Evaluation of sodium lauryl sulfate for the development of cow-side mastitis screening test

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Received: 09-04-2021, Accepted: 29-07-2021, Published online: 31-08-2021

How to cite this article: Tanni NS, Islam MS, Kabir M, Parvin MS, Ehsan MA, Islam MT (2021) Evaluation of sodium lauryl sulfate for the development of cow-side mastitis screening test, Veterinary World, 14(8): 2290-2295.

Abstract

Background and Aim: Subclinical mastitis (SCM) is an economically important disease for dairy cattle worldwide; therefore, regular screening is imperative to detect SCM at an early stage so as to control it. The study was conducted to compare the test characteristics of sodium lauryl sulfate (SLS) as a test reagent to detect SCM in dairy cows.

Materials and Methods: First, 106 milk samples of dairy cows were subjected to available indirect screening tests (white side test [WST], surf field mastitis test, Leucocytest, and Immucell) considering somatic cell count (SCC) as gold standard test. Then 273 milk samples were allowed to react with different concentrations of SLS with or without sodium hydroxide (NaOH) and indicators (bromothymol blue [BTB] and bromocresol purple [BCP]).

Results: SLS with or without NaOH yielded best reaction with the milk samples similar to Leucocytest. It was observed that the reaction of milk samples with SLS added with indicators (BTB and BCP) was easier to visualize than without indicators. SLS 3%+NaOH 1.5% with BTB and SLS 2% with BCP had high sensitivity, specificity, and substantial agreement with SCC. The area under the receiver operating characteristics curve of SLS 2% with BCP and SLS 3%+NaOH 1.5% with BTB was 0.917 and 0.875, respectively.

Conclusion: It may be concluded that SLS 3%+NaOH 1.5% with BTB and SLS 2% with BCP may be the potential reagents for the development of an effective cow-side test to detect SCM, as the main ingredient SLS is considerably cheap and readily available in developing countries.

Keywords: bovine subclinical mastitis, dairy cows, screening test, sensitivity, sodium lauryl sulfate, specificity.

Introduction

Mastitis is one of the most prevalent and costly diseases of dairy cows [1]. It occurs in two forms, clinical and subclinical mastitis (SCM) [1]. SCM is the foremost production disease of dairy cows, which can directly or indirectly affect the economy of the farmers globally, including in developing countries [2-4]. Earlier reports indicate that the cost of SCM is often greater than that of clinical mastitis [5,6]. SCM is the inflammation of the mammary gland that does not create any visible changes in the milk or of the udder but the quality and quantity of the milk is highly altered [1]. Both clinical and subclinical forms of mastitis have been considered as the major constraints for the development of the dairy industry in Bangladesh [7]. SCM in dairy cows is important because it (a) is 15-40 times more prevalent than the clinical form, (b) usually precedes the clinical form, (c) is of long duration, (d) is often undetected, (e) reduces milk production, (f) adversely affects milk quality, and (g) serves as reservoirs for the spread of the infection to other cows in the herd [8,9]. SCM causes 1.2-33% milk production losses in affected quarters; however, this loss per farm ranges from 1.3% to 13.5% [10,11]. The reduction in milk production attributed to SCM accounts for 32-70% of the total losses [5,12]. Like other countries, SCM is also a major problem for dairy cows in Bangladesh [13,14]. The prevalence of bovine SCM in Bangladesh is 20.2% [13]. The annual economic losses that occur due to reduced milk production caused by SCM in Bangladesh have been estimated to be Taka 122.6 million (US $ 2.11 million) [7].

Early detection of SCM is the key component of a mastitis control program [15]. SCM is not a problem of individual cow rather a problem at the dairy herd level [6]. Therefore, it is essential to test the dairy herd of cows periodically for the screening of SCM [14]. According to the International Dairy Federation recommendations, microbiological status of the quarters and the somatic cell count (SCC) are the most
Materials and Methods

Ethical approval and informed consent

Ethical approval was not required for this study because animal experimentation was not done. During milk sample collection, none of the cows were harmed. However, informed written consent was obtained from the owner of the cows to collect milk samples and data. Furthermore, the study protocol was approved by the board of studies of the department, and the Committee for Advanced Studies and Research of the university.

