Use of domestic detergents in the California mastitis test for high somatic cell counts in milk

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The California mastitis test (CMT) is used on farms to identify subclinical mastitis by an indirect estimation of the somatic cell count (SCC) in milk. Four commercially available detergents were compared with a bespoke CMT fluid for their ability to detect milk samples with a SCC above 200,000 cells/ml; differences between the interpretation of the results of the tests by eight operators were also investigated. The sensitivity and specificity of the test were affected by the type of detergent, and by the operators' interpretations. When used by the most sensitive operator, suitably diluted Fairy Liquid performed almost identically to CMT fluid in identifying milk samples with more than 200,000 cells/ml. The average sensitivities achieved by the eight operators for detecting this threshold were 82 per cent for Fairy Liquid and 84 per cent for CMT fluid, and the specificities were 93 and 91 per cent respectively. The other detergents contained less anionic surfactants and were less sensitive but similarly specific.

THE rapid identification of the quarters affected by subclinical mastitis provides valuable information for the control of mastitis in individual cows and for managing herd-level somatic cell counts (SCC). An early method for detecting subclinical mastitis, based on the 'Whiteside reaction' (Whiteside 1939), was developed by Schalm and Noorlander (1957) and became known as the California mastitis test (CMT). It is still widely used for identifying quarters affected by subclinical mastitis, both on commercial farms (Busato and others 2000), and in trials for research purposes (Fabre and others 2004). Whiteside (1939) described a reaction between sodium hydroxide and milk that resulted in the thickening and separation of mastitic milk (Whiteside 1939, Murphy and Hanson 1941). However, the test had limitations, because some positive reactions were difficult to detect, and because the reaction would eventually occur in normal milk. The CMT was developed by finding a reagent that would enhance the release of 'abnormal material', which later proved to be DNA from somatic cells (Carroll and Schalm 1962), and eliminate the confounding effect of milk fat on the reaction. Schalm and Noorlander (1957) identified suitable reagents as the sodium or potassium salts of long chain fatty acids, alkyl sulphates, alkyl sulphonates, alkyl arylsulphates or alkyl arylsulphonates. The combination of milk with such an anionic surface-active agent dissolves the walls of any cells in the milk, causing them to rupture and release their contents. The extent of this reaction increases with the cell count of the milk, and subjective scoring on an ordered categorical scale is used to quantify it. Differences between operators in the interpretation of the CMT are inevitable owing to the subjective nature of the test (Miller and Kearns 1966, Wesen and others 1968, Brolund 1985) but there have been no published investigations of these differences.

A special reagent for the test is marketed under the name 'CMT – Test' (Bovivet), but domestic detergents are quite frequently used by veterinary practitioners and farmers, because they are cheaper and more readily available. However, their performance has not been formally validated in the UK. This study investigated the performance of four proprietary detergents as reagents for the CMT, and the variations in the interpretations of the test by eight operators. The ability of the different reagents and operators to correctly identify milk samples with a SCC of more than 200,000 cells/ml was analysed.

MATERIALS AND METHODS

Four commercially available domestic detergents were diluted with water to produce a fluid with a consistency similar to that of milk; the products and dilution rates required are shown in Table 1. Red food colouring was added to each fluid at the rate of 1 ml in 200 ml test fluid, to improve the visibility of the reaction. Individual quarter samples of milk were collected from 40 cows (160 samples in total), during morning milking on a commercial farm with a rolling bulk SCC of approximately 200,000 cells/ml. The samples were stored at 4°C for seven hours. The CMT was then carried out on each of the quarter samples, using each of the test fluids and the CMT fluid (Bovivet) which contains water, anionic tenside, colour and preservative.

Approximately 3 ml of milk was placed in a CMT paddle and an equal volume of the test fluid was added. The paddle was gently agitated and the reaction was scored simultaneously from 0 to 3 by eight operators, who were blind to the identity of the samples and the test fluids. The descriptions of the reaction used to allocate the scores are given in Table 2. There was a slight modification of the original categories used by Schalm and Noorlander (1957), in that the 'trace' category was omitted to simplify the procedure. Five of the participants were experienced users of the CMT test and three had never previously performed the test. A single-page standard operating procedure was used to train the inexperienced operators, such as might be used to train farmers using the test for the first time. The four quarter samples of one cow with one test fluid were assessed in the same paddle but the order of cows and fluids was randomised.

