A study of the effectiveness of a detergent-based California Milk Test (CMT), using Ethiopian and Nigerian domestic detergents, for the detection of high somatic cell count (SCC) in milk (>400,000 cells/ml), and their reliability compared to the commercial UK CMT.

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Mastitis is a prevalent and economically important disease of the lactating dairy cow that can persist undetected in a subclinical state. Due to the infectious nature of mastitis-causing pathogens, it is important for the farmer to detect and control mastitis in order to maximize cow health and well-being to maintain herd health (*Kandeel et al., 2018*).

The California Milk Test (CMT) is an inexpensive, easily-applicable, cow-side test that allows for the subjective estimation of the number of inflammatory (somatic) cells present in milk samples as an assessment of the probability and severity of intramammary infection (*Kandeel et al., 2018*).

The CMT involves combining equal volumes (2-3 ml) of milk with a testing reagent (containing anionic-surface-active agents) (Figure 1). This dissolves the walls of somatic cells, releasing cellular DNA, which agglutinates to give varying degrees of a ‘slimy’ or mucoid appearance depending on the number of cells within the sample. The extent of the reaction increases with the somatic cell count (SCC) (*Leach et al., 2008*). The degree of visible agglutination can be subjectively scored based on an ordinal scale (Table 1) and used as a qualitative test to estimate the SCC of milk samples *(Moroni et al., 2018)*.

Figure 1: Implementing the California Milk Test (CMT) for the diagnosis and targeted control of mastitis in Uganda. Credits: MJ Christian.

Table 1: Descriptions of the reactions observed in the CMT according to each score category (*Leach et al., 2018).*

Category	Score	Description of Reaction
Negative	0	Mixture of milk and test fluid remains the same and can easily be shaken.
Weakly Positive	1	Mixture has a slight mucus appearance but can easily be shaken.
Positive	2	Unmistakable mucus formation can be seen, it is possible still to tip a small proportion of the mixture out.
Strongly Positive	3	Jelly-like consistency is formed and it is difficult to shake the mixture. It is no longer possible to tip out any surplus liquid.

Mastitis is the most widespread infectious disease of cattle in Nigeria and Ethiopia *(Ameh et al., 1999)*, and the most expensive bovine disease due to its capacity to reduce milk yield, profit margins, quality and quantity of milk *(DODD., 1985)*. UK CMT reagents are not easily sourced in Nigeria or Ethiopia, and the cost of importation limits the cost-benefit ratio in identifying and treating mastitis. However, as a domestic detergent-based CMT reagent is accepted in the UK as a cost-effective alternative (*Leach et al., 2008),* this project intends to research if domestic detergents available in Ethiopia and Nigeria can be utilised in the CMT, in place of the UK commercial CMT reagent, to achieve comparable results.

Dilution Study - Method

In the UK, an accepted detergent-based CMT reagent comprises of 40 ml Fairy Liquid to 160 ml water, with 1 ml dark food colouring to enhance visualisation (*Leach et al., 2008*). However, we cannot assume domestic detergents available in Ethiopia and Nigeria will have an equal concentration of anionic-surface-active agents. Therefore, this study undertook a series dilution test to determine the most effective concentration of each detergent for visualising high somatic cell count (SCC) (>400,000 cells per ml milk).

Figure 2: The Ethiopian and Nigerian commercially available domestic detergents and food colouring (indicator) selected for this study, alongside the CMT paddle and UK commercial CMT reagent.

This study takes six commercially-available detergents from Ethiopia (Reagents 1-3) and Nigeria (Reagents 4-6) (Figure 2), and created five different dilutions: 40 ml detergent to 160 ml water; 50 ml detergent to 150 ml water, 60 ml detergent to 140 ml water; 70 ml detergent to 130 ml water; and 80 ml detergent to 120 ml water. Each with 1 ml food colouring from Ethiopia/Nigeria, depending on the detergent country-of-origin.

All dilutions were then tested with 20 quarter milk samples (5 low SCC and 15 high SCC) to see which dilution of each reagent achieved results most equivalent to the UK CMT.

