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# Comparison of Cow-Side Diagnostic Tests for Estimating Bovine Subclinical Mastitis Prevalence in Sirajganj District, Bangladesh

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**ABSTRACT**

Subclinical mastitis (SCM) is a significant constraint on milk production and udder health because affected cows show no visible abnormalities in the udder or milk. In Bangladesh, dairy production is dominated by smallholder and semi-intensive farms where hand milking is common and routine access to somatic cell counting or bacteriological culture is limited. This cross-sectional on-farm study compared three practical cow-side tests for estimating SCM prevalence in Sirajganj district. A total of 380 clinically normal lactating cows from 72 dairy farms were examined and screened at quarter level using the California Mastitis Test (CMT; ImmuCell Corporation, USA), UdderCheck® milk test strips (India), and a Dramiński Mastitis Detector MD4X4Q2 (Dramiński S.A., Poland). A cow was positive by a test when at least one functional quarter met that test's positive criterion. Cow-level prevalence was 45.8% (95% CI: 40.8-50.8) by CMT, 41.1% (95% CI: 36.2-46.1) by UdderCheck®, and 37.9% (95% CI: 33.2-42.9) by Dramiński testing. Overall, 52.4% (95% CI: 47.3-57.3) were positive by at least one test. Agreement was substantial between CMT and UdderCheck® (κ = 0.63) and moderate between CMT and Dramiński (κ = 0.53) and between UdderCheck® and Dramiński (κ = 0.54). CMT yielded the highest screening prevalence; however, without SCC or culture, this does not indicate superior diagnostic accuracy. The tests are suitable for rapid herd-level screening, while repeated or discordant results should be confirmed by laboratory methods when available.

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## Introduction

Mastitis is a complex, multifactorial inflammatory disease of the mammary gland and remains one of the most economically important diseases of dairy cows worldwide. It occurs in clinical and subclinical forms. Clinical mastitis is recognized by visible changes in the udder or milk, whereas subclinical mastitis (SCM) is usually not accompanied by obvious abnormality in milk or udder appearance but is associated with increased somatic cell response, altered milk composition and reduced production (International Dairy Federation 1999; Viguier et al. 2009; Sharma, Singh and Bhadwal 2011). Because the milk appears normal, SCM often remains undetected by farmers and may persist as a source of intramammary infection within the herd.

Early detection of SCM is particularly important in Bangladesh, where dairy production is dominated by smallholder and semi-intensive farms. Many herds use tie-stall or restricted-housing systems; cows are commonly milked by hand; and udder preparation, individual towel use, post-milking teat disinfection, record-keeping, and routine laboratory testing are inconsistent. Wet floors, poor drainage, manure contamination, limited farmer awareness, and delayed recognition of udder disease can facilitate transmission and allow subclinical infections to persist. Previous Bangladeshi studies have reported substantial SCM burdens across production regions. Rahman et al. (2009) identified wet or soiled floors, udder cleanliness, season, and milk yield as important factors. Sarker et al. (2013) described animal- and management-related risks in smallholder dairy cows. Tripura et al. (2014) reported 51.8% cow-level SCM prevalence by CMT in Mymensingh and Lakshmipur, while Kabir et al. (2017) reported 51% prevalence in crossbred cows in the Baghabari milk-shed area of Sirajganj and Pabna.

Somatic cell count (SCC) and bacteriological culture provide stronger evidence of intramammary inflammation and infection, but their cost, turnaround time, and limited field availability constrain routine use on many Bangladeshi farms. Cow-side tests can therefore support rapid screening, farmer education, and decisions about follow-up testing. CMT is inexpensive and produces a visible gel reaction related to cellular content (Sargeant et al. 2001; Barbosa et al. 2002). UdderCheck® is an LDH-based strip that reflects mammary tissue injury and inflammation. Electrical conductivity testing detects changes in milk ionic composition following disruption of the blood-milk barrier and is rapid and reagent-free (Fernando, Spahr and Jaster 1985; Langer et al. 2014). Iraguha et al. (2017) demonstrated the practical value of comparing these field tests and showed that results differ because each test measures a distinct biological response.

Because these tests measure different biological responses, their positive proportions and agreement are expected to differ. In the absence of an SCC or culture reference standard, comparisons must be restricted to screening prevalence and agreement; sensitivity, specificity, predictive values, and claims of diagnostic superiority are not justified. This study, therefore, aimed to estimate farm-, cow-, and quarter-level SCM screening prevalence using CMT, UdderCheck®, and Dramiński testing, and to quantify pairwise agreement among the tests under field conditions in Sirajganj district, Bangladesh.

