Sensitivity and specificity of a hand-held milk electrical conductivity meter compared to the California mastitis test for mastitis in dairy cattle

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Abstract

Screening tests for mastitis can play an important role in proactive mastitis control programs to reduce the economic impact of this disease. The primary objective of this study was to compare the accuracy of milk electrical resistance (MER) to the California milk cell test (CMCT) in commercial dairy cattle of South Africa using Bayesian methods without a perfect reference test. A total of 1,848 quarter milk specimens were collected from 173 cows sampled during 6 sequential farm visits and 25.8% yielded pathogenic bacterial isolates. Most frequently isolated bacterial species were coagulase negative Staphylococci (n = 346), *Streptococcus agalactiae* (n = 54), and *Staphylococcus aureus* (n = 42). The overall cow-level prevalence of mastitis was 54% based on the Bayesian latent class analysis and the prevalence varied by quarter. The CMCT was more accurate than MER for classification of cows as having somatic cell counts >200,000/ml and isolation of a bacterial pathogen. Bayesian latent class analysis also suggested an overall benefit of CMCT over MER but the difference was not highly statistically probable. The Bayesian model estimated the sensitivity and specificity of MER at a cut-point of >25 mΩ/cm to be 89.9% and 86.8% respectively. The CMCT had a sensitivity and specificity of 94.5% and 77.7% respectively when evaluated at the slight positive cut-point. Milk electrical resistance was useful for identifying milk specimens harboring pathogens but was not able to differentiate among evaluated bacterial isolates. Screening tests can be used to improve udder health as part of a proactive management plan.

Key words: mastitis, diagnostic tests, Bayesian, dairy cow, South Africa

Introduction

Mastitis is the most costly diseases of dairy cattle and has major importance for the commercial dairy sector in South Africa. In 2009, there were 3,551 commercial milk producers in the country (MPO, 2009) with a median herd size of 145 cows (MPO, 2010). The recorded average daily milk production per cow was 17.8 kg and in 34% of herds, cows averaged between 15.6 and 20.6 kg and in 19.4% of herds more than 20.6 kg (MPO, 2010). Twenty-four percent of South African dairy cattle (129,511 cows in 656 herds) were monitored in 2008 by the national milk recording scheme and the average per lactation production for recorded cows was 7,271 kg and approximately 50% higher than the national herd average (du Toit, 2009). Holstein total 47.5% of the monitored dairy cattle and another 45.2% are Jersey with the remainder predominantly Ayrshire (5.5%) (du Toit, 2009).

In South Africa, *Streptococcus agalactiae* and *Enterococcus canis* have been associated with clinical mastitis problems within herds. Coagulase-negative staphylococci were the most frequently isolated bacteria in milk samples from both lactating and dry cows during 2000 to 2007, followed by *Staphylococcus aureus* and *Streptococcus agalactiae* (Petzer et al., 2009). *Staphylococcus aureus* can be considered the most important mastitis pathogen in South Africa because of the economic impact (Wilson et al., 1997) and potential for chronic infections and treatment failures.

A number of diagnostic options exist for the identification of mastitis but they have differences with respect to accuracy and cost (Emanuelson et al., 1987; Pyorala, 2003; Viguier et al., 2009). A difficulty in the estimation of diagnostic accuracy is the lack of a gold standard for the classification of cattle as having mastitis (Dohoo et al., 2011). The enumeration of somatic cells is a common method for identification of gland inflammation and is frequently approximated on an ordinal scale using the California milk cell test (CMCT) (Schalm and Noorlander, 1957). Electrical conductivity (or resistance as the inverse) has also

been employed to detect mastitis (Fernando et al., 1982; Nielen et al., 1992; Norberg et al., 2004) and hand-held meters have been promoted as a screening tool in South Africa. Milk electrical conductivity is mainly determined by type and concentration of ions, interactive influence of ions, and components contributing to milk viscosity including protein, fat, and lactose (Henningsson et al., 2005).