Study period and location

The study was conducted from February to May 2018. Study cows were selected from Mymensingh Sadar Upazila, and the laboratory works were conducted in the Population Medicine and AMR Laboratory of the Department of Medicine, Bangladesh Agricultural University, Mymensingh.

Collection and transportation of milk samples

Three hundred and seventy nine milk samples were collected on several occasions from apparently healthy crossbred dairy cows (Friesian × Indigenous and Sahiwal × Indigenous) from smallholder dairy farms of Mymensingh Sadar Upazila. The farms mainly practiced zero grazing. The cows were provided with green grasses such as Napier, roadside grass, and seasonal maize. However, cows were given free access to paddy straw as roughage. Besides, most of the cows were offered varying amounts of concentrates such as rice bran, wheat bran, and occasionally chickpea bran. Most of the farms had the brick floor. The practice of manure removal was on a daily basis. Hand milking was practiced in all the farms. Pre- or post-milking teat dipping was not practiced in any of the farms. Milk samples from cows at 1st week of lactation were excluded due to the presence of physiologically high concentration of somatic cells in milk during this period, which may affect the results of indirect screening tests. Grossly, dirty teats and udders were thoroughly washed, dried, and soaked with 70% ethanol immediately before collection of samples. Three or four streams of milk were discarded to minimize the chance of contamination. Approximately 15 mL of milk was collected from each quarter in a sterilized falcon tube and kept in a cooler box. Then, the samples were transported to the laboratory within 2-3 h, and screening tests and SCC were performed on the same day.

Study description

Initially, 106 milk samples were tested with four existing indirect screening tests (WST, SFMT, Leucocytest, and Immucell) and gold standard test (SCC). Among them, the Leucocytest with high Se was used in the next step. Later, 273 samples were tested with anionic surfactant (SLS) of different concentrations as a test reagent to detect SCM in dairy cows.

Materials and Methods

Ethical approval and informed consent

Ethical approval was not required for this study because animal experimentation was not done. During milk sample collection, none of the cows were harmed. However, informed written consent was obtained from the owner of the cows to collect milk samples and data. Furthermore, the study protocol was approved by the board of studies of the department, and the Committee for Advanced Studies and Research of the university.

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Indirect screening tests for SCM

Leucocytest

The test was conducted as per the manufacturer’s instruction (Synbiotics Corporation-2, rue Alexander Fleming-69007 Lyon, France). Briefly, 2.0 mL of quarter milk sample was mixed with an equal volume of reagent in a paddle. The mixing was accomplished by the gentle circular motion of the paddle in a horizontal plane for few seconds, and the changes in milk fluidity and viscosity were observed. The results were interpreted as 0 (negative), 1+ (weak positive), 2+ (distinct positive), and 3+ (strong positive).
**ImmuCell (Coburn Company, USA)**

To make working solution, CMT concentrate was added to the lower line marked on a dispensing bottle, and then water was added to fill the level line marked at top of the dispensing bottle. Milk samples were taken into four cups of a paddle, and then the paddle was tilted until milk was halfway between the inner and outer circles. CMT working solution was added into each cup slowly. In horizontal position, the paddle was rotated. The results were interpreted as negative (-), trace (T), weak positive (1), distinct positive (2), and strong positive (3).

**WST**

Fifty microliters (five drops) of milk were placed on a glass slide with a blue background. Subsequently, 20 µL (two drops) of 4% sodium hydroxide (NaOH) solution was added to the milk sample and the mixture was stirred rapidly with a toothpick for 20-25 s. The results were interpreted as strong (+++), distinct (++), weak (+), trace (+), and negative (-) based on the formation of coagulation.