## Materials and methods

### Study design and study area

A cross-sectional on-farm study was conducted in Potazia Union, Shahzadpur Upazila, Sirajganj district, Bangladesh, during July 2024 to June 2025. Sirajganj includes the Baghabarighat milkshed and is one of the country's major milk-producing areas. Farms in this production setting are predominantly smallholder or semi-intensive units with hand milking, locally available feed resources, and variable housing, floor drainage, hygiene, and access to veterinary and laboratory services. The sampled farms were located within Potazia Union in Shahzadpur Upazila under Sirajganj district. The location of the study area is shown in Figure 1.

### Farm and cow selection

Seventy-two dairy farms and 380 lactating cows were included. Farms were selected from accessible dairy-farming communities after farmers agreed to participate; consequently, the sample should be regarded as field-based rather than as a district-wide probability sample. The achieved sample comprised all eligible and available cows on participating farms during farm visits. Cows were eligible when lactating and free from visible signs of clinical mastitis. Cows with abnormal milk, marked udder swelling, heat, pain, or systemic illness were excluded from SCM screening, and farmers were advised to seek veterinary care. Farm-level prevalence used farms as the denominator; cow-level prevalence used all tested lactating cows. No retrospective adjustment or replacement of excluded cows was undertaken.

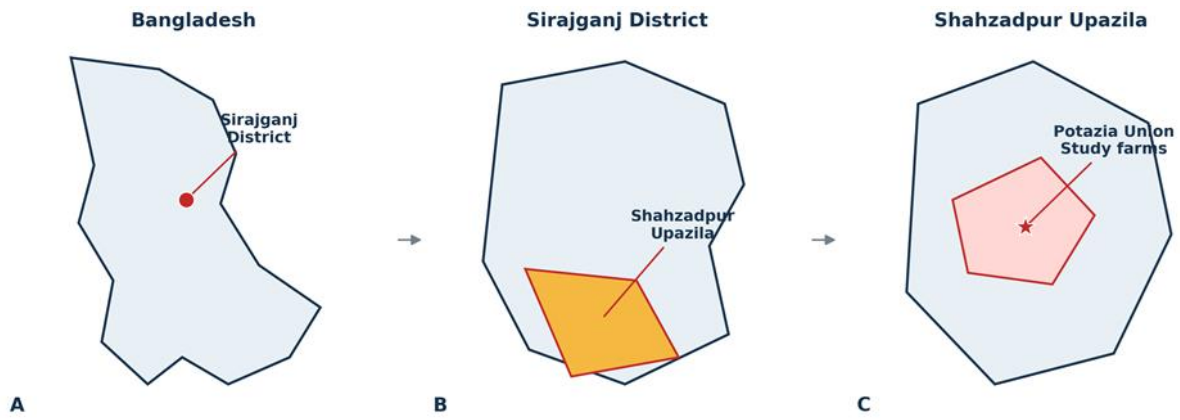


**Figure 1.** Location of the study

**Clinical examination and milk sampling**

Before testing, each cow was calmly restrained, and the udder and teats were inspected and palpated for injury, swelling, heat, pain, fibrosis, or other abnormalities. The first stream from each quarter was discarded into a strip cup and examined for clots, flakes, watery secretions, blood, or other clinical changes. Teats were cleaned and dried before testing. Fresh foremilk from each functional quarter was tested immediately on the farm. Operators followed a consistent sequence and recorded each test result separately to reduce transcription and interpretation errors.

**California Mastitis Test**

The California Mastitis Test was performed using the ImmuCell CMT kit (ImmuCell Corporation, Portland, Maine, USA) according to the manufacturer's instructions. Approximately equal volumes of milk and reagent were mixed in the corresponding wells of a four-compartment paddle. The paddle was gently rotated, and the reaction was read immediately under adequate lighting. Reactions were recorded as negative, trace, weak positive, distinct positive, or strong positive. For the primary analysis, weak positive or stronger reactions were classified as positive; trace reactions were recorded as suspect and classified as non-positive. The same operational threshold was used throughout the study.

**UdderCheck® milk test strip**

UdderCheck® milk test strips (India) were used as an LDH-based indicator of mammary tissue inflammation. Fresh quarter milk was applied according to the supplied instructions. Each strip was read after the recommended reaction interval and compared with the manufacturer's color chart. A reaction in the manufacturer's positive category was recorded as UdderCheck®-positive. Strips were kept dry and protected from excessive heat and direct sunlight during field visits, conditions that are particularly important in Bangladesh's warm, humid environment.