The objective of the study reported here was to compare the accuracy of milk electrical resistance (MER) for identification of mastitis to the California milk cell test (CMCT) in commercial dairy cattle of South Africa using Bayesian methods without a perfect reference test. Secondary objectives included the evaluation of diagnostic methods based on a microbiological classification of mastitis and to determine whether resistance values varied by microbiological results.

Materials and methods

Herd sampling

All farms were visited during 2008 upon request by the producer and were within a 100 km radius of Onderstepoort in Gauteng province. Herds were visited a maximum of 6 times to monitor effectiveness of interventions (data not presented). Quarter milk samples were collected after clinical examination using a strip cup. Samples were maintained on ice and transported to the Milk Laboratory, Production Animal Studies, Ondersterpoort for analysis. Diagnostic procedures were performed as a service to producers and statistical analyses were performed after extraction of recorded data.

Laboratory testing

Milk electrical resistance (MER). Approximately 5ml of milk was ejected into the cup of a hand-held conductivity meter (Mast-O-Test, Durotec, P.O. Box 12540 Centralhill, Port Elizabeth, 6006 South Africa) after collection of specimens for bacterial culture. Milk

electrical resistance readings, reported in milliohms per centimeter (mΩ/cm), were obtained independently from each quarter.

California milk cell test (CMCT). The California milk cell test was performed immediately after recording MER. The test was performed and results were recorded as 0, negative; 1, weak positive; 2, distinct positive; and 3, strong positive in accordance with the manufacturer's guidelines (California mastitis test kit, ImmuCell, Portland Me., USA).

Somatic cell counts (SCC). Quarter-level somatic cell counting was performed using a Fossomatic 90 cell counter (FOSS Analytical A/S, Hillerød, Denmark).

Organism isolation. Quarter milk samples were plated onto Columbia Agar base supplemented with 5% defibrinated bovine blood and incubated aerobically for 24-48 hours at 37 °C. Isolated bacteria were identified based on colony morphology, haemolysis, catalase, KOH test and Gram staining. Additional tests included the latex agglutination Strepkit (Quantum Biotechnologies (Pty) Ltd, Ferndale, South Africa), Staphylase Test (Quantum Biotechnologies (Pty) Ltd, Ferndale, South Africa) and the API 20E kit (Omnimed, P.O. Box 4328, Honeydew, 2040, South Africa).

Statistical Analysis

Diagnostic accuracy of the milk electrical resistance (MER) and California milk cell test (CMCT) were estimated relative to two classifications of mastitis: a quarter-level somatic cell count (SCC) of greater than 200,000/ml (Schukken et al., 2003) and the successful isolation of a pathogenic bacterial organism. Only cows sampled during the first herd visit were used to evaluate diagnostic accuracy. Sensitivity (Se) was estimated at the quarter-level

as the proportion of positive test results within those quarters that were classified as having mastitis. Specificity (Sp) was similarly estimated within quarters that were mastitis negative. The design effect (Ukoumunne, 2002) was estimated to adjust for the clustering of quarters within cows and available software (Epi Info version 6.04d for Windows, Centers for Disease Control and Prevention, Atlanta, Ga., USA) was used to calculate 95% confidence intervals (CI). A Monte Carlo simulation method was employed to estimate and compare area under the receiver-operating characteristic curve (AUC).

Diagnostic accuracy of MER and CMCT were estimated within a Bayesian framework using a two-test, four population model assuming conditional independence. Each quarter was considered a separate population to eliminate the problem of interdependence of mastitis among quarters (Barkema et al., 1997). Tests were evaluated as ordinal results rather than dichotomization as positive or negative. Milk electrical resistance was categorized into 3 ordinal levels (<25 mΩ/cm, 25-30 mΩ/cm, and >30 mΩ/cm) based on manufacturer's suggestion of a green light being a healthy udder (>30 mΩ/cm), orange light indicating a mastitis suspect (25-30 mΩ/cm), and red light a mastitis positive (<25 mΩ/cm). The CMCT was evaluated at the typical scores of 0-3. Only cows sampled during the first herd visit and with complete test information were included in this analysis. Non-informative prior probability distributions (beta 1,1) were employed for components of Se and Sp and a mildly informative prior was used for prevalence (beta 4.7, 10.4). The prior for prevalence was based on the quarter-level mastitis prevalence (31%; 212/683) using the number of cows with SCC >400,000/ml and isolation of bacterial pathogen as an approximate gold standard (Petzer et al., 2009). Receiver-operating characteristic (ROC) curves were plotted for evaluated cutoffs by connecting the points of the 1 – Sp (x-axis) by Se (y-axis). Area under the estimated ROC curve (AUC) was calculated by the trapezoid approximation method (Munem