**SFMT**

A shallow paddle having four cups was used and rinsed after each use. About 2 mL of milk sample and equal volume of 3% SFMT reagent (Surf Excel, Unilever, Bangladesh) was mixed in the cup of a paddle. Mixing was accomplished by gentle circular motion of the paddle in horizontal position for few seconds. The formation of gel indicated that the sample was positive for SCM. The peak of reaction was obtained within 30 s. The results were interpreted based on the gel formation as negative (-), trace (+), weak reaction (+), moderate reaction (++), and strong reaction (+++). For all the indirect screening tests, milk samples giving weak to strong reactions were considered positive for SCM.

**SCC**

Fifty microliters of milk sample and an equal volume of reagent C (1:1 dilution) were taken in an Eppendorf tube using micropipette and mixed using a vortex mixture. Then, SCC-Cassette was loaded with the diluted milk sample by immersing the tip of the cassette into the solution and pressing the piston. The SCC-Cassette was placed in the instrument (NucleoCounter, Chemometec, Denmark) and pressed the “Run” key. After 30 s, the SCC was presented on the instrument display. A milk sample was considered positive when the SCC value was ≥ 100 × 10³ cells/mL [32].

**Anionic surfactant as test solution to detect SCM in dairy cows**

SLS was selected as a test reagent for the diagnosis of SCM. Different concentrations of SLS were used with or without NaOH and indicators (bromothymol blue [BTB] and bromocresol purple [BCP]).

**Test procedure**

An equal volume (1 mL) of SLS solutions and milk sample was mixed on a shallow paddle having four cups, and the mixing was properly done by the gentle circular motion of the paddle for few seconds. The reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of reaction was obtained within 10 s and any change in the milk, such as viscosity, consistency was observed as mentioned below.

**Reaction characteristics**

| Reaction characteristics | Score     | Infection          |
|--------------------------|-----------|--------------------|
| Consistency and viscosity| Negative (0) | Absent             |
| Very slight thread formation | Trace (±) | Slight infection risk |
| and/or precipitation disappearing after stirring | Distinct positive (+) | Subclinical mastitis |
| Distinct precipitation and threads formation but no gel formation | Weak positive (+) | Subclinical mastitis |
| Mixture become more viscous and formation of gel | Distinct positive (+) | Subclinical mastitis |
| Distinct gel formation, which is thick and has tendency to adhere to the bottom of the cup of the paddle | Strong positive (+++) | Subclinical mastitis near the clinical stage |

**Statistical analysis**

SCC was considered as the gold standard test for the determination of epidemiological test characteristics of different indirect screening tests as well as test anionic surfactant - SLS. Five quality parameters: Se, Sp, positive predictive value (PPV), negative predictive value (NPV), and kappa-value were calculated. The results were weighted on the basis of kappa value, which ranges from 1 to -1, where a value of -1 indicates complete disagreement between tests, 0 indicates agreement by chance only, 0.01-0.20 indicates slight agreement, 0.21-0.40 indicates a fair amount of agreement, 0.41-0.60 indicates moderate agreement, 0.61-0.80 indicates substantial agreement, and 0.81-1 indicates almost perfect agreement [33]. The SPSS v.20 was used for all the analyses.

**Receiver operating characteristic (ROC) curve**

A graph of Se against 1-Sp, that is, a ROC curve was generated and the performance of a diagnostic test was quantified by calculating the area under the ROC curve (AUROC). The ideal test would have an AUROC of 1.