SCC analysis

After 48 hours storage at 4°C the milk samples were analysed for SCC by the Fossomatic method at an accredited laboratory.

Statistical analysis

The numbers and proportions of test scores recorded with each fluid reagent by each operator were calculated (Figs 1, 2). Data on the effects of the reagents and operators were also analysed with a threshold test score of 'low' for scores of 0 and 1, and 'high' for scores of 2 and 3.

The SCC of each quarter was $\log_{10}$-transformed to normalise the data, and the transformed data were used in the analysis. A scatter plot of $\log_{10}$ SCC against test score was made to assess the relationship between the two measurements visually (Fig 3). The purpose of cow-side tests is to identify infected quarters, and the SCC was used as a threshold measurement; a SCC of more than 200,000 cells/ml was taken to indicate the likely presence of a bacterial infection in the mammary gland (Schepers and others 1997, National Mastitis Council 1999).

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TABLE 1: Comparison of reagents with the special California mastitis test (CMT) fluid, and the dilutions used

Reagent	Product	Fluid:water ratio	Cost/l as used
1	CMT fluid	100:0	£6.00
2	ASDA smartprice washing up liquid	80:20	11·2p
3	Fairy Liquid (P&G)	20:80	46·8p
4	Tesco value washing up liquid	80:20	11·2p
5	ASDA original detergent	20:40	38·6p

TABLE 2: Descriptions of the reactions observed in the California mastitis test used to allocate the milk samples to the four categories and scores

Category	Score	Description of reaction
Negative	0	Mixture of milk and test fluid stays unchanged and can easily be shaken
Weakly positive	1	Mixture is slightly mucous but can still be shaken
Positive	2	With movement of the mixture an unmistakable mucous formation can be seen. It is still possible to tip a small portion of the mixture out
Strongly positive	3	Jelly-like, mucous consistency is formed and it is difficult to shake the mixture. It is no longer possible to tip out any surplus liquid

Using a SCC of more than 200,000 cells/ml as a positive outcome and a test score of 'high' as a positive CMT result, the sensitivity, specificity, positive predictive value and negative predictive value (Petrie and Watson 1999) of the CMT test for identifying infected quarters were calculated. This procedure was applied to all the test scores together and also to the test scores grouped by reagent and by operator.

Statistical models To assess the differences between the reagents and the operators in their ability to identify the specified SCC threshold correctly, general linear models were constructed. A Bernoulli outcome (1 or 0), calculated as shown in Table 3, was used to provide an indicator of 'correct' SCC. Operator 6 and reagent 1 were taken as reference categories against which to compare the performance of the other operators and reagents.

Two models were specified. First, conditional logistic regression (Hosmer and Lemeshow 1989) was used to compare the effects of the reagents and operators with scores being matched within quarter, and secondly a random effects model was applied to allow for repeated measures within a quarter within a cow (Snijders and Bosker 1999, Burton and others 1999). Both models resulted in similar covariate coefficients (and therefore biological interpretation), but the second model is presented because it made it possible to compare the variations between quarters and between cows.

The modelling was performed in a Bayesian context. The general linear mixed model for the Bernouilli response was specified in a standard manner (Zeger and Karim 1991, Burton and others 1999) as an unconditional logistic regression model with random effects. The modelling strategies have been described by Green and others (2004) and the models were constructed by using Markov chain Monte Carlo methods with Gibbs sampling and flat prior distributions to represent no strong prior estimates of the parameters (Gilks and others 1996, Spiegelhalter and others 2004) in WinBUGS, v 1.3 (Spiegelhalter and others 2000). 'Reagent' and 'operator' were fitted as fixed effects and the 95 per cent credibility interval of the odds ratio was used to assess the relationship between an explanatory covariate and the response variable. The validity and fit of the model were examined as described by Green and others (2004). Three versions of each model were run in parallel, starting from different values. Their convergence was examined by informal visual assessments of the growing chains (Gilks and others 1996) and the Gelman Rubin convergence diagnostic (Brooks and Gelman 1998). The fit of the versions was assessed visually using plots of amalgamated Pearson residuals and higher level Bayesian residuals (Green and others 2004). Outlying data points were investigated to check whether they had a large influence on the values of the parameters and, when deemed necessary, the models were re-run after they had been excluded; none affected the interpretation of the model.