and Foulis, 1984). More information concerning latent class models based on ordinal classifications can be found elsewhere (Fosgate et al., 2007; Fosgate et al., 2010).

Markov chain Monte Carlo (MCMC) techniques were employed using available statistical software (WinBUGS Version 1.4, MRC Biostatistics Unit, Cambridge, UK). Autocorrelation among iterate values was assessed and only every fifth value was retained. Convergence was assessed by evaluating plots of model parameter iterates and by calculating the Gelman-Rubin statistic. The first 200,000 iterations were discarded as the burn-in and inferences were made based on the subsequent 40,000. Median values and percentiles were used as point estimates and probability intervals, respectively.

A random effects variance component analysis was performed to determine the proportion of variability in MER measurements due to bacterial isolate, cow, and quarter-level factors incorporating results from all herd visits. Mixed effects linear regression was used to determine if MER values varied by bacterial isolate while adjusting for cow as a random effect and quarter as a fixed effect with Bonferroni adjustment for multiple post-hoc pairwise comparisons. For this component of the analysis isolates were grouped as none, coagulase negative Staphylococci, *Streptococcus agalactiae*, *Staphylococcus aureus*, and other organism based on the empirical distribution of counts in the sample. Contaminated (n=3) and mixed growth (n=3) cultures were excluded. Statistical modeling was performed in commercially available software (SPSS version 17.0, SPSS Inc, Chicago, Ill., USA) and results interpreted at the 5% level of significance.

Results

A total of 1,858 quarter milk specimens with complete data were collected from 173 cows sampled during 6 sequential farm visits. Four hundred and seventy-seven specimens (25.8%) yielded pathogenic bacterial isolates and test results varied descriptively among

isolations (Table 1). One hundred and sixty-eight cows sampled during the first visit had complete test information for all four quarters. During the first visit, 81% of sampled cows had at least one quarter with SCC >200,000/ml and 59% of cows yielded pathogenic bacterial isolates from at least one quarter (Table 2). The overall cow-level prevalence of mastitis during the first visit was estimated to be 54% based on the Bayesian latent class analysis and the prevalence varied by quarter with the right side of the udder more likely to be affected.

Sensitivity and specificity of MER and CMCT were estimated over the ordinal categories and descriptively varied based on the evaluated definitions of mastitis (Table 3). Sensitivities were noticeably higher when estimated via the Bayesian latent class analysis. Latent class analytic results also suggested that the tests overall were more accurate when contrasted with the other definitions of mastitis (Table 4). The CMCT was more accurate than MER for classification of quarters as having SCC >200,000/ml and isolation of a pathogen. The overall accuracy of CMCT was also better than MER based on the latent class analysis but this difference was not statistically probable (AUC 0.931 versus 0.904; P = 0.257). Receiver-operating characteristic curves plotted at the evaluated cut-points did not suggest large differences in accuracy for MER and CMCT (Figure 1).

Variance components analysis of all 1858 quarter-milk specimens estimated the amount of variability in MER due to cow factors, quarter factors, and bacterial isolation results as 20.4%, 0.1%, and 2.5%, respectively. Seventy-seven percent of the variability in MER was unexplained by these variables. Bacterial species were categorized as none (n = 1371), coagulase negative Staphylococci (n = 346), *Streptococcus agalactiae* (n = 54), *Staphylococcus aureus* (n = 42), and other (n = 39). Mixed effects linear regression of MER estimated significant effects for cow (P < 0.001) and bacterial category (P < 0.001) but not for quarter (P = 0.222). Post-hoc pairwise comparisons demonstrated lower MER for coagulase

negative Staphylococci and *Streptococcus agalactiae* compared to no bacterial isolation ($P < 0.05$ after Bonferroni adjustment; see Table 1 for descriptive results).