Dilution Study - Result

Ethiopian Reagents, 1-3, Figure 3, are seen to achieve 100% comparability to the UK CMT at different dilutions: 70 ml detergent per 200 ml solution for Reagent 1; 60 ml per 200 ml for Reagent 2; and both 50 ml and 60 ml per 200 ml, in which this study takes an average of 55 ml per 200 ml, for Reagent 3, Table 2.

Nigerian Reagents, 4-6, Figure 3, are seen to be less efficacious, however there are clear optimal dilutions that achieve results most comparable to the UK CMT: 70 ml detergent per 200 ml solution for Reagents 4 and 5; and 60 ml per 200 ml for Reagent 6, Table 2.

Figure 3: The percentage of detergent-based CMT results that achieved the same result as the commercial UK CMT for each different dilution, when testing 20 milk samples.

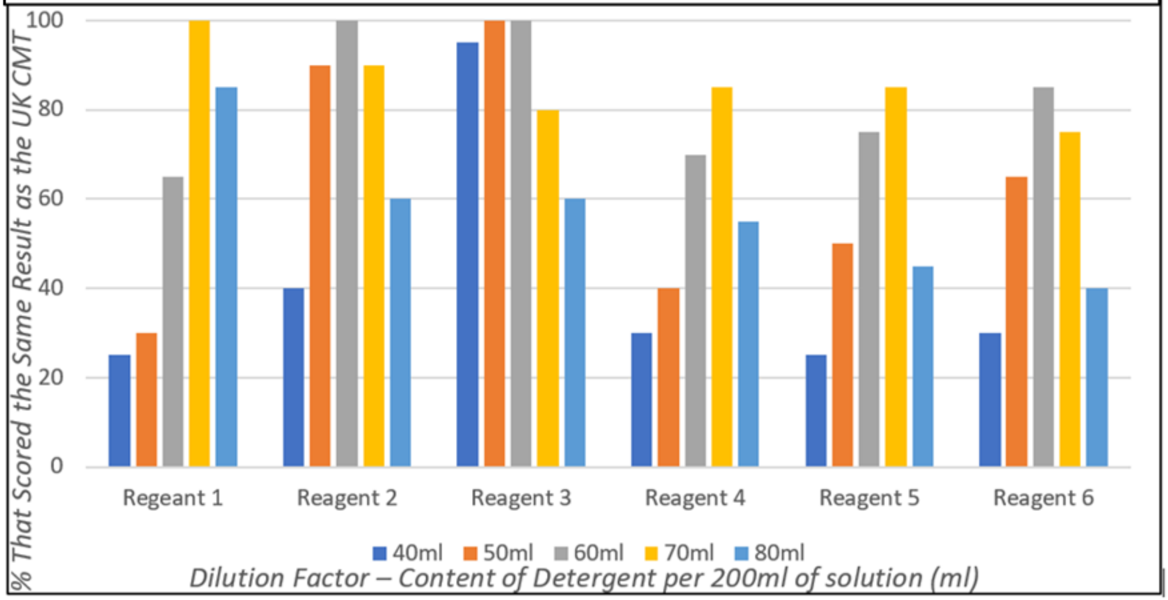

Table 2: The optimal dilution factor of each detergent to achieve results most comparable to the UK CMT that are then used in the validation study.

Reagent 1	70 ml detergent to 130 ml water
Reagent 2	60 ml detergent to 140 ml water
Reagent 3	55 ml detergent to 145 ml water
Reagent 4	70 ml detergent to 130 ml water
Reagent 5	70 ml detergent to 130 ml water
Reagent 6	60 ml detergent to 140 ml water

Validation Study

To verify that the Ethiopian and Nigerian detergent-based CMT reagents reliably indicate a high SCC (>400,000 cells per ml), each reagent was tested against a further 132 milk samples. Two additional operators tested all samples, and samples were submitted for a laboratory SCC reading to affirm the accuracy of the CMT as a diagnostic tool.