**Results**

**Comparison of existing indirect screening tests with SCC**

Four popularly used indirect screening tests (Leucocytest, WST, SFMT, and Immucell) along with SCC as gold standard test were employed on 106 milk samples of dairy cows to diagnose SCM and to compare the test characteristics. Leucocytest had comparatively higher Se (64.9%) than that of WST (45.9%), Immucell (56.8%), and SFMT (63.9%) (Table-1). Kappa-value indicated substantial agreement of Leucocytest with SCC. Therefore, Leucocytest was selected for further comparison with test anionic surfactant SLS.
Evaluation of SLS at different concentrations

SLS with or without NaOH yielded the best reaction with the milk samples similar to Leucocyst. It was observed that the reaction of milk samples with SLS added with indicators (BTB and BCP) was easier to visualize than without indicators. Se, Sp and kappa-value of SLS 3%+NaOH 1.5% with BTB and SLS 2% with BCP were higher than other concentrations of SLS (Table-2). The Se and Sp of both Leucocyst and 2% SLS with BCP was 1, and the area under the curve (AUROC) for both the tests was 0.917, which was higher than AUROC of other concentrations of SLS (Figure-1). The AUROC of 3% SLS+1.5% NaOH with BTB was also high (0.875). As SLS 3%+NaOH 1.5% with BTB and SLS 2% with BCP gave the best reactions in terms of Se, Sp and diagnostic agreement they can be potential reagents to be used as indirect screening tests to diagnose SCM in dairy cows. The test results are further summarized in Table-3.

Discussion

SCM is not a problem of individual cows rather a problem at the dairy herd level; therefore, it is essential to test the dairy cows of the herd periodically for the screening of SCM [6]. Se and Sp are intrinsic values of a test and are useful for comparing one diagnostic test with another [34]. However, they cannot be used to determine the probability that a given test result reflects the true disease status of the animal [35]. Se and Sp can be calculated only if there is an objective, independent means of determining whether the disease is truly present [34].

Here, an anionic surfactant, SLS was evaluated based on the Se and Sp taking SCC as the gold standard for the detection of SCM in dairy cows. SLS with or without NaOH at different concentrations gave better reaction with the milk samples similar to existing indirect screening test, Leucocytest. SLS decreases surface tension, change the structure and conductivity of cell membrane and nucleus, interfere osmotic balance, block oxidation and stimulate proteolytic enzymes, and increases milk viscosity. NaOH degrades somatic cells; as a result, the DNA of the cells reacts with the milk and increases milk viscosity. Indicator is a halochromic chemical compound that changes color in different pH reactions in terms of Se, Sp and diagnostic agreement with the reaction of the solution can be determined visually [36,37]. Therefore, SLS with or without NaOH affected quarters as positive and from truly not-affected quarters as negative. Further evaluation was done by adding indicators to SLS to observe the reaction of milk. Indicator is a halochromic chemical compound added in small amounts to a solution so that the reaction of the solution can be determined visually [36,37]. Therefore, SLS with or without NaOH

Table-1: Sensitivity, specificity, and agreement of four indirect screening tests with somatic cell count for the diagnosis of subclinical mastitis (n=106).

| Screening tests | Se  | Sp  | PPV | NPV  | Kappa-value |
|-----------------|-----|-----|-----|------|-------------|
| WST             | 45.9| 98.6| 97.4| 77.5 | 0.505       |
| SFMT            | 63.9| 85.1| 69.7| 81.4 | 0.499       |
| Leucocytest     | 64.9| 95.7| 92.5| 84.1 | 0.646       |
| ImmuCell        | 56.8| 80.8| 80.4| 81.0 | 0.455       |

n=Number of milk samples, WST=White side test, SFMT=Surf field mastitis test, Se=Sensitivity, Sp=Specificity, PPV=Positive predictive value, NPV=Negative predictive value, Kappa-value=Measurement of agreement

Table-2: Sensitivity, specificity, and agreement of sodium lauryl sulfate with and without indicators with somatic cell count for the diagnosis of subclinical mastitis (n=273).

