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A study of the effectiveness of a detergent-based California mastitis test (CMT), using Ethiopian and Nigerian domestic detergents, for the detection of high somatic cell counts in milk and their reliability compared to the commercial UK CMT

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RESEARCH ARTICLE

A study of the effectiveness of a detergent-based California mastitis test (CMT), using Ethiopian and Nigerian domestic detergents, for the detection of high somatic cell counts in milk and their reliability compared to the commercial UK CMT

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Abstract

Background: The California mastitis test (CMT) is a simple cow-side indicator of the somatic cell count (SCC) in milk, providing a useful tool in identifying cases of subclinical mastitis in cattle. Mastitis, and in particular subclinical mastitis, is a major concern in Ethiopia and Nigeria, yet detection is challenging due to cost and access to commercial CMT reagents.
Methods: Commercially available domestic detergents from Ethiopia and Nigeria were compared (n = 3 for each country) with the UK commercial CMT reagent in their ability to detect high SCC (>400,000 cells/ml milk). Sensitivity and specificity of the CMT test were calculated for the different detergents and positive and negative predictive values were established.
Results: The average sensitivities of the tests ranged from 28-75% for the Ethiopian detergents and 68-80% for the Nigerian detergents, compared to 76% for the UK domestic detergent. Test specificities were 84-98%, 93-97% and 96%, respectively.
Conclusions: Overall, the detergents demonstrated higher specificity than sensitivity. Nigerian detergents performed better than the Ethiopian products, however, the study identified suitable domestic detergents from both Ethiopia and Nigeria, comparable to the UK commercial CMT reagent, and we recommend their use as alternative CMT reagents for livestock-keepers to aid in cost-effective diagnosis of

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mastitis.

This article is included in the AgriKnowledge

gateway.

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Introduction

Mastitis is regarded globally as the most common infectious disease in dairy cattle, as well as the most economically important (Halasa *et al.*, 2007; Makolo *et al.*, 2019; Shittu *et al.*, 2012; Suleiman *et al.*, 2013). The disease can be both clinical and subclinical in nature. Clinical mastitis can be visibly diagnosed and treated on routine physical examination, while subclinical mastitis often remains undetected (Reddy *et al.*, 2014). An array of causative pathogens can be involved, entering the teat canal and multiplying in the udder, however most cases of mastitis are caused by a small group of bacteria including *Staphylococcus aureus*, *Streptococcus uberis*, *Escherichia coli* and *Mycoplasma species* (Carrillo-Casas & Miranda-Morales, 2012).

Due to the infectious nature of mastitis-causing pathogens, it is important for the livestock keeper to detect cases of subclinical mastitis in order to maximize cow health and well-being, as well as maintaining herd health (Kandeel *et al.*, 2018). Subclinical cases of mastitis tend to show no visible changes in udder or milk, however the production of milk reduces and its composition changes, characterised by high somatic cell counts (SCCs) (Erskine, 2001).

SCC has been used as an indicator of mastitis in dairy herds since the 1960s (Pyörälä, 2003). Somatic cells contribute to natural defence mechanisms and include lymphocytes, macrophages, polymorphonuclear and epithelial cells (Pillai *et al.*, 2001) and as such, these cells indicate an inflammatory process in intramammary infection. Counting somatic cells is used to distinguish between infected and uninfected quarters (Carrillo-Casas & Miranda-Morales, 2012), and has long been considered the gold standard in mastitis detection (Duarte *et al.*, 2015). The California Mastitis Test (CMT) is an inexpensive, easily-applicable, cow-side test that enables the subjective assessment of the number of somatic cells present in a milk sample, as an estimation of the probability and severity of intramammary infection (Schalm & Noorlander, 1957).

The CMT involves combining equal volumes (2–3 ml) of milk and testing reagent, generally the sodium or potassium salts of long chain fatty acids, alkyl sulphates, alkyl sulphonates, alkyl arylsulphonates or alkyl arylsulphates (Leach *et al.*, 2008). The gentle agitation of milk with these anionic-surface-active agents within a CMT paddle causes lysis of somatic cells and the release of cellular DNA. The DNA then agglutinates, giving the sample 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 SCC of the milk (Leach *et al.*, 2008). The degree of visible agglutination can be subjectively scored based on an ordinal scale and used as a qualitative test to estimate the SCC of milk samples (Moroni *et al.*, 2018). Sensitivity, specificity and positive predictive values are important test parameters to assess test accuracy, validity and to minimize error, and are calculated at varying thresholds (Schepers *et al.*, 1997; Thrusfield, 2018a).