Discussion

The definition of mastitis will have an important effect on measures of test accuracy and validation procedures should be performed in the population that accurately represents the population in which tests will be employed. Producers in the current study believed that their herds had mastitis problems and requested investigations to be performed. The reported accuracies should more closely represent what would be expected within South African farms with mastitis problems. However, accuracies might not adequately reflect cows within herds with low incidence of clinical mastitis. The fact that latent class analyses do not define the criteria for classification of animals as being affected or unaffected is both a strength and a limitation. The strength is that it prevents circular logic that might develop when the outcome is defined based on test results or surrogates measuring similar qualities. For example, the use of SCC for the classification of mastitis will likely lead to overestimation of accuracy of other tests, such as the CMCT, similarly based on the detection of milk cells. The limitation is that the statistical procedure cannot incorporate biological criteria and findings simply represent statistical probabilities rather than the formal classification of subjects as affected and not affected.

The prevalence of mastitis estimated via the latent class analysis was 54% and very similar to the prevalence of cows with pathogenic bacterial isolations. The right front quarter had the highest prevalence of successful bacterial isolations (56%) whereas the Bayesian latent class analysis suggested an equal prevalence in both right quarters of approximately 33%. The latent class analysis further suggested that the left hind quarter had the lowest prevalence and there was a statistically probable difference between the prevalence of mastitis

on the left and right sides of the udder. The reason for this difference is unknown but a higher prevalence of subclinical and clinical mastitis in the right quarters has been previously described (Barkema et al., 1997; Walsh, 1985) and is likely related to the milking practices within sampled herds. These previous studies either did not identify a difference between front and rear quarters or rear quarters were more likely to be affected than front, whereas in our study the left front quarter was more likely to be affected when compared to the left rear. Difference in prevalences between front and rear quarters of sampled cows within these herds could be related to management or conformation that would predispose cows to injuries and subsequent bacterial invasions.

The evaluation of MER and CMCT within sampled herds also suggested that Se and Sp of these tests could be adequate for use as cow-side tests. An earlier meta-analysis (Nielen et al., 1992) estimated the Se and Sp of electrical conductivity as 66% and 94%, respectively. Mansell and Seguya (2003) estimated the Se and Sp of a different hand-held conductivity meter to be 51% and 71%, respectively for the detection of subclincal mastitis. A study conducted using an in-line conductivity system estimated the Se and Sp of electrical conductivity to be 70% and 98%, respectively for the detection of clinical mastitis (Steeneveld et al., 2010). The present study included a population of cows with subclinical and clinical mastitis and the results of our Bayesian model are different but closer to the estimates derived from the latter study. A noticeable difference, however, is the higher Se but lower Sp that might be a consequence of using a different cutoff for classification as test positive.

The usefulness of presented results for management of mastitis must be evaluated in respect to the quality of information. Validity should be evaluated in respect to the likelihood of bias in the study design and statistical analysis. The herds sampled in this study were not a random sample of herds with mastitis problems and should therefore be considered a

convenience sample and possibly affected by selection bias. As such, internal comparisons would not be affected but it might be difficult to infer results to the general population of herds within South Africa.

Validity of the statistical results should be assessed by evaluating latent class modeling assumptions (Toft et al., 2005). These assumptions include different disease prevalence among sampled populations, equal diagnostic accuracy across populations, conditional independence between tests, and appropriate specification of prior probabilities. It is unlikely that presented results were biased due to inaccurate prior probability specification since only a mildly informative prior was employed for prevalence and all other parameters were noninformative, or flat priors. The assumption of different prevalences among populations might have been violated since both right quarters appeared to have equal prevalences of mastitis. However, since the model contained 14 unknown parameters for estimation (4 prevalences, 3 sensitivity and 3 specificity components for CMCT, 2 sensitivity and 2 specificity components for MER) and each population contained 12 degrees of freedom for estimation then even if both right quarters should have been considered a single population there were still adequate data to estimate all unknown parameters even without the addition of informative prior information. The assumption of conditional independence between tests is a concern and the base model did not allow for relaxation or evaluation of this assumption. Convergence diagnostics suggested that the model was an adequate representation of the data but it is unknown if a different modeling approach that incorporated conditional dependence would have affected model inferences.