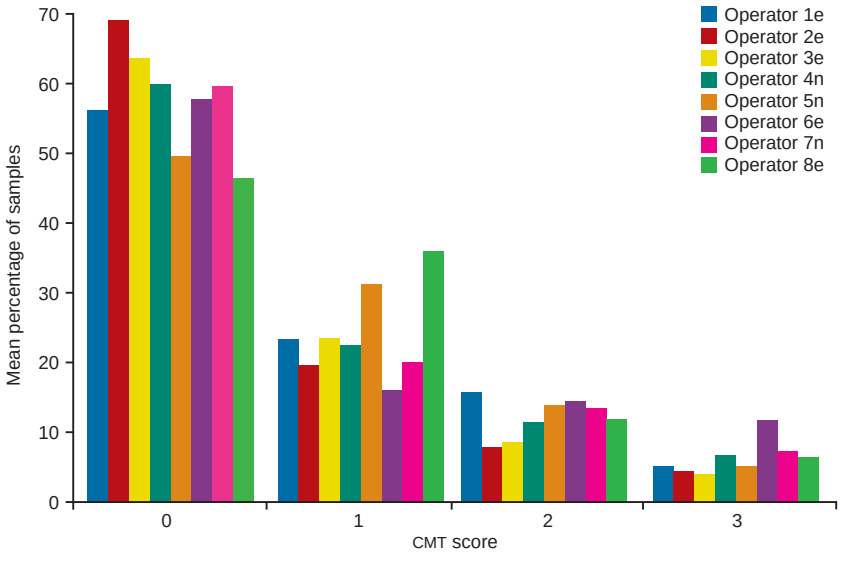

FIG 2: Mean percentage of 160 samples of milk allocated different scores in the California mastitis test (CMT) using five reagents by eight operators. e Experienced operator, n New operator

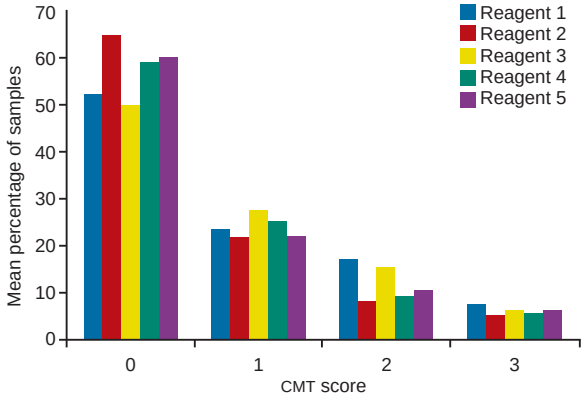

FIG 1: Mean percentage of 160 samples of milk allocated different scores in the California mastitis test (CMT) by eight operators using five different reagents

RESULTS

Fig 1 shows the mean percentages of the milk samples given different CMT scores by all eight operators with each of the five reagents. The percentages with CMT fluid and reagent 3 were very similar. Fig 2 shows the mean percentages of the milk samples allocated different scores by each of the operators with all the reagents. Operators 1, 5 and 8 tended to score fewer samples

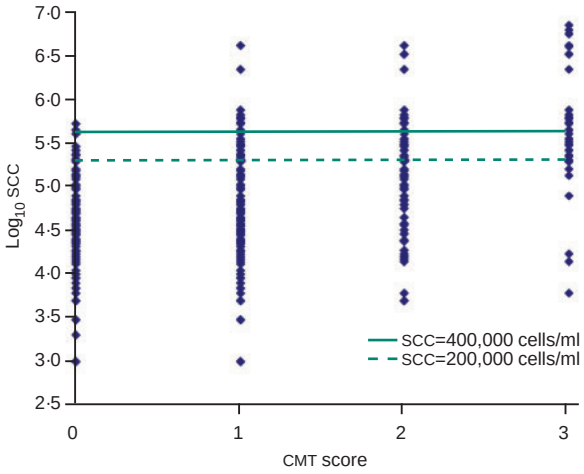

FIG 3: Scatter plot of the $\log_{10}$ somatic cell count (SCC) (cells/ml) of the samples of milk allocated different scores in the California mastitis test (CMT) by eight operators using five different reagents

TABLE 3: Calculation of the outcome variable for the Bernoulli models

SCC	Test score	Outcome	Reason
>200,000	2 or 3	1	Correct identification of SCC >200,000
>200,000	0 or 1	0	Incorrect
≤200,000	2 or 3	0	Incorrect
≤200,000	0 or 1	1	Correct identification of SCC ≤200,000

