the prevalence of subclinical mastitis.

Milk conductivity can change during milking because of changes in electrolyte or fat concentration of the milk.[12] Use of in-line systems to monitor EC of milk is reportedly a more sensitive indicator of subclinical mastitis than is use of milk collected at the beginning or middle of milking.[16,17] In the present study, milk collected at the beginning of milking was used for convenience and to duplicate how this meter would likely be used in the field. This standardized sampling reduced variability associated with changes in milk composition during milking.

As milk fat concentration increases, EC decreases.[3] Fat globules form a physical hindrance to migrating ions and reduce the volume of conducting medium (fat is a nonconductor).[25] The negative correlation between milk fat concentration and EC of milk may be the major factor in the disparity in absolute EC scores among animals of different breeds (Holstein vs Jersey) and species (cows vs goats) with subclinical mastitis. Use of correction factors has been suggested when measuring EC of milk from breeds or species with high milk fat concentrations.[11,26,b] Most cows and herds in this study consisted of a heterogenous mixture of Brahman, Jersey, and Holstein. In this type of situation, use of a constant threshold, rather than use of various correction factors, would be most practical.

Different types of EC scores may be used for diagnosis of subclinical mastitis: absolute conductivity, differential conductivity, parallelism, mixed conductivity, and 1-, 2-, and 3-way out-of-balance conductivity.[4,5] In this study, we used absolute and differential EC scores at a single visit, because these scores could be easily determined with a hand-held EC meter. Highest absolute and differential EC scores for cows with subclinical mastitis were significantly different from those for cows without, reflecting pathologic changes in mammary glands of cows with subclinical mastitis.[3,6,23]

Previously reported sensitivity and specificity of absolute EC scores range from 16 to 100% (median, 57%) and from 1 to 99% (median, 91%), respectively.[3] As with all diagnostic tests, changing the cutoff between positive and negative test results will affect the test's characteristics, and differences in cutoffs may help explain some of the inconsistencies among results of previous studies. The manufacturer of the EC meter used in this study suggests that absolute EC scores ≥ 5 be considered indicative of subclinical mastitis.[b] In this study, use of this cutoff for absolute EC resulted in high sensitivity and predictive value of a negative result; however, 71% (159/223) of the cows with EC scores ≥ 5 did not have subclinical mastitis (Tables 1 and 3). In a previous study[8] on a herd of Holsteins, sensitivity and specificity of using an absolute EC score ≥ 7 as indicative of subclinical mastitis were similar to values obtained in the present study; use of an EC score ≥ 7 maximized test characteristics. The effect of the cutoff value on sensitivity and specificity can be appreciated by studying Table 3 and 4. The appropriate cutoff will depend largely on the application to which the test is being used (ie, to identify cows with subclinical mastitis or to identify those who do not have the disease) and the cost of an erroneous diagnosis. These circumstances will vary among farms and veterinarians and will determine the cutoff value that should be used.

Many factors affect EC of milk from one milking to the next; however, only intramammary infection is likely to influence EC of individual quarters.[9] Thus, differential EC score may be more useful for predicting whether cows have subclinical mastitis than is absolute EC score.[4,8-10] Previously reported sensitivity and specificity of differential EC scores range from 6 to 100% (median, 58%) and from 32 to 100% (median, 96%), respectively.[3] Our results, using the manufacturer's recommendation that a differential EC score ≥ 2 be considered indicative of subclinical mastitis, were consistent with results of a previous study[11] and with the manufacturer's reported results[b] (Table 4). A potential drawback to use of differential EC score is that it assumes that at least 1 quarter is healthy. In this study, of the 96 cows with absolute EC score ≥ 7, 16 had the same absolute EC score for all quarters and 20 had a difference of only 1 between the highest and lowest absolute EC scores for all 4 quarters. This suggests that these cows may not have had at least 1 healthy quarter. In a previous study,[20] 7.3% of cows with subclinical mastitis had 4 infected quarters and 12.1% had 3 infected quarters.

In Costa Rica, indirect methods of detecting subclinical mastitis are mainly limited to use of hand-held EC meters or the California Mastitis Test. Somatic cell counts of individual cows are generally not available to producers at this time. The EC meter provides a quick test with an objective outcome, and in situations where resources and economics preclude determination of somatic cell counts, measurement of EC of milk may be a practical and useful screening test to identify cows that should undergo further testing (eg, bacteriologic culture of milk samples). In the present study, we did not find any advantage in using differential EC scores, as opposed to using absolute EC scores.

[a] Mas-D-Tec, Wescor, Logan, Utah.
[b] Mas-D-Tec operator's manual, Wescor, Logan, Utah.
[c] API 20 STREP, bioMerieux Vitek Inc, Hazelwood, Mo.
[d] API STAPH, bioMerieux Vitek Inc, Hazelwood, Mo.
[e] API CORYNE, bioMerieux Vitek Inc, Hazelwood, Mo.
[f] API 20E, bioMerieux Vitek Inc, Hazelwood, Mo.
[g] API NFT, bioMerieux Vitek Inc, Hazelwood, Mo.
[h] Proc FREQ, SAS Institute Inc, Cary, NC.
[i] Proc NPAR1WAY, SAS Institute Inc, Cary, NC.

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