**Electrical conductivity testing with Dramiński Mastitis Detector**

Electrical conductivity/resistance-related screening was performed using a Dramiński Mastitis Detector MD4X4Q2 (Dramiński S.A., Olsztyn, Poland). Fresh milk from each quarter was placed in the clean measuring cup, and the displayed value was recorded. The cup was emptied and cleaned between measurements following the manufacturer's instructions. Values were treated as Dramiński device display units and were not reported as laboratory mS/cm measurements. A quarter was classified as abnormal using the manufacturer's inter-quarter comparison rule, whereby a meaningful reduction in the displayed value relative to the other quarters indicates possible inflammatory change. Because physiological factors can influence conductivity, the result was interpreted as a screening indicator rather than laboratory confirmation.

**Case definitions**

At the quarter level, positivity was defined separately according to each test's operational criterion. At cow level, a cow was positive by a particular test when at least one functional quarter was positive. At farm level, a farm was positive when at least one tested cow was positive. A composite 'any test' screening outcome classified a cow or farm as positive when at least one of the three tests was positive. This composite outcome was used only to describe field-screening yield and was not considered a diagnostic gold standard or confirmed SCM.

**Statistical analysis**

Farm-, cow-, and quarter-level positive proportions were calculated with 95% confidence intervals using the Wilson binomial method. Pairwise agreement among cow-level test results was assessed using observed agreement and Cohen's kappa (Landis and Koch 1977; Iraguha et al. 2017). Kappa was interpreted as slight (0.00-0.20), fair (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80), or almost perfect (0.81-1.00). McNemar's test compared paired cow-level positive proportions. The analysis was descriptive and did not model clustering of cows within farms or quarters within cows. Because SCC and bacteriological culture were unavailable as reference methods, diagnostic sensitivity, specificity, predictive values, and likelihood ratios were not calculated.

**Ethical and animal welfare considerations**

Procedures were non-invasive and limited to routine clinical examination and collection of milk during normal milking. Animals were handled calmly to minimize distress, and verbal informed consent was obtained from each participating farmer before sampling. Individual farm and farmer identities were kept confidential.

## Results

A total of 380 lactating cows from 72 dairy farms were screened under field conditions. The mean number of cows tested per farm was 5.3, consistent with the smallholder nature of many Bangladeshi dairy units. The reported quarter-level denominator was 1,520, corresponding to 4 quarters per tested cow; this total should be checked against the original field records before submission. CMT produced the highest positive proportions at farm, cow, and quarter levels, followed by UdderCheck® and Dramiński testing.

**Table 1. Farm-level SCM-positive status by cow-side screening test**

| Cow-side test | Positive farms / tested farms | Farm-level prevalence (%) | 95% CI (%) |
|---|---|---|---|
| CMT | 61/72 | 84.7 | 74.7-91.2 |
| UdderCheck® | 57/72 | 79.2 | 68.4-86.9 |
| Dramiński EC | 53/72 | 73.6 | 62.4-82.4 |
| Any of the three tests | 64/72 | 88.9 | 79.6-94.3 |

Farm-level positive status means at least one tested cow on the farm was positive by the respective test. CI = confidence interval.

**Table 2. Cow-level prevalence of subclinical mastitis estimated by three cow-side tests**

| Cow-side test | Positive cows / tested cows | Cow-level prevalence (%) | 95% CI (%) |
|---|---|---|---|
| CMT | 174/380 | 45.8 | 40.8-50.8 |
| UdderCheck® | 156/380 | 41.1 | 36.2-46.1 |
| Dramiński EC | 144/380 | 37.9 | 33.2-42.9 |
| Any of the three tests | 199/380 | 52.4 | 47.3-57.3 |

Cow-level positive status was defined as at least one positive functional quarter by the respective test. 'Any of the three tests' is a screening outcome and not a gold standard.