SCC Somatic cell count per ml

TABLE 4: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of using a California mastitis test (CMT) score of 2 or 3 to identify somatic cell counts (SCCs) above 200,000 and above 400,000 cells/ml, across all eight operators and all five reagents

SCC (cells/ml)	Sensitivity	Specificity	PPV	NPV
>400,000	0·87	0·89	0·48	0·98
>200,000	0·72	0·95	0·78	0·93

as 0 and operator 6 scores more samples as 3. Full agreement between all eight operators in allocating the same score with a single reagent was low, ranging from 30·6 per cent to 42·5 per cent of samples, depending on the reagent. The most common discrepancy was between a score of 0 and a score of 1.

When the data on the effects of the reagents and operators were classified with a threshold score of either 'low', that is, scores of 0 and 1, or 'high', that is, scores of 2 and 3, reagents 1 and 3 gave a larger percentage of the samples in the high category than the other reagents. There were still wide variations between operators (Fig 4). However, the percentage of full agreement between the operators using a single reagent was much higher than with the four scores (76·9 to 81·9 per cent). Taking both operator and reagent into consideration, the lowest apparent prevalence of a high score (6·9 per cent) was reported by operator 2 using reagent 2 and the highest (29·4 per cent) was reported by operator 6 using reagent 1 (Fig 4). The prevalence of a SCC of more than 200,000 cells/ml measured by the Fossomatic method in the individual quarter samples was 20 per cent.

The CMT scores across all the reagents and all the operators were compared with the SCC results, to evaluate the accuracy of the CMT in predicting the SCC as measured by the Fossomatic method. In tests on samples with a SCC of more than 200,000 cells/ml (256 test results across all the operators and reagents), 4·4 per cent were scored 0, 23·9 per cent were scored 1, 41·1 per cent were scored 2 and 30·6 per cent were scored 3. Thus 71·7 per cent of the samples with more than 200,000 cells/ml were given a 'high' CMT score. For the 10 per cent of samples with a SCC of more than 400,000 cells/ml the percentages in categories 0 to 3 were 0·8, 12·0, 33·6 and 53·6 per cent, respectively.

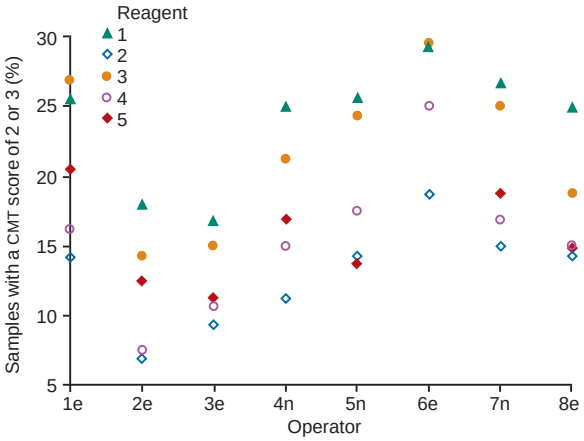

FIG 4: Percentages of the 160 samples of milk allocated a score of 2 or 3 in the California mastitis test (CMT) by eight operators using five different reagents; 20 per cent of the samples had a somatic cell count (SCC) of more than 200,000 cells/ml. e Experienced operator, n New operator

The sensitivity, specificity, positive predictive value and negative predictive value of using a CMT score of 2 or 3 to identify a SCC of more than 200,000 or 400,000 cells/ml by using the data from all the reagents and all the operators are shown in Table 4. A 'high' CMT score had a sensitivity of 87 per cent for a SCC of more than 400,000 cells/ml and 72 per cent for a SCC of more than 200,000 cells/ml; the equivalent specificities were 89 per cent and 95 per cent. As expected, the sensitivity for predicting a SCC of more than 200,000 cells/ml was lower than for more than 400,000 cells/ml and the positive predictive value for samples with more than 200,000 cells/ml was considerably higher than for more than 400,000 cells/ml.

The sensitivity of the operators in determining a SCC of more than 200,000 cells/ml by scoring the sample 'high' ranged from 54 per cent to 89 per cent and the positive predictive value ranged from 68 per cent to 90 per cent (Table 5). Operator 6 identified the highest proportion of the 'true positives' (highest sensitivity) and had the highest negative predictive value. Reagents 1 and 3 produced very similar results (Table 6) and had the highest sensitivity.