In Ethiopia and Nigeria, dairy cattle are essential contributors to the agricultural industry and national economy, yet the productivity and profitability of the dairy subsector remains below potential in both countries (Abebe *et al.*, 2016; Ameh *et al.*, 1999; Shittu *et al.*, 2012; Tangka *et al.*, 2002).

Economic losses to the dairy industry due to mastitis include reduced milk quality and quantity, veterinary costs, culling and deaths (Degraves & Fetrow, 1993; Seegers *et al.*, 2003). Subclinical mastitis is considered the most damaging and expensive due to the challenge in early detection (Adamu *et al.*, 2020) although studies on the economic impact of mastitis in both Ethiopia and Nigeria are lacking (Adamu *et al.*, 2020; Beyene & Tolosa, 2017; FAO, 2014; Mekonnen *et al.*, 2019; Moru *et al.*, 2018).

Commercial CMT reagents are not easily sourced in either Ethiopia or Nigeria, 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 aims to investigate whether domestic detergents available in Ethiopia and Nigeria can also be utilised in the CMT, in place of the UK commercial CMT reagent, to achieve comparable results. This would thereby provide a cost-effective, widely available means of screening for mastitis in both Ethiopia and Nigeria, allowing for targeted treatment and disease control. In turn, this will decrease disease prevalence and production loss, increase herd health and welfare, and maximize milk quantity, quality, and economic value.

Methods

Milk samples

The milk samples came from Holstein Friesian cattle, with the majority of samples with high SCC (>400,000) originating from a farm in Medan Vale, Nottinghamshire, based on the known high number of mastitis cases and their willingness to engage with research projects. Other samples were collected from the dairy farm operated by the University of Bristol, based on ease of collection during COVID-19 restrictions. Samples were predominantly collected by veterinarians using aseptic technique (cleaning the teat, wearing gloves and discarding foremilk) (SEBI, 2019), with a few (approximately 20) collected by the respective farmers.

Samples were collected between August 2020 and February 2021. All samples collected for the purpose of California Mastitis Testing were collected in sterile 50 ml sample pots provided by Scarsdale veterinary practice, Derby, were stored at room temperature and tested within 48 hours of collection. All samples collected for the purpose of SCC analysis were collected in sterile 30 ml QMMS sample pots containing a milk preservative and posted within 48 hours of collection. Samples were identified with numbers in chronological order of collection so as to maintain blinding of details such as sample location. Two of the three operators in the validation study were blind to

sample location and number as well as CMT reagents used. All operators were inexperienced in regards to performing the CMT prior to the study, and were trained using educational videos produced by SEBI (2019). All laboratory SCC results were sent to the respective veterinarians and farmers.

Dilution study
An accepted UK detergent-based CMT fluid is comprised of 40 ml Fairy liquid detergent (Proctor & Gamble), diluted with 160 ml water, with 1 ml of dark food colouring to enhance visualisation (Leach *et al.*, 2008). As one cannot assume the domestic detergent available in Ethiopia and Nigeria will have an equal concentration of the anionic-surface-active agent required to lyse inflammatory cells in milk, the study first undertook a series dilution test to determine the most effective concentration of each detergent for visualising high SCC (>400,000 cells per ml milk[1]) and achieving the most comparable results to the UK CMT.

The study used six commercially available domestic detergents from Ethiopia (Reagents 1–3) and Nigeria (Reagents 4–6), 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 dilution had the addition of 1 ml of commercially available food colouring from Ethiopia/Nigeria, depending on the detergent country of origin, to enhance visibility.

The five dilutions for each of the six detergents were then tested with 20 quarter milk samples. All samples were screened for inclusion in the study using the commercial UK CMT to ensure 15 samples with known high SCC values (CMT score 2–3) were included as well as five samples with known low SCC (CMT score 0–1) to test for false positives. Scores were based on reaction descriptions shown in Table 1.

Validation study
To verify that the Ethiopian and Nigerian domestic detergent-based CMT reagents reliably indicate a high SCC (>400,000

somatic cells per ml milk), indicative of mastitis, each reagent was tested with a further 132 quarter milk samples. These samples were screened using the UK CMT upon entry into the study to ensure a range of CMT scores (0, 1, 2 and 3) were present in the validation study.

An additional two operators also tested the 132 samples using each of the six detergent-based CMT reagents, as well as the UK CMT. Both operators were blind to the origin of each reagent so as to reduce bias. Additionally, all samples were submitted for a laboratory SCC reading to confirm the accuracy of the CMT as a diagnostic tool for indicating high SCC samples.