The statistical modeling of MER suggested that the majority of variability in the measures was unaccounted for by cow, quarter, and microbiological results. In fact, only 2.5% of the variability could be attributed to bacterial isolate and MER was only different when comparing coagulase negative Staphylococci and *Streptococcus agalactiae* to no

bacterial isolation. Therefore, there was no evidence that MER could be used to distinguish among mastitis pathogens. Bacterial isolate was a significant predictor of MER but is unlikely to be accurate for identification of specific pathogens.

The overall accuracy of CMCT was greater than MER for identifying cows with SCC >200,000/ml and presence of a pathogen but comparisons based on the latent class analytical approach suggested less of a difference. The accuracy of MER (or MEC) is generally considered to be poor (Hovinen and Pyorala, 2011) but the improvement with CMCT might not be as large as commonly suspected. A reason for the discrepancy could be that a typical definition of mastitis is based on increased SCC (Pyorala, 2003) and the fact that CMCT is an indirect measure of cell counts (SCC).

Conclusions

Cow-side screening tests can be used by producers to identify quarters with higher probabilities of harboring pathogenic bacteria and milk specimens could be collected and stored frozen for shipping to a laboratory for microbiological testing. In-line milking systems frequently have the capacity for measuring MER and these data could be used to improve udder health at the herd-level through the implementation of a proactive management plan despite the limitations of this tool compared to other potential screening tests.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Table 1. Descriptive statistics for quarter-level mastitis tests for 173 dairy cattle in South Africa sampled during 2008.

	Somatic cell count (1000/ml)	**California milk cell test**	**Milk electrical resistance (mΩ/cm)**
Isolated organism (n)	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
None (1371)	117 (35, 519)	0 (0, 1)	32.0 (29.0, 34.0)
Mixed growth (3)	312 (32, 740)	0 (0, 0)	32.0 (31.0, 32.0)
Contaminated (3)	1377 (240, 2978)	1 (0, 3)	29.0 (20.0, 32.0)
Coagulase negative Staphylococci (346)	294 (87, 1151)	0 (0, 1)	31.0 (27.0, 33.0)
Streptococcus agalactiae (54)	1969 (353, 9947)	1 (0, 2)	31.0 (25.0, 34.0)
Staphylococcus aureus (42)	398 (69, 4211)	0.5 (0, 2)	31.5 (28.0, 33.0)
Streptococcus dysgalactiae (17)	660 (97, 15153)	1 (0, 2.5)	32.0 (27.5, 36.0)
Micrococcus spp. (8)	140 (53, 864)	0 (0, 1)	33.5 (32.0, 35.0)
Strepococcus uberis (6)	1116 (276, 8304)	1 (0, 1.5)	31.5 (29.0, 33.3)
Enterococcus faecalis (5)	6025 (1162, 13790)	2 (0.5, 3)	29.0 (22.0, 33.0)
Enterobacteriae spp. (2)	10428 (1306, 19550)	1.5 (0, 3)	25.5 (22.0, 29.0)
Escherichia coli (1)	331 (N/A)	0 (N/A)	32.0 (N/A)

IQR = interquartile range. N/A = not applicable.

Table 2. Mastitis prevalence estimates based on a mastitis definition as somatic cell count (SCC) >200,000 cells/ml (SCC>200), successful isolation of a pathogenic bacterial species, and Bayesian latent class analysis for 173 dairy cattle in South Africa sampled during 2008.