The ability of operator 6, using reagents 1 and 3, to correctly categorise samples with more than 200,000 cells/ml or 200,000 cells/ml or less, using CMT scores of 0 or 1 for 200,000 cells/ml or less, and 2 or 3 for more than 200,000 cells/ml, was good. Across all the reagents, this operator achieved 89 per cent accuracy in identifying samples with more than 200,000 cells/ml by scoring them 2 or 3. With the original CMT reagent, operator 6 correctly identified 86 per cent of samples with 200,000 cells/ml or less (scored 0 or 1), 81 per cent of samples with more than 200,000 to 400,000 cells/ml (scored 2 or 3) and 100 per cent of samples with more than 400,000 cells/ml (scored 2 or 3). Using reagent 3, the closest in overall performance to the CMT fluid, the same operator produced almost identical results for samples with 200,000 cells/ml or less (87 per cent correctly identified as 0 or 1), and correctly identified all the samples in the other two categories (scored as 2 or 3).

TABLE 5: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the five reagents and eight operators: a California mastitis test (CMT) score of 2 or 3 is taken as a positive test; a somatic cell count of >200,000 cells/ml is taken as a positive result

	Sensitivity	Specificity	PPV	NPV
Operator				
1 (experienced)	0·78	0·93	0·75	0·94
2 (experienced)	0·54	0·99	0·91	0·90
3 (experienced)	0·57	0·98	0·90	0·90
4 (new)	0·73	0·96	0·82	0·93
5 (new)	0·74	0·95	0·78	0·94
6 (experienced)	0·89	0·89	0·68	0·97
7 (new)	0·77	0·94	0·75	0·94
8 (experienced)	0·72	0·96	0·82	0·93
Reagent				
1	0·84	0·91	0·70	0·96
2	0·57	0·98	0·87	0·90
3	0·82	0·93	0·75	0·95
4	0·67	0·98	0·87	0·92
5	0·68	0·95	0·79	0·92

TABLE 6: Random effects logistic regression model for the outcome of a 'high' California mastitis test (CMT) score (2 or 3) and a somatic cell count (SCC) of >200,000 cells/ml, that is, the 'correct' identification of a high SCC*

	Odds ratio	Lower 2·5%	Upper 97·5%
Reagent (reference is reagent 1 [CMT fluid])			
2	1·71	1·35	2·17
3	0·84	0·67	1·05
4	1·35	1·07	1·70
5	1·46	1·15	1·83
Operator (reference is operator 6 [experienced])			
2 (experienced)	1·46	1·07	1·98
3 (experienced)	0·97	0·72	1·30
4 (new)	0·89	0·66	1·20
5 (new)	0·39	0·29	0·52
1 (experienced)	0·71	0·53	0·95
7 (new)	0·90	0·67	1·21
8 (experienced)	0·31	0·23	0·41
Between quarter variance		0	1·65
Between cow variance		3·08	5·68

* Intercept 1·609, Between quarter variance 0·474, Between cow variance 4·243

DISCUSSION

The CMT is commonly used to identify infected quarters that do not show clinical signs. It is important for farmers to be able to identify which quarters of a cow are infected and producing milk with a high cell count, to help decide whether and how they should be treated, whether the milk should be withheld from sale, whether the cows or quarters should be dried off, and ultimately whether the cows should be culled. The test can also be used to evaluate the success of treatments when clinical signs are no longer apparent, and it is often used to identify the infected quarter or quarters of a cow with a high SCC. However, it is also used in the absence of cell counting facilities to give a qualitative indication of cell count and infection. It can be of great value, but the cost of the special reagent still prevents some farmers from using the test. Some farmers use proprietary household detergents as a cheaper alternative, but there is no published information on how they perform in comparison with the special CMT fluid.

In this experiment the main objective was to compare the ability of five different reagents and eight operators to detect a threshold of 200,000 cells/ml, which was considered to be biologically meaningful on the basis of results reported by Schepers and others (1997) and is commercially important. A SCC or more than 200,000 cells/ml has become widely accepted as an indication of the likely presence of a bacterial infection within the mammary gland (National Mastitis Council 1999). The division of the CMT scores into low (0 or 1) or high (2 or 3) gave good sensitivity and specificity for the identification of samples with a cut-off point of 200,000 cells/ml; the cut-off point with the greatest practical relevance is therefore between scores of 1 and 2, and the most important aspect of the test is its ability to differentiate between scores of 0 or 1 and 2 or 3. The parameters calculated demonstrate the relative performance of the different reagents and operators in identifying infected quarters, and their absolute performance in relation to a SCC threshold identified by the Fossomatic method. There are limitations to the sensitivity and specificity of SCC in relation to bacteriological results and neither is a perfect predictor of mammary infection, but the SCC remains the most practical, commonly used and economically influential indicator of infection.