This study identifies the SCC point of significance as 400,000 cells per ml, with SCCs >400,000cells/ml indicating a strong positive result and thus an expected CMT score of 2-3, and samples ≤400,000cells/ml we deem as negative to weakly positive, with an expected CMT score of 0-1.

Figure 4 shows the percentage of each reagent, including the UK CMT, to correctly identify samples with a SCC ≤400,000 as score 0-1. The majority of reagents score similarly to the UK CMT, above 95% specificity. Notably, Reagent 3 scored lowest of the reagents, with an average of 84.2% between the three operators, however, this is still an acceptable level of specificity.

Figure 4: The efficacy of each reagent to score negative to weakly positive CMT results (0-1) across 80 samples laboratory-tested as ≤400,000 SCC per ml.

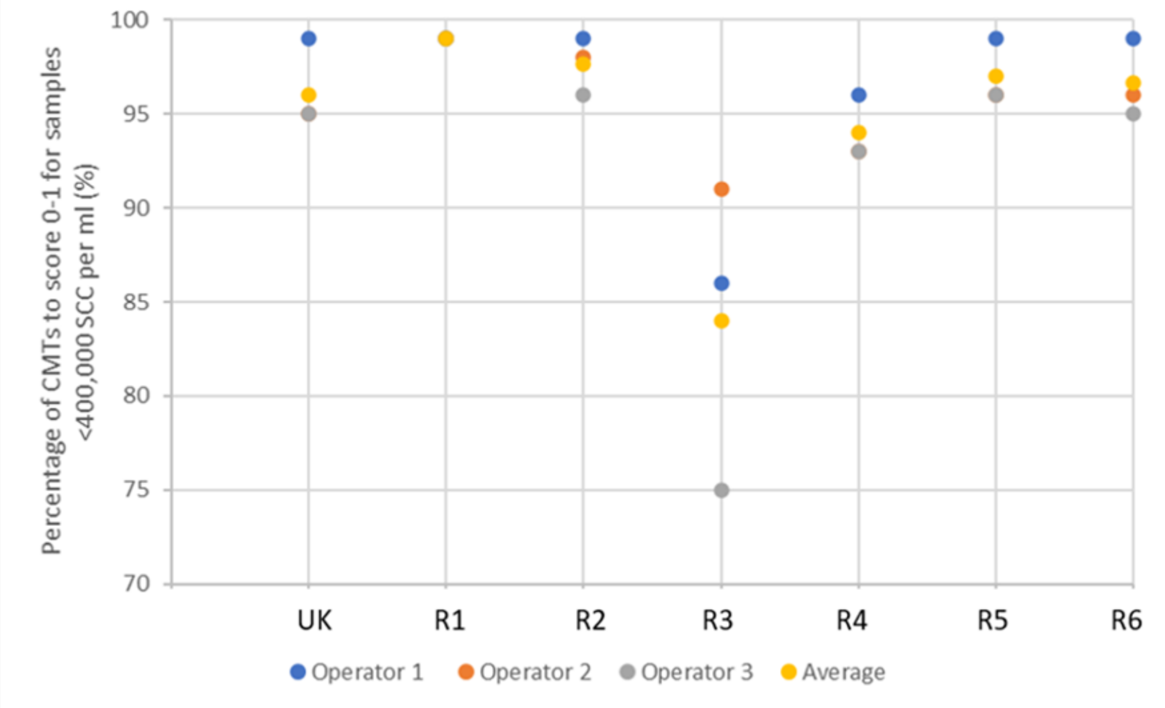

Figure 5, shows the percentage of high SCC (>400,000) samples each reagent correctly identified as score 2-3. Notably, Reagents 1 and 2 prove to be poor reagents in the identification of samples with SCC >400,000, with 28.3% and 53.3% of samples correctly identified respectively. With the UK CMT indicated 76.3% of high SCC samples as score 2-3, Reagents 3 and 4 prove to be of similar efficacy, achieving 75.7% and 81.0% respectively; and Reagents 5 and 6 achieve slightly lower with 68.7% and 74.3% of high SCC samples identified as score 2-3 respectively.

