EVALUATION OF A NEW 'ON FARM TEST' FOR DETECTION OF BOVINE SUBCLINICAL MASTITIS

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ABSTRACT

Diagnosis of subclinical mastitis is problematic as the milk and udder appears normal but generally has an elevated somatic cell count. In the present study, a new 'on farm test' kit "TANUCHEK SCC kit" was evaluated to diagnose subclinical mastitis. Efficiency of this 'on farm test' kit was initially compared with California mastitis test (CMT). Further, the results of these indirect tests (TANUCHEK SCC kit and CMT) were compared with the direct test i.e. direct microscopic somatic cell count which was used as reference. Milk samples (n=610) were simultaneously subjected to direct microscopic somatic cell count, CMT and TANUCHEK SCC kit for the presence of subclinical mastitis. The prevalence of subclinical mastitis was 80.33%, 72.95% and 68.85% for the direct test, CMT and TANUCHEK SCC kit, respectively. Kappa statistics was used to compare the results. The TANUCHEK SCC kit had showed substantial agreement (k = 0.8612) and moderate agreement (k=0.5749) with CMT and the reference direct microscopic somatic cell count, respectively. Results of this study revealed that the TANUCHEK SCC kit can be a simple, easily adoptable, on farm screening test to diagnose SCM in field condition.

Keywords: California mastitis test, Direct microscopic somatic cell count, 'On farm test', Subclinical mastitis, TANUCHEK SCC Kit

Subclinical mastitis (SCM) is the form of the disease responsible for more than 80% of financial losses estimated to occur as a result of mastitis (Giesecke *et al.*, 1994). Hence, screening and prevention of SCM should be the key focus in proactive udder health management. Dairy cows infected with SCM usually have an elevated somatic cell count (SCC), thus monitoring of milk somatic cells is a reliable tool to diagnose SCM.

SCC can be monitored by several direct or indirect tests (Langer *et al.*, 2014). Field tests (cow-side /on farm tests) are preferred to monitor the SCC because they are easy to use, rapid and their easy adoptability and applicability under field conditions. The TANUCHEK SCC kit is a simple, user friendly, on farm test kit, recently developed by Translational Research Platform for Veterinary Biologicals (TRPVB), Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, India. This kit detects the SCC by the development of color in the milk sample analyzed. Thus far, this new on farm test kit was evaluated in comparison with the commonly used field-based indirect diagnostic test i.e. CMT (Nithya *et al.*, 2017; Yasothai, 2017). Direct microscopic somatic cell count (DMSCC) technique was chosen as a reference direct test, to compare the results of indirect tests evaluated in this study. Hence, in this investigation, the efficiency of this TANUCHEK SCC kit was also compared with the direct test (DMSCC) along with the indirect test (CMT) for its adoptability in field condition.

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MATERIALS AND METHODS

Samples: A total of 610 milk samples from 153 crossbred cows (2 non milking quarters) were collected from various dairy farms located at Dindigul district, Tamil Nadu, India and screened for SCM in this study. Milk samples were collected from apparently healthy cows, not showing any clinical signs of mastitis. The udder and teats were physically examined before collecting milk samples. Cows with abnormal milk were ruled out as clinical mastitis cases and those samples were excluded. Prior to sampling, the udder and teats were cleaned with water and wiped with potassium permanganate ($KMnO_4$) solution to have aseptic environment. Milk samples were collected (~10 ml) from all four quarters after stripping first 3 squirts and then transported to laboratory in sterile labeled containers under refrigerated condition. Milk samples were analyzed within 12 hours of collection.

Tests: The milk samples from each quarter were simultaneously subjected to DMSCC, CMT and TANUCHEK SCC kit to screen for the SCM.

The SCC of milk samples were determined using direct microscopic count as per the standard protocol (Schalm *et al.*, 1971). Ten micro liter of milk was spread over 1 cm^2 marked square area on a glass slide and dried at room temperature. Then the slide was fixed in methanol for 5 min and stained with Newman's stain (Nice chemicals, India). Subsequently, the slides were examined microscopically under oil-immersion to calculate SCC/ml of milk.

CMT (DeLaval, Sweden) was performed as described by Schalm *et al.* (1971). Briefly, two ml of milk from each quarter of the udder was placed into the appropriate chamber of the CMT paddle and mixed with an equal amount of CMT reagent by gentle circular rotation of the paddle for 20-30 seconds. The CMT reaction scores which are directly related to average somatic cell counts were scored visually as: negative, trace, 1, 2, and 3 based on the change of viscosity.