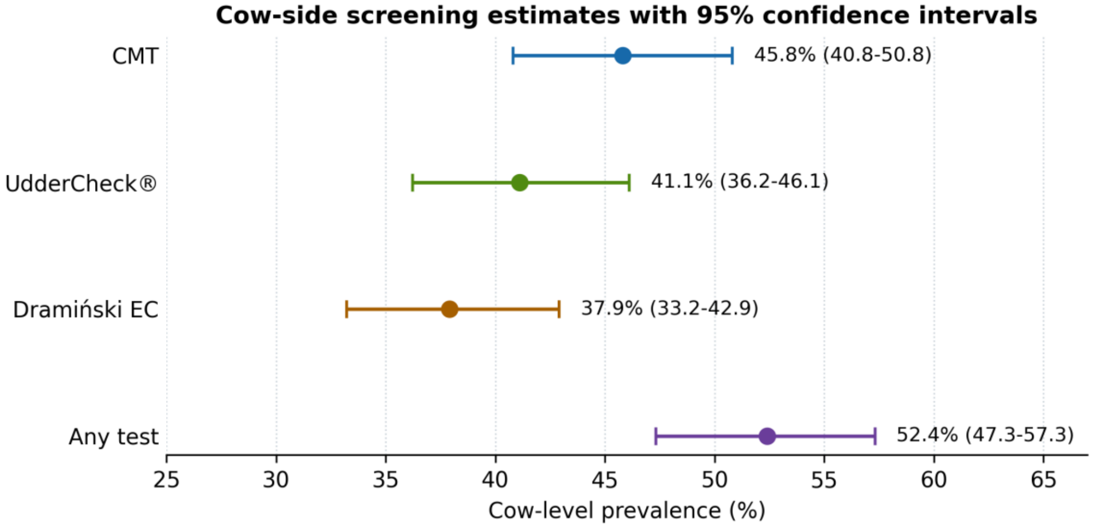


**Figure 2. Cow-level prevalence estimates from the three cow-side screening tests and the composite any-test outcome. Error bars represent 95% Wilson confidence intervals.**

**Table 3. Quarter-level prevalence of subclinical mastitis estimated by cow-side tests**

| Cow-side test | Positive quarters / tested quarters | Quarter-level prevalence (%) | 95% CI (%) |
|---|---|---|---|
| CMT | 356/1520 | 23.4 | 21.4-25.6 |
| UdderCheck® | 318/1520 | 20.9 | 19.0-23.0 |
| Dramiński EC | 287/1520 | 18.9 | 17.0-20.9 |

The reported denominator of 1,520 corresponds to four quarters for each of 380 cows and should be verified against the original field records. If blind, non-functional, missing, or invalid quarters occurred, the denominator and confidence intervals must be recalculated.

**Table 4. Pairwise cow-level agreement among cow-side diagnostic tests.**

| Comparison | +/+ | +/- | -/+ | -/- | Observed agreement (%) | Kappa | McNemar p-value |
|---|---|---|---|---|---|---|---|
| CMT vs UdderCheck® | 130 | 44 | 26 | 180 | 81.6 | 0.63 | 0.041 |
| CMT vs Dramiński EC | 115 | 59 | 29 | 177 | 76.8 | 0.53 | 0.002 |
| UdderCheck® vs Dramiński EC | 108 | 48 | 36 | 188 | 77.9 | 0.54 | 0.230 |

+/+ = positive by both tests; +/- = positive by first test only; -/+ = positive by second test only; -/- = negative by both tests. P-values were calculated from paired discordant cow-level results.

CMT detected the highest cow-level positive proportion (45.8%), followed by UdderCheck® (41.1%) and Dramiński testing (37.9%) (Figure 2). The composite 'any test' outcome identified 199 of 380 cows (52.4%), but these cows should not be interpreted as laboratory-confirmed SCM cases. Agreement was substantial between CMT and UdderCheck® ($\kappa$ = 0.63) and moderate between CMT and Dramiński ($\kappa$ = 0.53) and UdderCheck® and Dramiński ($\kappa$ = 0.54). McNemar's test indicated different paired positive proportions for CMT versus UdderCheck® ($p$ = 0.041) and CMT versus Dramiński ($p$ = 0.002), whereas UdderCheck® and Dramiński did not differ significantly ($p$ = 0.230). These results demonstrate differences in screening yield, not comparative diagnostic accuracy.

## Discussion

This study provides a Bangladesh-focused comparison of three cow-side SCM screening methods under routine farm conditions in Sirajganj district. Positive proportions varied by test, with CMT identifying the largest proportion of cows, followed by UdderCheck® and Dramiński testing. This ordering is biologically plausible because CMT reflects somatic-cell-associated viscosity, UdderCheck® reflects LDH activity related to mammary tissue injury, and conductivity testing reflects inflammatory changes in milk electrolytes (Fernando, Spahr and Jaster 1985; Viguier et al. 2009). The differences must not be interpreted as evidence that CMT was more sensitive or accurate, as no independent reference standard was used. Iraguha et al. (2017) similarly found differences among cow-side tests when evaluated against a portable SCC-based reference.