	SCC >200	Pathogen recovery	Latent-class
Quarter	**Prevalence (95% CI)**	**Prevalence (95% CI)**	**Prevalence (95% PI)**
Right front	0.72 (0.64, 0.78)	0.56 (0.49, 0.64)	0.33[a] (0.25, 0.42)
Right hind	0.57 (0.49, 0.64)	0.37 (0.30, 0.44)	0.32[a] (0.24, 0.41)
Left front	0.40 (0.33, 0.48)	0.21 (0.16, 0.28)	0.23[b] (0.17, 0.31)
Left hind	0.34 (0.27, 0.41)	0.13 (0.08, 0.18)	0.16[c] (0.10, 0.22)
Overall (cow-level)	0.81 (0.75, 0.87)	0.59 (0.52, 0.66)	0.54 (0.45, 0.63)

Latent-class estimated prevalences without superscripts in common are different at $P < 0.05$
CI = confidence interval. PI = probability interval.

Table 3. Accuracy of California milk cell test (CMCT) and milk electrical resistance (MER) for the detection of mastitis at the quarter level based on a quarter-level somatic cell count >200,000 cells/ml (SCC>200), successful isolation of a pathogenic bacterial species, and Bayesian latent class analysis of 173 dairy cattle in South Africa sampled during 2008.

Test	Cut-off (score)	SCC>200		Pathogen recovery		Latent class analysis	
		Sensitivity-% (95% CI)	Specificity-% (95% CI)	Sensitivity-% (95% CI)	Specificity-% (95% CI)	Sensitivity-% (95% PI)	Specificity-% (95% PI)
CMCT	0	100	0	100	0	100	0
	1	67.4 (58.2, 75.6)	86.9 (82.7, 90.2)	77.3 (70.0, 83.3)	76.5 (70.4, 81.7)	94.5 (85.8, 99.7)	77.7 (73.0, 82.1)
	2	35.2 (30.2, 40.5)	97.9 (95.6, 99.1)	48.1 (41.3, 55.0)	94.8 (92.3, 96.6)	68.9 (58.5, 79.2)	98.7 (96.1, 99.8)
	3	11.2 (6.8, 17.8)	100 (98.6, 100)	15.7 (5.9, 33.8)	98.9 (97.4, 99.6)	22.1 (16.0, 29.4)	99.7 (98.7, 100)
MER	<55 mΩ/cm	100	0	100	0	100	0
	<30 mΩ/cm	52.0 (45.1, 58.9)	86.9 (82.7, 90.2)	63.4 (49.6, 75.4)	81.4 (77.5, 84.8)	89.7 (81.8, 97.7)	86.8 (82.6, 91.0)
	<25 mΩ/cm	20.2 (16.2, 24.9)	97.9 (95.6, 99.1)	25.9 (16.8, 37.6)	95.7 (93.3, 97.3)	41.2 (33.1, 49.6)	98.9 (97.1, 99.9)

CI = confidence interval. PI = probability interval.

Table 4. Area under the receiver-operating characteristics curve (AUC) for the California milk cell test (CMCT) and milk electrical resistance (MER) for the detection of mastitis at the quarter level based on a somatic cell count >200,000 cells/ml (SCC>200), successful isolation of a pathogenic bacterial species, and Bayesian latent class analysis of 173 dairy cattle in South Africa sampled during 2008.