Reagents

The results show that some domestic detergents can be used to detect differences in SCC but not all the detergents performed equally well. Reagents 2, 4 and 5 (the cheaper detergents) were less likely to give a reaction with samples of lower SCC than with reagent 3 or the CMT fluid. This may be related to the fact that the cheapest domestic detergents contained lower concentrations of anionic surfactants (5 to 15 per cent in reagents 2 and 4 compared with 15 to 30 per cent in reagents 3 and 5). All five reagents indicated that a similar proportion of the samples had a score of 3. The 'cheaper' reagents could detect samples with scores of 2 or 3, and the highest SCC, but they were less sensitive than CMT fluid in their ability to differentiate between samples with scores between 1 and 2, closer to the 200,000 cells/ml threshold. The type of reagent affected the apparent prevalence of samples with a high SCC considerably; with the least sensitive reagent (reagent 2), the prevalence of samples with scores of 2 or 3 was estimated as 13 per cent, compared with 24 per cent with CMT fluid. However, reagent 3 gave a prevalence of 21 per cent, very close to the results of the CMT fluid and the Fossomatic tests. The relative performance of the different test fluids when used by the eight operators was quite consistent (Fig 4).

For the purposes of identifying infected quarters, the sensitivity of the test takes priority. In fact there was more variation between the sensitivity than between the specificity of the five reagents. Household detergents can be obtained more easily and more cheaply than CMT fluid, and therefore may be used more readily by farmers. These results show that the use of cheap products may result in an underestimation of the prevalence of samples with high cell counts, and farmers should be aware of this possibility.

It is important that the test fluid and the milk sample are well mixed, and the ease of mixing depends on the viscosity of the test fluid. All the household detergents needed to be diluted, but by different amounts in accordance with their initial viscosities. The reaction was visible much more clearly when red colouring was added to the test fluids.

Operator differences

There were differences in the interpretation of the CMT by the eight operators, most often between samples with scores of 0 or 1, which, it could be argued, would not be likely to affect management decisions. When the reaction was stronger, there were fewer differences. Agreement on the difference between samples with 'low' and 'high' scores was much better than on the allocation of the samples to the four scores. However, there were still differences between the operators which resulted in differences in their sensitivity, specificity, predictive values and their estimates of the prevalence of samples with a high SCC. Their sensitivity varied more than

their specificity, suggesting that the greatest risk from the variations would be the under-detection of samples with a high SCC. There were statistically significant differences between the operators in their ability to detect the 200,000 cells/ml SCC threshold (Table 6), and different operators might therefore make different management decisions if they were based on their interpretation of the CMT. There was no clear distinction between the experienced and the new operators, suggesting that a written and illustrated protocol is suitable for training purposes. Operators 2 and 3, who had a considerably lower sensitivity and higher specificity than the other operators, were, in fact, experienced. They may have developed their own idiosyncrasies of scoring, unlike the newly trained operators who followed the standard operating procedure, or they may have had experience of more extreme reactions to the test, reducing their sensitivity to milder reactions. Several operators felt that the score given to a sample could be influenced by the appearance of the adjacent samples in the paddle.

Reagent 3, sufficiently diluted, was the most suitable alternative to the special CMT reagent, being closest in sensitivity and specificity, and resulting in a similar prevalence of samples with scores of 2 or 3. The cheaper detergents identified the samples with the highest cell counts in a similar way to CMT fluid, but tended to underestimate the incidence of samples with a lower SCC. Products with a higher content of anionic surfactants appear to be preferable. There were differences in the interpretation of the test by the eight operators, but the newly trained operators performed more consistently as a group than the experienced operators, suggesting that periodic retraining of operators might be advisable. There was about 80 per cent agreement between the operators in their scoring of samples with 'high' or 'low' SCC, and the different reagents had little effect on this agreement, but their allocation of samples to individual CMT scores was more variable.