The study identifies the point of significance as 400,000 cells per ml, determined by the laboratory SCC. Samples with SCC >400,000 were determined as a positive indicator of mastitis and thus we can expect a CMT score of 2–3, and samples <400,000 were deemed as weakly positive to negative, and thus we can expect a score of 0–1 on the CMT.

Statistical analysis
Validity of the CMT results using different detergents was assessed by comparing test sensitivity and specificity. Sensitivity is the proportion of animals that test positive that truly have the disease, while specificity is the proportion of animals that test negative that indeed do not have the disease. The results of each CMT were validated against a SCC, and sensitivity and specificity were calculated using the formula in Table 2 (Thrusfield, 2018b). High sensitivity minimises the number of false-negative results, while high specificity minimises the number of false-positive results. Confidence intervals were calculated using Wilson's method (Thrusfield, 2018a)

Positive and negative predictive values were also assessed (Table 2, Thrusfield, 2018a). These take into account disease prevalence to show the probability of a test detecting a positive, or negative, animal. All calculations were performed in Microsoft Excel (Version 2108).

Results
Dilution study
Individual detergents have differing concentrations of anionic-surface-active agent, and therefore, different detergents have

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[1]The legal limit of bulk milk SCC is 400,000 cells per ml in the European Union

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Table 1. Description of the reactions observed in the CMT according to each score category (Leach *et al.*, 2008).

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.

varying optimal dilutions to achieve results most comparable to the UK CMT (Figure 1) (Vance, 2021).

Reagents 1–3 (Figure 1), made with commercially available Ethiopian detergents, achieved 100% agreement with UK CMT results at varying 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.

Reagents 4–6 (Figure 1), made with commercially available Nigerian detergents, are seen to be less efficacious, with none achieving 100% equivalent results to the UK CMT. However, there are clearly 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.

Results obtained from the dilution study are summarised in Table 3, demonstrating the optimum dilution of each detergent selected for the validation study.

Validation study

In the validation study, the majority of reagents scored similarly to the UK CMT, demonstrating greater than 95% specificity (Figure 2). Reagent 3, however, was a notable exception, scoring lowest, with an average of 84.2% between the three operators. This result, however, is still an acceptable level of specificity.

Reagents were tested for sensitivity, by establishing the percentage of high SCC (>400,000) samples each reagent correctly identified as score 2–3 (Figure 3). It was observed that detergent-based CMT reagents have a higher specificity than sensitivity, and as such are more able to identify low SCC samples. It was, therefore, possible to best differentiate test validity of different detergent-based reagents by their ability to identify high SCC. Reagents 1 and 2 proved to be particularly poor reagents in the identification of samples >400,000 SCC, with 28.3% and 53.3% of samples correctly identified as high SCC, respectively. This is an insufficient proportion of high SCC samples correctly scored, due to the high number of false negative results. With the UK CMT indicating 76.3% of high SCC samples as score 2–3, Reagents 3 and 4 proved to be of similar efficacy, achieving 75.7% and 81.0%, respectively. Reagents 5 and 6 achieved slightly lower sensitivities with 68.7% and 74.3% high SCC samples identified as score 2–3, respectively.

The percentage of detergent-based CMTs that achieved the same interpreted results as the UK CMT, i.e., negative to weakly

Table 2. Calculation of sensitivity and specificity (Thrusfield 2018a, Thrusfield, 2018b).