Table 3 shows how all reagents have a high specificity, above 95%, with the exception of Reagent 3 which achieves slightly lower specificity at 84.2%. However, there are greater differences in sensitivity, ranging from 28.2% for Reagent 1, to 80.8% for Reagent 4. This is reflected in the PPV and NPV, where again Reagent 3 is shown to have the lowest PPV, indicating increased false positives; and Reagents 1 and 2 show lower NPV, increased false negatives.

Of the Ethiopian reagents (1-3), Reagent 3 is of highest sensitivity (75.6%, CI% 63.9 - 86.3) and NPV, with the fewest false negatives, and so is most reliable for detecting mastitis. Whereas, Nigerian reagents (4-6), are more efficacious at identifying high SCC milk samples than Ethiopian, with highest sensitivity achieved by Reagent 4 (80.8%, CI% 68.1 – 89.2), which had the fewest false negatives, Table 2.

Figure 5: The efficacy of each reagent to score positive to strongly positive CMT results (score 2-3) across 52 samples laboratory tested as >400,000 SCC per ml.

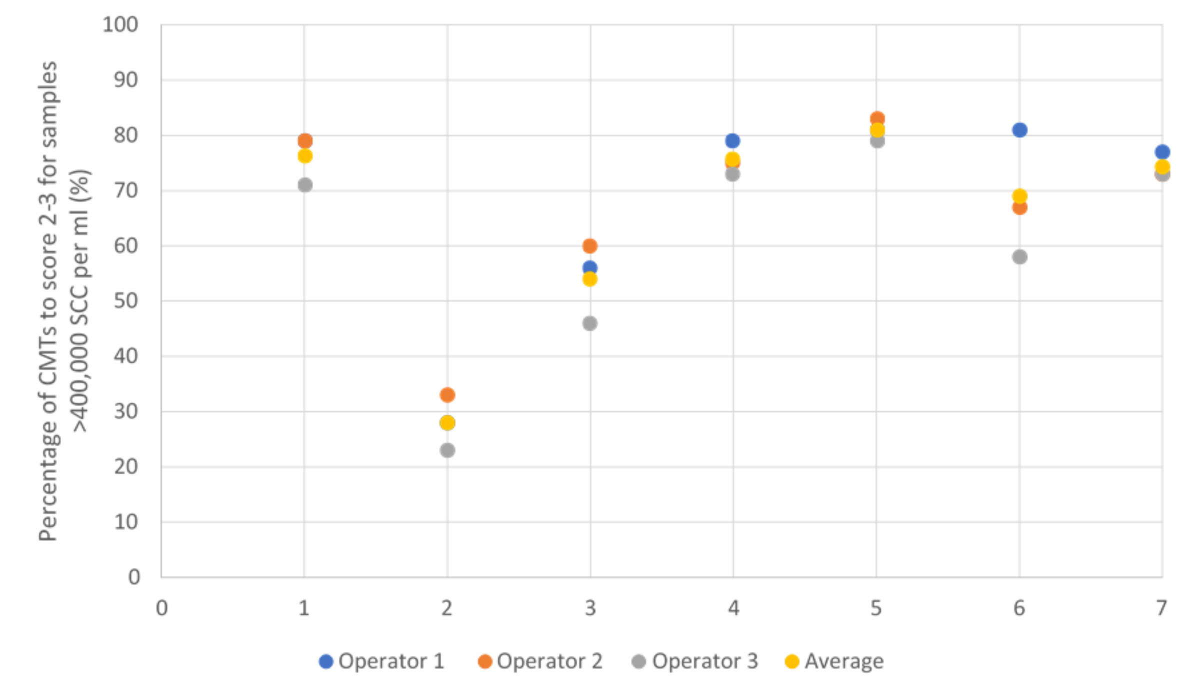

Table 3: The average sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of using the CMT to determine high SCC (>400,000 cells per ml) across different domestic-based CMT reagents and operators.