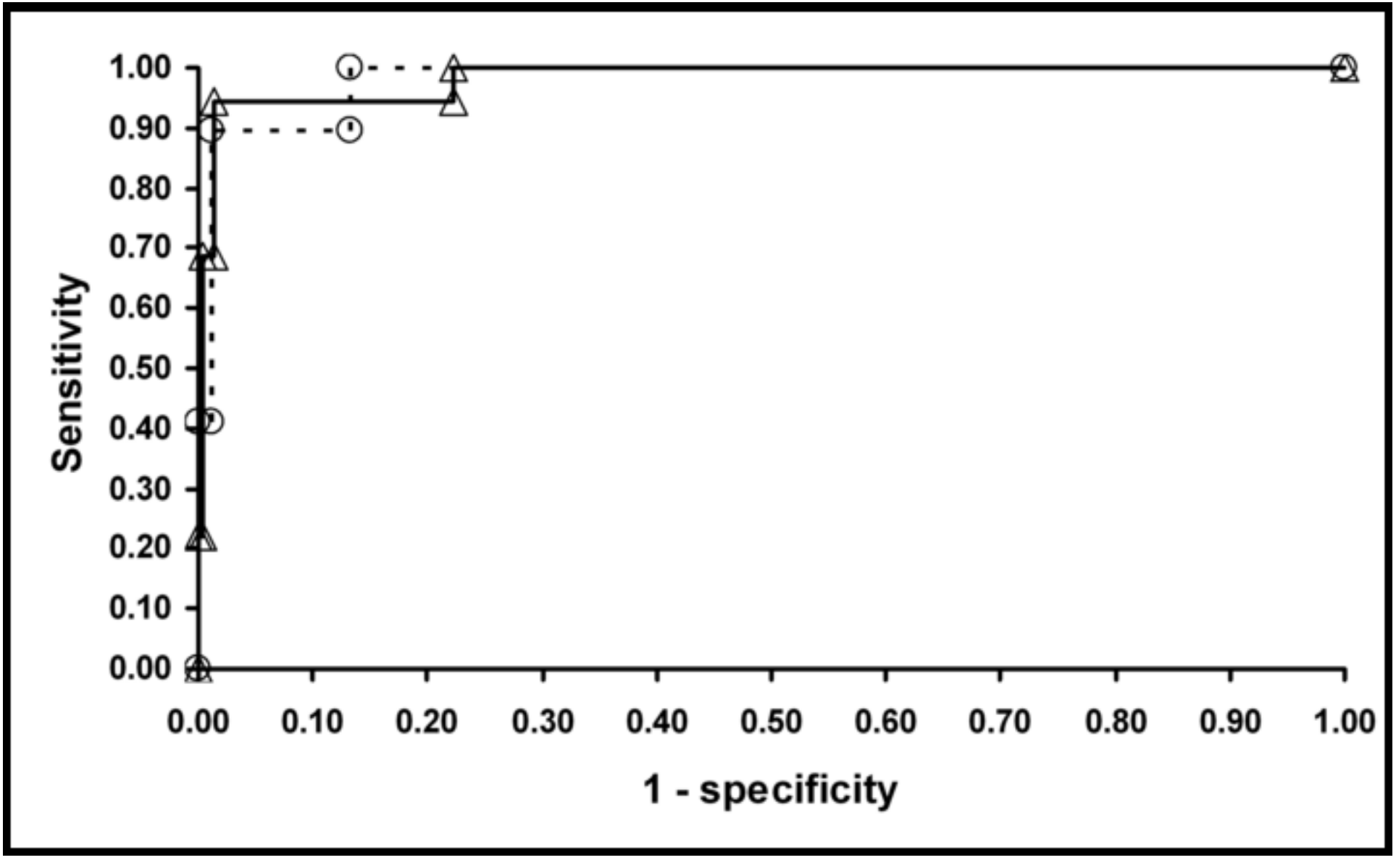
	SCC>200	Pathogen recovery	Latent class
Test	**AUC (95% CI)**	**AUC (95% CI)**	**AUC (95% PI)**
CMCT	0.790 (0.730, 0.845)	0.810 (0.745, 0.865)	0.931 (0.878, 0.967)
MER	0.705 (0.640, 0.760)	0.735 (0.670, 0.795)	0.904 (0.859, 0.950)
P value†	0.006	0.019	0.257

CI = confidence interval. PI = probability interval.

†Based on Bayesian modeling or Monte Carlo simulations

Figure legends

Figure 1. Receiver-operating characteristic curve to diagnose mastitis based on the California milk cell test (solid line, triangles) and milk electrical resistance scores (dashed line, circles) based on a Bayesian latent class analysis without assuming a gold standard within 173 dairy cattle in South Africa sampled during 2008.


Sensitivity
1.00
0.90
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1 - specificity