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# Comprehensive Assessment of Raw Milk Quality: Techniques for Identifying Mastitis and Monitoring Bacterial Growth at 37°C

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**ABSTRACT: Milk quality is fundamental to dairy safety and public health, significantly influencing the nutritional value and safety of dairy products. Mastitis, an inflammatory condition of the mammary gland caused by bacterial infections, is a major factor affecting milk quality by increasing the somatic cell count (SCC), a crucial indicator of infection and inflammation. Elevated SCC levels can lead to milk rejection by processors and pose health risks if not properly treated. This study evaluates raw milk quality using SCC, California Mastitis Test (CMT), and Hotis test to detect mastitis and monitor bacterial growth during storage at 37°C. The prevalence of mastitis was 27%, with visible clots in CMT and canary yellow colonies in the Hotis test indicating significant somatic cell presence and Streptococcus agalactiae infection, respectively. Bacteriological quality assessment revealed a substantial increase in bacterial counts during storage at 37°C, emphasizing the need for proper refrigeration to inhibit microbial growth. Initial standard plate counts were 5.664±0.287 log10 cfu/ml, rising to 8.526±0.090 log10 cfu/ml after six hours, while coliform counts increased from 3.885±0.093 log10 cfu/ml to 6.495±0.301 log10 cfu/ml. These findings highlight the effectiveness of SCC, CMT, and Hotis tests in mastitis detection and milk quality assessment, underscoring the importance of maintaining optimal storage conditions to ensure dairy product safety.**

**Keywords:** Raw Milk Quality, Somatic Cell Count (SCC), California Mastitis Test (CMT), Hotis Test, Mastitis Detection, Bacterial Growth, Milk Storage, Bacteriological Assessment, Streptococcus agalactiae, Microbial Contamination.

## INTRODUCTION

Milk is a fundamental component of human diets, providing a rich source of essential nutrients. However, its perishable nature and susceptibility to microbial contamination present significant challenges to dairy safety and public health. While raw milk initially contains low numbers of microorganisms, it can rapidly become contaminated with spoilage bacteria and pathogens from various sources, including animal feces, soil, air, feed, water, bedding material, animal hides, and milking equipment (Muehlhof *et al*., 2013; Moatsou & Moschopoulou 2015). Such contamination can severely compromise milk quality and pose potential health risks to consumers. The quality of milk at the farm level is heavily influenced by farm management practices and hygienic standards. Ensuring milk safety requires effective control of microbial contamination through proper farm hygiene, correct milking procedures, and timely refrigeration (Owusu-Kwarteng *et al*., 2020; Omore *et al.,* 2005). The initial microbial load of milk can impact the quality of dairy products throughout the supply chain, as high bacterial counts may not be completely eliminated by subsequent heat treatments (Zucali *et al.,* 2011; Nacul & Revoredo-Giha 2022). Major sources of microbial contamination in bulk milk include the external surfaces of the udder and teats, milking equipment, and mastitis organisms from within the udder (Elmoslemany *et al*., 2010).

To safeguard milk quality, various diagnostic techniques are employed to monitor and manage bacterial contamination. Total Bacterial Count (TBC) and Coliform Count (CC) are commonly used to assess the hygienic quality of raw milk (Piepers *et al*., 2014). TBC measures the total number of aerobic bacteria present and reflects the overall cleanliness of the milking environment and equipment (Jayarao & Wolfgang 2003; Bava *et al*., 2011). Elevated TBC levels are associated with unsanitary conditions, including dirty udders and equipment, and can lead to defects in milk texture and flavor (Chambers, 2002; Gleeson *et al*., 2017). CC measures the number of coliform bacteria, which indicate fecal contamination and poor hygiene practices (Pantoja *et al*., 2009; Elmoslemany *et al*., 2009).

Mastitis, an inflammatory condition of the mammary gland caused by bacterial infections, significantly affects milk quality. Mastitis raises somatic cell count

(SCC), a key indicator of infection and inflammation, which can result in milk being rejected by processors and pose health risks if not properly treated (Omore *et al*., 2005; Zucali *et al.,* 2011). The California Mastitis Test (CMT) and Hotis test are essential tools for detecting mastitis and specific pathogens, offering rapid and cost-effective methods for assessing milk quality (Jayarao *et al.,* 2004; Martin *et al*., 2016).

Monitoring bacterial growth in milk during storage is also critical, as raw milk is highly perishable and can deteriorate rapidly if not stored at appropriate temperatures. Studies have shown that bacterial contamination increases significantly when milk is stored at ambient temperatures, highlighting the need for proper refrigeration to prevent microbial growth (Mhone *et al.,* 2011; Jooste & Anelich 2008).

This study aims to provide a comprehensive assessment of raw milk quality by evaluating mastitis using SCC, CMT, and Hotis tests, and by monitoring bacterial growth during storage at 37°C. By analyzing these parameters, the study seeks to enhance understanding of factors affecting milk quality and underscore the importance of proper storage practices to ensure the safety and quality of dairy products.

## MATERIALS AND METHODS

**Sample Collection.** Fresh milk sample were collected from cattle yard, National Dairy Research Institute, Karnal, India. Milk samples were collected from individual animals free from visible blood and pus cells. The samples were transported to the laboratory under chilled conditions to minimize microbial growth (Singh *et al*., 2012).