Test result	True status	
	Positive	Negative
Positive	a	b
Negative	c	d
Calculations		
Sensitivity	$a / (a + c)$	
Specificity	$d / (d + b)$	
Positive predictive value	$a / (a + b)$	
Negative predictive value	$d / (c + d)$	
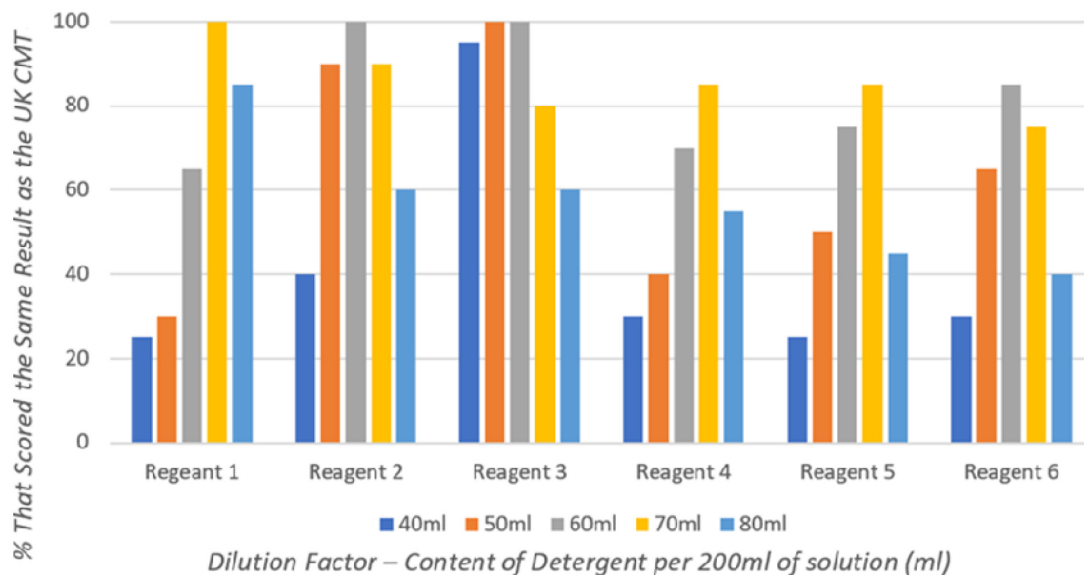
Figure 1. The percentage of detergent-based CMT results that achieved the same result as the commercial UK CMT for each different dilution when testing 20 milk samples.

Table 3. The optimal dilution factor of each detergent to achieve results most comparable to the UK CMT that are then used in the validation study.

Detergent	Country of Origin	Reagent ID	Optimal Dilution
Princess	Ethiopia	Reagent 1 (R1)	70 ml detergent to 130 ml water
Rotana	Ethiopia	Reagent 2 (R2)	60 ml detergent to 140 ml water
Shagan	Ethiopia	Reagent 3 (R3)	55 ml detergent to 145 ml water
Sunlight	Nigeria	Reagent 4 (R4)	70 ml detergent to 130 ml water
Morning Fresh	Nigeria	Reagent 5 (R5)	70 ml detergent to 130 ml water
Mama Lemon	Nigeria	Reagent 6 (R6)	60 ml detergent to 140 ml water
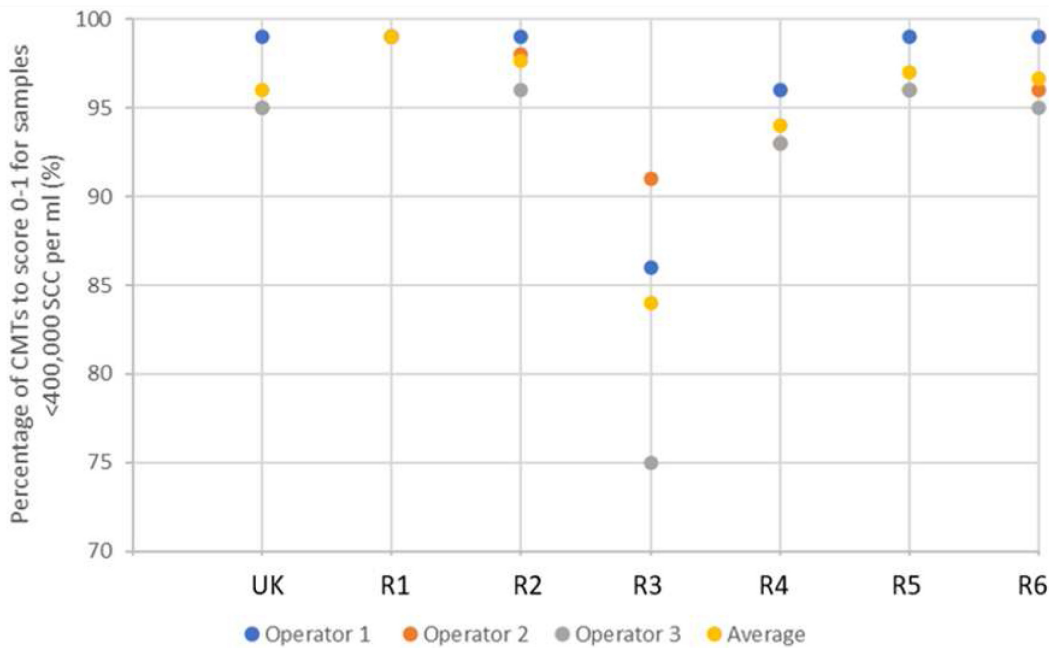
Figure 2. The efficacy of each reagent to score negative to weakly positive CMT results (0–1) across 80 samples, laboratory tested as <400,000 SCC per ml. 'UK' indicates UK commercial CMT reagent; 'R' indicates reagent.