Milk samples were also screened using the 'on farm test' kit "TANUCHEK SCC kit" (TRPVB, TANUVAS, Tamil Nadu, India) as recommended by manufacturer. Substrate and enhancer solution needed for the test were provided with the test kit. A drop of substrate solution and three drops of enhancer solution were added to the microfuge tube (provided in the kit) and mixed well. Further, one drop of milk sample was added to the same tube, mixed well and incubated at room temperature for 20-30 minutes for the development of color. The blue colour developed was matched for the intensity with the color card given in the kit outer wrapper. The detection range for this kit is from 1, 00,000 to 9, 00,000 somatic cells/ ml of milk. SCC per ml of milk was obtained by multiplying the value matched by 1000 i.e. 100×1000 cells/ml, 300×1000 cells/ml, 500×1000 cells/ml, 700×1000 cells/ml, 900×1000 cells/ml (Yasothai, 2017).

Results were recorded for individual udder quarters, i.e. left fore (LF), left hind (LH) right fore (RF) and right hind (RH), for the all the three tests.

Statistics and data analysis: The tests were compared for their ability to detect the subclinical mastitis based on the SCC. The SCC obtained by direct microscopic count was considered as reference to calculate test properties of CMT and TANUCHEK SCC kit. A cut-off SCC count of ≥ 200000 cells/mL of milk was used to interpret SCM. The sensitivity, specificity, diagnostic accuracy and the predictive values (at 95% Confidence interval) of CMT and TANUCHEK

SCC kit were calculated and compared with the reference test (DMSCC). Likewise, the results obtained using TANUCHEK SCC kit was also compared with the results of CMT, as the CMT is a most commonly used cow side test worldwide. The agreement between the tests was calculated using kappa statistics. The interpretation of kappa results was carried out as poor (< 0.00), slight (0.00–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–80), almost perfect (0.81–1.00) (Landis and Koch, 1977). MEDCALC® statistical software was used for statistical analysis.

RESULTS AND DISCUSSION

In the present study, 610 milk samples of crossbred dairy cows were screened for SCM by DMSCC, CMT and TANUCHEK SCC kit.

The prevalence of SCM recorded was 80.33%, 72.95% and 68.85%, with DMSCC, CMT and TANUCHEK SCC kit, respectively (Table 1). Earlier studies also showed that SCC of $\geq 200\ 000$ cells/mL of milk indicates an inflammatory response and that quarter is likely to be infected (Dohoo and Leslie, 1991; Laevens *et al.*, 1997; Schepers *et al.*, 1997; Smith *et al.*, 2001). Similarly quarter-wise prevalence of SCM is presented in Table 1. The left hind quarter (LHQ) revealed highest percentage positivity of SCM as 84.31% for DMSCC, 81.70% for CMT and 78.43% for TANUCHEK SCC kit.

Salvador *et al.* (2014) has successfully used SCC as a reference to compare other field tests. Similarly in this study, the DMSCC results were taken as reference to compare and analyze the results of CMT and TANUCHEK SCC kit. The results of TANUCHEK SCC kit were compared with the reference values (DMSCC) and with CMT. Two by two tables were generated comparing the results of DMSCC Vs CMT (Table 2), DMSCC Vs TANUCHEK SCC kit (Table 3) and CMT Vs TANUCHEK SCC kit (Table 4). Sensitivity, specificity, diagnostic accuracy and Cohen's kappa value of CMT and

Table 1

Quarter-wise prevalence of subclinical mastitis by different methods

Methods	LHQ (153)		LFQ (153)		RHQ (153)		RFQ (151)		Total (610)	
	No. of samples positive	% positivity	No. of samples positive	% positivity	No. of samples positive	% positivity	No. of samples positive	% positivity	No. of samples	% positivity
DMSCC	129	84.31	109	71.24	129	84.31	123	81.46	490	80.33
CMT	125	81.70	105	68.63	110	71.90	105	69.54	445	72.95
TANUCHEK SCC Kit	120	78.43	105	68.63	95	62.09	100	66.23	420	68.85

LHQ –Left hind quarter; LFQ – Left fore quarter; RHQ – Right hind quarter; RFQ – Right fore quarter; DMSCC - Direct microscopic somatic cell count; CMT – California mastitis test

Table 2

Two-by-Two table of DMSCC Vs CMT results

		DMSCC		Predictive values
		Positive	Negative	
CMT	Positive	420	25	Positive predictive value - 94.38% (92.21% -95.98.0%)*
	Negative	70	95	Negative predictive value -57.58% (51.75% -63.20%)*