| Screening tests | Se  | Sp  | PPV | NPV  | Kappa-value |
|-----------------|-----|-----|-----|------|-------------|
| SLS 3%          | 77.8| 91.7| 93.3| 73.3 | 0.667       |
| SLS 3% with BTB | 61.1| 100 | 100 | 63.1 | 0.557       |
| SLS 3%+NaOH 1.5%| 83.3| 91.7| 93.7| 78.6 | 0.730       |
| SLS 3%+NaOH 1.5%| 83.3| 91.7| 93.7| 78.6 | 0.730       |
| SLS 2% with BTB | 67.8| 83.3| 87.5| 71.4 | 0.675       |
| SLS 2% with BCP | 100 | 83.3| 90  | 100  | 0.857       |
| SLS 2%+NaOH 1.5%| 72.2| 100 | 100 | 70.6 | 0.675       |
| SLS 2%+NaOH 1.5%| 77.8| 91.7| 93.3| 73.3 | 0.667       |
| SLS 2% with BCP | 72.2| 83.3| 86.7| 66.7 | 0.533       |
| Leucocytest     | 100 | 83.3| 90  | 100  | 0.857       |

n=Number of milk samples, SLS=Sodium lauryl sulphate, BTB=Bromothymol blue, NaOH=Sodium hydroxide, BCP=Bromo cresol purple, Se=Sensitivity, Sp=Specificity, PPV=Positive predictive value, NPV=Negative predictive value, Kappa-value=Measurement of agreement

Table-3: Sensitivity, specificity, and agreement of sodium lauryl sulphate with indicators with somatic cell count for the diagnosis of subclinical mastitis.

| Screening test | SCC | Se  | Sp  | PPV | NPV  | Kappa-value |
|----------------|-----|-----|-----|-----|------|-------------|
| SLS 3%+NaOH 1.5% with BTB | 83.3| 91.7| 93.7| 78.6 | 0.730       |
| SLS 2% with BCP | 100 | 83.3| 90  | 100  | 0.857       |
| Leucocytest     | 100 | 83.3| 90  | 100  | 0.857       |

SLS=Sodium lauryl sulfate, BTB=Bromothymol blue, NaOH=Sodium hydroxide, BCP=Bromo cresol purple, Se=Sensitivity, Sp=Specificity, PPV=Positive predictive value, NPV=Negative predictive value, Kappa-value=Measurement of agreement
were subjected to further evaluation with or without indicators (BTB and BCP). It was observed that the reaction of milk samples with SLS added with indicators (BTB and BCP) was easier to visualize than without indicators. S Sensitivity and specificity-value of SLS 3%+NaOH 1.5% with BTB and SLS 2% with BCP were higher than other concentrations of SLS. Among the available screening tests, the Se was very low in case of WST and SFMT. Muhammad et al. [24], Sarker [38], and Badiuzzaman et al. [25] also reported the lower Se of WST and SFMT. Although Leucocytest had high Se and Sp it is somewhat costly as well as not readily available whenever needed in our country. On the other hand, WST and SFMT are not appreciated due to its low Se.

Conclusion

SLS 3%+NaOH 1.5% with BTB and SLS 2% with BCP may be the potential reagents to be used as test solutions to detect SCM in dairy cows as SLS is available and relatively cheap in our country. However, field evaluation of these reagents is needed to validate their characteristics as a screening test to detect SCM in dairy cows.

Authors’ Contributions

NST and MTI: Conceptualization. NST, MSI, and MK: Sample collection, data collection, and data curation. NST, MSI, MK, and MSP: Formal analysis. MTI: Funding acquisition. NST: Investigation. NST: Methodology. MTI and MAE: Project administration. NST, MSP, and MTI: Resources. MTI and MAE: Supervision. NST, MSP, and MTI: Validation and visualization. NST: Writing – original draft. NST, MSI, MK, MSP, MAE, and MTI: Writing – review and editing. All authors read and approved the final manuscript.

Acknowledgments

The research was financially supported by the Bangladesh Agricultural University Research System (Grant No. 2017/237/BAU). We gratefully thank the smallholder dairy farmers for their generous support during the collection of milk samples.

Competing Interests

The authors declare that they have no competing interests.

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