positive tests score 0–1, and positive to strongly positive tests score 2–3, were established (Figure 4). Reagent 2 of the Ethiopian detergents (R1-3) is shown to have the highest percentage of similarity to the UK commercial CMT reagent with an average of 74.0%, despite Reagent 3 demonstrating the greatest sensitivity (of the Ethiopian reagents) (Figure 3). Of the Nigerian reagents (R4-6), Reagent 4 is shown as achieving the greatest percentage of score similarity to the UK CMT reagent, with an average percentage of 73.3% (Figure 4).

It can be seen that the UK CMT itself is an imperfect diagnostic tool due to the subjective nature of scoring, achieving an average of 96.3% and 76.3% efficacy at identifying negative to weakly

positive, and positive to strongly positive samples, respectively (demonstrated in Figure 2 and Figure 3). Nonetheless, it is accepted for use in the field as an indicator of mastitis and, therefore, alternative detergent-based reagents should be held to similar standards. This suggests, therefore, that a better measure of test validity is achieved through each reagent's ability to identify high SCC, rather than achieving the same interpreted score as the UK CMT.

As well as establishing the sensitivity and specificity for each reagent, positive predictive value (PPV) and negative predictive value (NPV) at identifying high SCC (>400,000) samples for each of the detergent-based CMT reagents and the commercial UK CMT reagent were calculated (Table 4). Almost all reagents
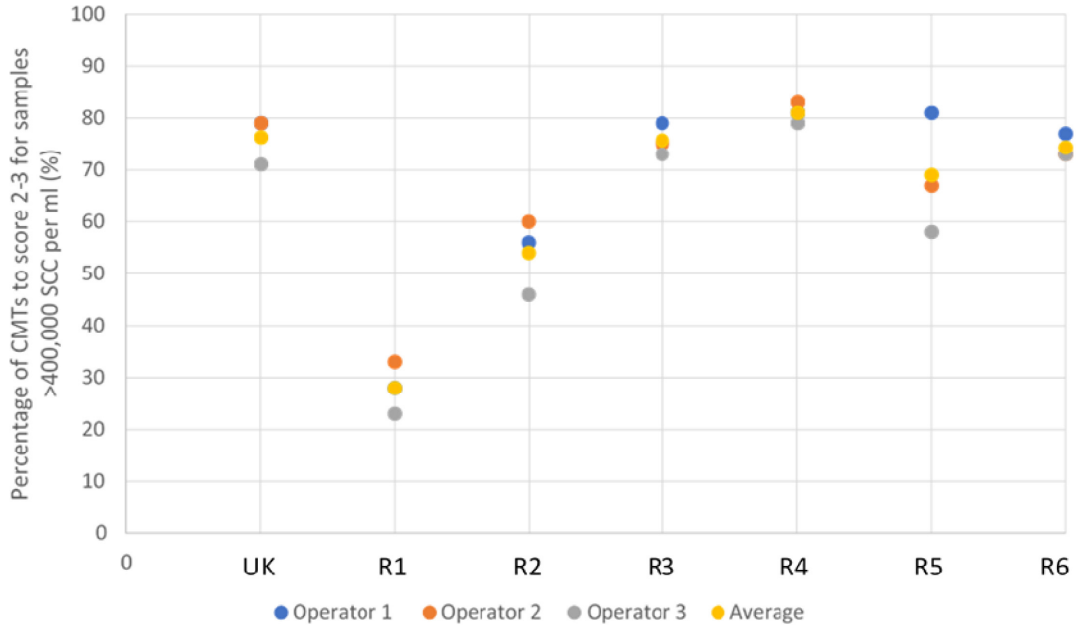
Figure 3. The efficacy of each reagent to score positive to strongly positive CMT results (score 2–3) across 52 samples, laboratory tested as >400,000 SCC per ml. 'UK' indicates UK commercial CMT reagent; 'R' indicates reagent.
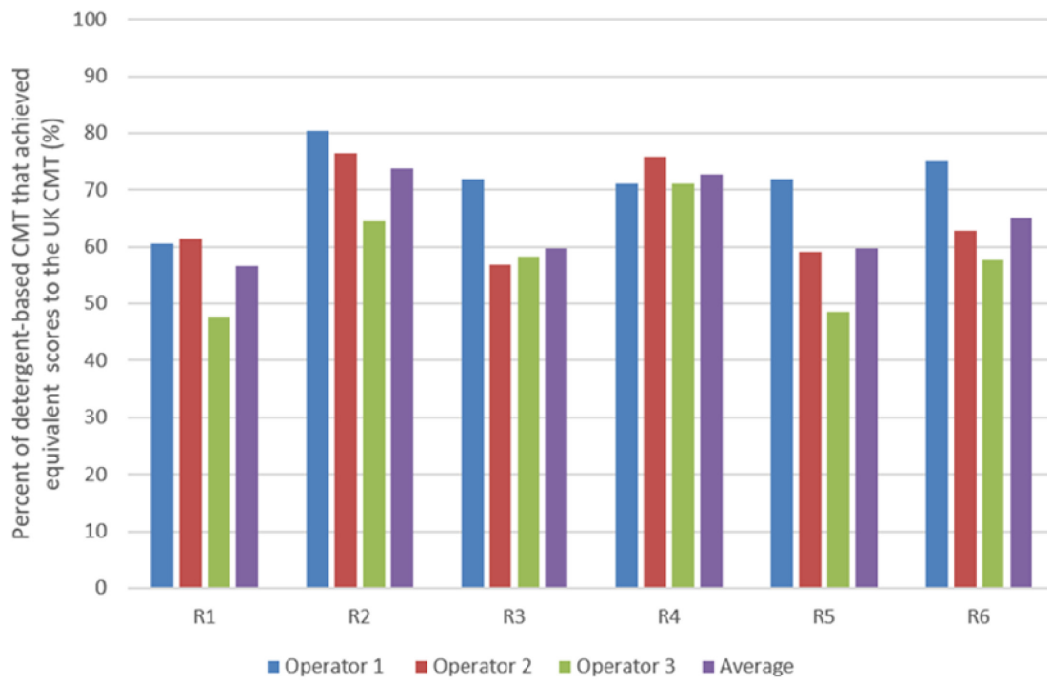
Figure 4. The percentage of detergent-based CMT results that indicated the same SCC (<400, 000 or >400,000) as the commercial UK CMT.