* - Values at 95% CI; DMSCC - Direct microscopic somatic cell count; CMT – California mastitis test

Table 3

Two-by-Two table of DMSCC Vs TANUCHEK SCC kit results

		DMSCC		Predictive values
		Positive	Negative	
TANUCHE K SCC KIT	Positive	405	15	Positive predictive value - 96.43% (94.38% - 97.75%)*
	Negative	85	105	Negative predictive value -55.26% (50.16% - 60.25%)*

* - Values at 95% CI; DMSCC - Direct microscopic somatic cell count

Table 4

Two-by-Two table of CMT Vs TANUCHEK SCC kit results

		CMT		Predictive values
		Positive	Negative	
TANUCHE K SCC KIT	Positive	415	05	Positive predictive value - 98.81% (97.22% - 99.49%) *
	Negative	30	160	Negative predictive value -84.21% (79.04% - 88.29%) *

* - Values at 95% CI; CMT – California mastitis test

Table 5

Comparison of the results of three methods

Parameters (95% CI)	Methods		
	DMSCC Vs CMT	DMSCC Vs TANUCHEK SCC Kit	CMT Vs TANUCHEK SCC Kit
Sensitivity	85.71 %(82.30 %-88.69%)	82.65 %(79.0 -85.90%)	93.26 %(90.52 % - 95.41%)
Specificity	79.17 %(70.80% - 86.04%)	87.50 %(80.22% - 92.83%)	96.97 %(93.07% - 99.01%)
Diagnostic accuracy	84.43 %(81.30% -87.21%)	83.61 %(80.43% - 86.46%)	94.26 %(92.11% - 95.97%)
Cohen's Kappa value	0.5683(0.4922 – 0.6444)	0.5749(0.5031 – 0.6466)	0.8612(0.8178 – 0.9056)

DMSCC - Direct microscopic somatic cell count; CMT – California mastitis test

TANUCHEK SCC kit with 95% confidence interval were calculated and presented in Table 5. The agreement between the tests was obtained using kappa statistics. TANUCHEK SCC kit (k=0.5749) and CMT (k=0.5683) showed a moderate agreement with the reference DMSCC values. Comparison between both the indirect on farm tests (CMT Vs TANUCHEK SCC kit) showed a substantial agreement with a k value of 0.8612 as shown in Table 5.

TANUCHEK SCC kit showed favorable sensitivity with CMT (93.26 %) and DMSCC (82.65%). Similarly, TANUCHEK SCC kit also revealed 87.50% and 96.97% specificity with DMSCC and CMT, respectively, as indicated in Table 5. This higher sensitivity with CMT

could possibly be due to the similar kind of subjective interpretation of results in both of the indirect test methods compared. Similar observation was also reported earlier by Nithya *et al.* (2017) and Yasothai (2017).

The obtained results suggested that both CMT and TANUCHEK SCC kit are reliable diagnostic methods for use in field conditions to screen SCM. The CMT and TANUCHEK SCC Kit, both diagnose SCM based on the concentration of somatic cells in the milk. Though, the cost involved / test was almost equal (Rs. 2.60/sample for CMT and Rs. 2.50/sample for TANUCHEK SCC kit) in both the tests, the time taken to analyses the sample was high in TANUCHEK SCC kit (20-30 minutes) than CMT (20-30 seconds). However, the TANUCHEK SCC kit is practically applicable in field condition because of its easy availability and interpretability as compared to the reagents used in CMT. TANUCHEK SCC kit can be easily procured from TRPVB, TANUVAS, Chennai, India. Moreover, in CMT, scoring of viscosity change is subjective so that, the scoring and interpretation of the results may differ between testers. On the other hand, the results of TANUCHEK SCC kit will be read based on the intensity of colour development which is proportionate to the SCC. Thus, the results of this kit can easily be differentiated and interpreted by a less trained dairy man without involving any technical skill.

In summary, the adoptability of a new 'on farm test' "TANUCHEK SCC Kit" intended to diagnose SCM was evaluated in comparison with the existing widely used indirect cow side test i.e. CMT and also compared with the reference values of DMSCC. This study showed that TANUCHEK SCC kit had comparable sensitivity, specificity, diagnostic accuracy and substantial agreement with CMT. The results suggested that the investigated "TANUCHEK SCC kit" is an easy to use "on farm test kit" suitable for regular monitoring of the herd for SCM.

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