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References

BROLUND, L. (1985) Cell counts in bovine milk. Causes of variation and applicability for diagnosis of subclinical mastitis. *Acta Veterinaria Scandinavica Supplement* **80,** 1-123

BROOKS, S. P. & GELMAN, A. (1998) Alternative methods for monitoring convergence of iterative simulations. *Journal of Computational and Graphical Statistics* **7,** 434-455

BURTON, P. R., TILLER, K., GURRIN, L. C., MUSK, A. W., COOKSON, W. O. C. M. & PALMER, L. J. (1999) Genetic variance components analysis for binary phenotypes using generalized linear mixed models (GLMMs) and Gibbs sampling. *Genetic Epidemiology* **17,** 118-140

BUSATO, A., TRACHSEL, P., SCHALIBAUM, M. & BLUM, J. W. (2000) Udder health and risk factors for sub-clinical mastitis in organic dairy farms in Switzerland. *Preventive Veterinary Medicine* **44,** 205-220

CARROLL, E. J. & SCHALM, O. W. (1962) Effect of deoxyribonuclease on the California Test for Mastitis. *Journal of Dairy Science* **45,** 1094-1097

FABRE, J. M., GARDEY, L., BOUSQUET, E., BOSQUET, G., LEPOUTRE, D., SEEGERS, H. & BERTHELOT, X. (2004) Treatment of sub-clinical mastitis in lactating cows: evaluation of a new protocol called 'simultaneous treatment'. Proceedings of the British Mastitis Conference. Stoneleigh, UK, October 13, 2004. pp 1-18

GILKS, W. R., RICHARDSON, S. & SPIEGELHALTER, D. J. (1996) Markov Chain Monte Carlo in Practice. London, Chapman and Hall

GREEN, M. J., BURTON, P. R., GREEN, L. E., SCHUKKEN, Y. H., BRADLEY, A. J., PEELER, E. J. & MEDLEY, G. F. (2004) The use of Markov Chain Monte Carlo for analysis of correlated binary data: patterns of somatic cells in milk and the risk of clinical mastitis in dairy cows. *Preventive Veterinary Medicine* **64,** 157-174

HOSMER, D. W. & LEMESHOW, S. (1989) Applied Logistic Regression. Chichester, John Wiley and Sons

MILLER, D. D. & KEARNS, J. V. (1966) Effectiveness of the California mastitis test as a measurement of the leucocyte content of quarter samples of milk. *Journal of Dairy Science* **50,** 683-686

MURPHY, J. M. & HANSON, J. J. (1941) A modified Whiteside test for the detection of chronic bovine mastitis. *Cornell Veterinarian* **31,** 47-55

NATIONAL MASTITIS COUNCIL (1999) Laboratory Handbook on Bovine Mastitis. Madison, National Mastitis Council. p 157

PETRIE, A. & WATSON, P. (1999) Statistics for Veterinary and Animal Science. Oxford, Blackwell Science. pp 168-169

SCHALM, O. W. & NOORLANDER, D. O. (1957) Experiments and observations leading to the development of the California mastitis test. *Journal of the American Veterinary Medical Association* **130,** 199-204

SCHEPERS, A. J., LAM, T. J. G. M., SCHUKKEN, Y. H., WILMINK, J. B. M. & HANEKAMP, W. J. A. (1997) Estimation of variance of components for somatic cell counts to determine thresholds for uninfected quarters. *Journal of Dairy Science* **80,** 1833-1840

SNIJDERS, T. & BOSKER, R. (1999) Multilevel Analysis: an Introduction to Basic and Advanced Multilevel Modelling. London, Sage Publications

SPIEGELHALTER, D. J., ABRAMS, R. K. & MYLES, J. P. (2004) Bayesian approaches to clinical trials and health-care evaluation. In Statistics in Practice. Chichester, John Wiley and Sons

SPIEGELHALTER, D., THOMAS, A. & BEST, N. (2000) WinBUGS Version 1.3. Cambridge, MRC Biostatistics Unit, Institute of Public Health

WESEN, D. P., LUEDECKE, L. O. & FORSTER, T. L. (1968) Relationship between California Mastitis Test reaction and bacteriological analyses of stripping samples. *Journal of Dairy Science* **51,** 679-684

WHITESIDE, W. H. (1939) Observations on a new test for the presence of mastitis in milk. *Canadian Public Health Journal* **30,** 44

ZEGER, S. L. & KARIM, M. R. (1991) Generalized linear models with random effects; a Gibbs sampling approach. *Journal of the American Statistical Association* **86,** 79-86