The cow-level prevalence detected by CMT (45.8%) was consistent with the high SCM burden previously reported in Bangladesh. Rahman et al. (2009) reported a high prevalence of mastitis in dairy cows, particularly in the wet season, and highlighted the contributions of wet or soiled floors and udder cleanliness. Tripura et al. (2014) reported 51.8% cow-level SCM prevalence by CMT in Mymensingh and Lakshmipur. Kabir et al. (2017) reported 51% SCM prevalence in crossbred cows at the Baghabarighat milk-shed area of Sirajganj and Pabna. The slightly lower CMT prevalence in the present study may reflect differences in farm selection, season, breed composition, lactation stage, CMT threshold, and hygiene status.

The relatively high detection yield, low cost, rapid reaction, and simple equipment make CMT a practical first-line screening option for smallholder and semi-intensive farms. Its usefulness is especially relevant where farmers depend on hand milking and veterinary or laboratory services are not readily available. Nevertheless, reader subjectivity, reagent-to-milk ratio, rotation time, lighting, and classification of trace reactions can alter results. Standardized demonstrations and periodic supervision are therefore important if CMT is incorporated into farmer training, cooperative milk collection, or herd-health programs.

UdderCheck® produced a slightly lower positive proportion than CMT but showed substantial agreement with it. The strip format may be convenient for veterinarians and trained field workers, although storage, milk volume, reaction time, and color interpretation can influence performance. Protection from heat, humidity, and sunlight is especially important under Bangladeshi field conditions. UdderCheck® may therefore be useful as a supportive screening method when the product is available, and its storage requirements can be maintained.

The Dramiński detector produced the lowest positive proportion but remained moderately concordant with the other tests. It offers immediate results, is reusable, and requires no reagent, which may reduce recurring screening costs. However, its readings can be influenced by milk temperature, parity, stage of lactation, mineral concentration, time since milking, and physiological inter-quarter variation. It should therefore be used for repeated monitoring and identification of suspicious quarters rather than as a stand-alone confirmatory test (Iraguha et al. 2017). Consistent cleaning and measurement techniques are essential when the device is used across multiple cows and farms.

For Bangladesh dairy herds, cow-side screening is most useful when linked to practical control measures. Farmers with positive or repeatedly suspicious cows should receive guidance on clean, dry housing; drainage; handwashing; use of individual clean towels; foremilk examination; post-milking teat disinfection; keeping cows standing briefly after milking; recordkeeping; and prompt veterinary evaluation. Recurrent or discordant cow-side results should be confirmed by SCC and bacteriological culture when accessible. Treatment decisions, especially antimicrobial use, should not be based solely on a cow-side screening result.

This study has several limitations. First, SCC and bacteriological culture were not included; therefore, the results describe positive proportions and inter-test agreement rather than diagnostic accuracy or confirmed infection. Second, farms were selected from accessible communities with farmer consent, so the estimates may not represent all farms in Sirajganj or Bangladesh. Third, the analysis did not adjust for clustering of quarters within cows or cows within farms, which may make quarter- and cow-level confidence intervals too narrow. Fourth, cow and farm characteristics such as breed, parity, stage of lactation, yield, housing, floor condition, drainage, hygiene, and previous mastitis were not incorporated into the present comparison. Finally, operator technique, strip storage, milk temperature, reaction thresholds, and physiological variation may have affected results. Future studies should use probability-based sampling, document invalid and non-functional quarters, collect management and animal-level covariates, and include SCC, culture, pathogen identification, and antimicrobial susceptibility testing.

## Conclusion

In this field study, positive proportions differed among CMT, UdderCheck®, and Dramiński testing in lactating cows from Sirajganj district. CMT produced the highest cow-level screening estimate, while UdderCheck® and Dramiński testing provided supportive information and showed moderate-to-substantial agreement with CMT. More than half of the cows were positive by at least one test, indicating a substantial need for structured udder-health surveillance; however, this composite result does not represent laboratory-confirmed SCM. CMT is suitable as a practical first-line farm screening method, particularly where resources are limited. Repeated or discordant findings should be confirmed using SCC and culture, and screening should be combined with improved milking hygiene, dry housing, farmer training, records, and responsible veterinary decision-making.

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## Competing interests

The authors declare no conflicts of interest.

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