	Sensitivity		Specificity		PPV	NPV	Range of False Positives	Range of False Negatives
	%	95% CI	%	95% CI				
UK CMT	76.3%	63.9 - 86.3%	96.3%	89.6 - 98.7%	93%	86%	1 - 4	11 - 15
Reagent 1	28.2%	18.3 - 42.3%	98.8%	93.3 - 99.8%	94%	68%	1 - 1	35 - 40
Reagent 2	53.2%	40.5 - 66.7%	97.5%	91.3 - 99.3%	93%	76%	1 - 3	21 - 29
Reagent 3	75.6%	63.9 - 86.3%	84.2%	74.2 - 90.3%	76%	84%	7 - 20	11 - 14
Reagent 4	80.8%	68.1 - 89.2%	93.8%	96.2 - 97.3%	89%	88%	3 - 6	9 - 11
Reagent 5	68.6%	55.7 - 80.1%	97.1%	91.3 - 99.3%	94%	83%	1 - 3	10 - 22
Reagent 6	74.4%	61.8 - 84.8%	96.7%	89.6 - 98.7%	94%	85%	1 - 4	12 - 14

Limitations

Figure 6: Approximately 400 milk samples have been collected from Holstein-Friesian cattle for use in this study.

The CMT is subjective in nature which allows room for bias and operator error. A Laboratory SCC reduces operator error by automatically counting cells, removing bias, but is not without a margin of error.

Ideally all testing in the validation study would have been conducted by the same three operators, however, due to COVID-19 guidelines, this was not feasible.

All samples are taken from UK Holstein-Friesians cows (Figure 6), we can assume indigenous breeds native to Ethiopia and Nigeria, though managed differently, will respond similar to the CMT.

This study identified some areas for further research. Firstly, there may have been differences in reagent efficacy in relation to how long the reagent had been made and left standing. Secondly, the Nigerian food colouring was not dark enough and limited visibility, a darker UK food colouring was added to enable testing. Future studies should look at Nigerian food colouring available for their suitability for use in the CMT.

Conclusion

In the absence of cell counting laboratories, the CMT offers an easy, inexpensive, cow-side test to indicate the presence of intramammary infection and subsequent high SCC. This study investigates the use of six detergents from Ethiopia (Reagents 1-3) and Nigeria (Reagent 4-6) to determine the optimal dilution for use in the CMT, and to validate the sensitivity and specificity comparable to the UK CMT, as an alternative means of screening for mastitis.

All Ethiopian detergent-based CMT reagents (1-3) proved to be less effective than the UK CMT, however Reagent 3 had the highest sensitivity (75.6%, CI% 63.9 - 86.3) and NPV (84%), and therefore, despite the lowest specificity and PPV, is the best at ruling out disease and identifying cows of likely high SCC to treat. Thus, this study recommends the use of detergent 'Shagan' (Reagent 3) at a dilution of 55 ml detergent to 145 ml water, with 1 ml of indicator, for use in the CMT as an alternative to the UK CMT.

All Nigerian detergent-based reagents (4-6) proved to be more effective than the Ethiopian reagents at identifying high SCC in milk. However, Reagent 4 proved to be the highest sensitivity (80.8%, CI% 68.1 – 89.2), and NPV (88%), and therefore, this study recommends the use of the detergent 'Sunlight' (Reagent 4) at a dilution of 70 ml detergent to 130 ml water, with 1 ml indicator, for use in the CMT to identify high SCC.

The use of detergent-based CMTs will improve the health and wellbeing of Ethiopian and Nigerian cattle through the diagnosis and control of mastitis, thereby increasing production and quality of milk, and improving the economic value of the dairy subsector, Figure 7.

This research is contributing to a larger ongoing study into mastitis in both countries, and will be used to produce educational pamphlets and videos to promote the CMT for the control and management of mastitis, for the benefit of Nigerian and Ethiopian cattle and people.

Figure 7: A Fulani herder tending his cattle in Nigeria. Herds have high prevalence of mastitis and herders are looking for new ways to measure, control and prevent mastitis infection to improve cattle health and productivity, Credits J.C. Townsley, (*Vance and Suarez-Martinex., 2020).*

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