Table 4. 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.

Reagent	Sensitivity		Specificity		PPV	NPV	Range of False Positives	Range of False Negatives
	%	95% CI	%	95% CI				
UK CMT	76.30%	63.9 - 86.3%	96.30%	89.6 - 98.7%	93%	86%	1 – 4	11 – 15
Reagent 1	28.20%	18.3 - 42.3%	98.80%	93.3 - 99.8%	94%	68%	1 – 1	35 – 40
Reagent 2	53.20%	40.5 - 66.7	97.50%	91.3 - 99.3%	93%	76%	1 – 3	21 – 29
Reagent 3	75.60%	63.9 - 86.3%	84.20%	74.2 - 90.3%	76%	84%	7 – 20	11 – 14
Reagent 4	80.80%	68.1 - 89.2%	93.80%	96.2 - 97.3%	89%	88%	3 – 6	9 – 11
Reagent 5	68.60%	55.7 - 80.1%	97.10%	91.3 - 99.3%	94%	83%	1 – 3	10 – 22
Reagent 6	74.40%	61.8 - 84.8%	96.70%	89.6 - 98.7%	94%	85%	1 - 4	12 - 14

demonstrate a high specificity, greater than 95%, with slightly lower specificity for Reagent 4 at 93.8%, and Reagent 3 at 84.2%. Greater differences in sensitivity were observed, 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 showing lower NPV, or increased false negatives.

It would appear overall that Ethiopian detergents are less effective in performing the CMT. However, of the three Ethiopian detergents investigated, Reagent 3 is of highest sensitivity (75.6%, CI% 63.9 - 86.3) and NPV, with the fewest false negatives, and so can be considered the most reliable for detecting mastitis, and thus individual cows requiring treatment, in the Ethiopian setting. In contrast, all Nigerian commercially available domestic detergents (Reagents 4–6), are more efficacious at identifying high SCC milk samples in a detergent-based CMT, with highest sensitivity achieved by Reagent 4 (80.8%, CI% 68.1 – 89.2), which had the fewest false negatives.

Discussion

The CMT is a common test to indicate infected quarters in cases of subclinical mastitis. Mastitis is a significant disease in both Ethiopia and Nigeria due to its high prevalence and damaging effects on cattle health and productivity. It is, therefore, important for Ethiopian and Nigerian livestock keepers to be able to identify intramammary infections in order to control disease, whether that be to treat, reject the milk, dry off or cull high SCC individuals (Kandeel *et al.*, 2018).

Due to the absence of cell counting laboratories, the CMT offers an easy, inexpensive, cow-side test to indicate the presence of infection and subsequent high SCC. However, the cost of reagent importation limits the cost-benefit ratio of the UK Commercial CMT. As a domestic detergent-based CMT is accepted in the UK, this study has investigated the use of six detergents, from Ethiopia (Reagents 1–3) and Nigeria (Reagents 4–6) to determine

the optimal dilution for use in the CMT, and to validate the sensitivity and specificity these achieve compared to the UK CMT.

All of the Ethiopian detergent-based CMT reagents (R1-3) 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, was considered the best at ruling out disease and identifying cows of likely high SCC to treat. Thus, the authors recommend the use of detergent 'Shagan' (Reagent 3), commercially available in Ethiopia, at a dilution of 55ml detergent to 145ml water, with 1ml of indicator, for use in the CMT as an alternative to the UK CMT.

All of the Nigerian detergent-based reagents (R4-6) proved to be more effective than the Ethiopian reagents at identifying high SCC in milk. Reagent 4 proved to have the highest sensitivity (80.8%, CI% 68.1 – 89.2), and NPV (88%), and therefore, the authors recommend the use of the detergent 'Sunlight' (Reagent 4), commercially available in Nigeria, at a dilution of 70ml detergent to 130ml water, with 1ml indicator, for use in the CMT to identify high SCC.

Availability and cost are both important factors in being able to purchase reagents. In selecting detergents for the study, national availability was a selection criterion, along with detergents being in the higher end of the market, as better-quality products had been found to be more effective by Leach *et al.* (2008). For the CMT, the required volume of test fluid per cow is 12ml (3 ml per quarter); one litre of test fluid would therefore be sufficient for 83 cows. At the time of writing, the recommended Ethiopian detergent 'Shagan' costs 92–138 Ethiopian Birr (ETB) (equivalent to 2.0–3.1 USD) for one litre. To make one litre of the CMT fluid at the recommended dilution, 275 ml of Shagan is required, equivalent to 25–38 ETB (0.6–0.8 USD), equating to 0.1 ETB per quarter (0.002 USD). The recommended

Nigerian detergent 'Sunlight' costs 800–866 Nigerian Naira (NGN) (equivalent to 1.9-2.1 USD) for one litre. To make one litre of CMT fluid at the recommended dilution, 350 ml of Sunlight is required, equivalent to 280–303 NGN (0.6-0.7 USD). This equates to around 0.9 NGN per quarter (0.002 USD). Commercial reagent for the UK CMT costs between 6.9–8.3 USD for one litre. The recommended local detergents, therefore, cost around one tenth of the commercial product and are both readily available.

The study does carry some limitations. Undertaking the study during the COVID-19 pandemic, and ensuring relevant guidelines were adhered to, was an ongoing challenge. In particular, the collection and testing of milk samples proved difficult, with many farms not allowing external access, vet practices frequently only open for emergency cases, and the difficulty in multiple operators testing the samples due to social distancing. This meant the project was very stop-start and took much longer than initially anticipated. Ideally, all samples in the validation study would have been tested by the same three operators, for consistency and to minimise bias, however due to restrictions, this was not possible.

The CMT is subjective in nature, which allows room for bias as well as operator error. A laboratory SCC automatically counts cells within a sample, reducing operator error and removing bias, but it is not without an error margin. The prolonged retention of samples, postal disturbances, transport and delivery may all contribute to sample modification. Additionally, the SCC also does not take away the value of a clinical exam, as mastitis and other mammary disease may not, in the rare occasion, be reflected in high SCC. All samples in this study are taken from UK Holstein Friesians cows. This study, therefore, assumes that indigenous breeds native to Ethiopia and Nigeria, though managed differently, will respond similarly to the CMT.

The study identified areas for further research. Firstly, there may have been differences in the efficacy of detergent-based CMT reagents in relation to how long the reagent had been made and left standing; further research is needed to clarify this. Secondly, the Nigerian food colouring was not dark enough and limited visibility, therefore a darker UK food colouring was added to enable testing. Future studies should look at food colouring and

indicator dye available in Nigeria for their suitability for use in the CMT.

Conclusion

This study supports the use of detergent-based CMTs to 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. The most suitable local detergent and dilution was established for both countries and are recommended for use by livestock keepers to aid cost-effective diagnosis of mastitis and importantly reduce herd-level prevalence. This research is contributing to a larger ongoing study into mastitis in both Ethiopia and Nigeria, and will be used to produce educational pamphlets and videos as part of outreach activities to further the promotion of the CMT for the control and management of mastitis.

Data availability

Underlying data

Harvard Dataverse: Replication Data for: A study of the effectiveness of a detergent-based California mastitis test (CMT), using Ethiopian and Nigerian domestic detergents, for the detection of high somatic cell counts in milk and their reliability compared to the commercial UK CMT. https://doi.org/10.7910/DVN/PULPBI (Vance, 2021).

This project contains the following underlying data:

- Dilution_Study_RAW DATA.tab

- Dilution_Study_Satistical_Analysis.tab

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

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Open Peer Review

Current Peer Review Status: ?

? **Katharine A. Leach** (iD)

Quality Milk Management Services, Easton Hill, UK

This is a useful study providing practical information that makes detection of subclinical mastitis more accessible to Ethiopian and Nigerian dairy farmers. I have a few suggestions:

The clarity of reporting could be improved by making clear in each section of methods, results and discussion what is being used as the gold standard to assess the performance of the individual reagents - in some cases it is the UK commercial CMT reagent and in others it is the laboratory determined SCC.

Traditionally, bacterial culture has been taken as the ultimate gold standard for detection of clinical mastitis (Dohoo *et al.*, 2011[1]), rather than counting somatic cells as suggested in paragraph 3 - this is standard for subclinical mastitis.

Methods:
The Methods section needs a clearer explanation of the testing for SCC. It is not immediately clear whether the same samples were used for CMT/detergent tests and somatic cell counting. Was a single sample divided, part in sterile pot without preservative and part in "QMMS sample pot" (whatever that is) with preservative? This would be expected, but is not perfectly clear. The laboratory method used to determine SCC counts (Fossomatic? Coulter?...). is not mentioned at all in the methods, just that the samples were "posted within 48 hours of collection". Please explain more.

Dilution study section in the methods section - the first paragraph gives the impression that the objective was to evaluate the ability to detect samples with >400,000 cells/ml. However, the third paragraph states that the selection of 20 (why 20?) samples to include in this exercise was based on CMT results. I think this will be because the detergent testing needed to be done before the SCC results were available. It would be useful to explain this.

Everything would be clearer if the word "chosen" was used in reference to the "point of significance" in the last paragraph of the methods, and, in fact, if this point was made earlier in the

methods section e.g. "In this study 400,000 cells/ml, as determined by somatic cell counting (XX Method) was chosen as the point of significance, as a positive indicator of infection."

Statistical Analysis:
Your definition at present is not correct: "Sensitivity is the proportion of animals that test positive that truly have the disease, while specificity is the proportion of animals that test negative that indeed do not have the disease". should read: "Sensitivity is the proportion of animals that truly have the disease that test positive, while specificity is the proportion of animals that do not have the disease that test negative."

Results:
I think there should be some description (e.g. boxplots), of the distribution of somatic cell counts (done by machine) for all of these results as the distribution of SCCs will have a bearing on CMT "accuracy". The operating characteristics of a test will vary with prevalence of a disease (Bentley *et al.*, 2012[2], Martin *et al.*, 1987[3]).

Table 1:
Mucous membranes secrete mucus. "Mucus" is the noun and "mucous" is the adjective. You need the adjective here, the substance you have is not mucus, it merely has similar properties.

Figure 2 & Figure 3:
Could the circles be offset? At present it appears that not all operators used all reagents, but are the circles overlapping so that an operator is sometimes obscured?

Table 4:
Are these results *for* different reagents, *across* operators? The meaning of "range of false positives" and "range of false negatives" is not clear - I presume it is across operators. Also, to evaluate the importance of the range of false positives and false negatives we need to be given the number of samples tested in the title please.

Discussion:
The financial information is useful and illustrates the advantage of a diluted detergent reagent. It would also be interesting if you could give an indication of the value of any milk that a producer in Nigeria or Ethiopia might be selling, at the time of the study.

In the discussion you may also wish to comment on any differences in pathogens and (hence) chronicity between a "typical" UK case of mastitis and a "typical" African case. Stage of disease, i.e. how chronic the mastitis is at the time of sampling, will also affect the SCC and sensitivity and specificity. For example, if a large proportion of African cases are more chronic than UK cases, then sensitivity and specificity will be different when this is used in the field in Africa.

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Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Dairy herd health; mastitis diagnostics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.