**Somatic Cell Count (SCC).** SCC was measured using the Ekomilk scan as per Galdhar *et al*. (2005). Milk samples were filtered through muslin cloth to ensure cleanliness. A mixture of 5.0 ml of Ekoprime reagent and 10.0 ml of milk was prepared. The Eko milk analyzer measured the viscosity of the mixture, which correlates with SCC. Samples with SCC 250 × $10^3$ cells/ml were classified as disease-free.

**California Mastitis Test (CMT).** CMT was conducted by mixing 3 ml of milk with 3 ml of CMT reagent (NaOH 1.5%, Teepol 0.5% v/v, Bromothymol blue 0.01% w/v). The mixture was gently swirled for 10-20 seconds. Results were scored from 0 to 3 (Table1), with scores of 2 or 3 indicating a positive result (Shuster *et al.,* 2004).

**Table 1: CMT Score Card to Identify Extent of Infection in California Mastitis Test.**

| CMT Score | Interpretation | Visible Reaction | Probable SCC/ml | Neutrophils (%) |
|---|---|---|---|---|
| 0 | Negative | Milk fluid and normal | 0 - 200,000 | 0 - 25 |
| T | Trace | Slight precipitation | 15,000 - 500,000 | 30 - 40 |
| 1 | Weak Positive | Distinct precipitation but no gel formation | 40,000 - 1,500,000 | 40 - 60 |
| 2 | Distinct Positive | Mixture thickens with gel formation | 80,000 - 5,000,000 | 60 - 70 |
| 3 | Strong Positive | Viscosity increased and strong gel formed that is cohesive with a convex surface | >5,000,000 | 70 - 80 |

**Hotis Test** Hotis test was performed as described in IS: 1479 Part III (1977). A mixture of 0.5 ml bromocresol purple solution (0.5 g in 100 ml distilled water) and 9.5 ml of milk was incubated at 37±0.5°C for 24 hours. Presence of canary yellow colonies indicated Streptococcus agalactiae infection (Galdhar *et al.,* 2005).

**Bacteriological Quality Assessment** Milk from healthy animals was stored at 37°C, with samples drawn hourly for six hours. Standard plate count and coliform count were measured using standard plate count agar and violet red bile agar, respectively. Bacterial counts were recorded as log10 colony-forming units per milliliter (cfu/ml) (Vargova *et al*., 2011).

## RESULTS

**Prevalence of Mastitis.** The prevalence of mastitis among the buffalo milk samples, based on the California Mastitis Test (CMT) and Hotis test as shown in Fig. 1 and Table 2, was found to be 27%. The CMT results showed visible clots in the milk samples, indicating a significant presence of somatic cells and, thus, mastitis. Specifically, scores of 2 or 3 on the CMT were observed, reflecting a moderate to severe mastitis infection. These results are consistent with previous research indicating that elevated SCC correlates with mastitis severity (Shuster *et al.,* 2004). Healthy animals had a mean somatic cell count (SCC) of 74.928 × $10^3$ ± 1.894 cells/ml, which is consistent with normal values reported in similar studies (Halasa *et al.,* 2009).

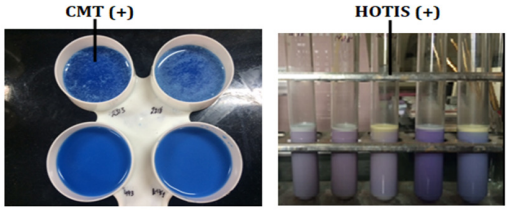


**Fig. 1: (a)** California mastitis test and **(b)** Hotis test positive samples.

**Hotis Test Results.** The Hotis test, used to identify Streptococcus agalactiae, involved the incubation of milk with bromocresol purple solution at 37±0.5°C. After 24 hours of incubation, positive results were indicated by the appearance of canary yellow colonies on the surface of the test samples. The color change to yellow is a clear, visible indication of the presence of Streptococcus agalactiae, as the bromocresol purple changes color in response to the metabolic activity of the bacteria. This colorimetric reaction provides a highly specific method for detecting this pathogen and

is supported by previous studies on its effectiveness (Galdhar *et al*., 2005; Vargova *et al*., 2011).

**California Mastitis Test (CMT) Results.** In the California Mastitis Test, visible clots were observed upon mixing the milk samples with the CMT reagent. The presence of these clots is indicative of high somatic cell counts, which correlates with mastitis infection. Scores of 2 and 3 on the CMT correspond to a gel-like consistency, reflecting an increased concentration of somatic cells due to inflammation or infection. This reaction is crucial for diagnosing the severity of mastitis in dairy cattle and aligns with the findings of Shuster *et al.* (2004) and other similar research (Halasa *et al.,* 2009).

**Table 2: Somatic Cell Count (SCC), California Mastitis Test (CMT), and Hotis Test for Selected Animals.**

| Sr. No. | SCC ($\times 10^3$) | CMT Score | CMT Interpretation | Hotis Test Result | Inference |
|---|---|---|---|---|---|
| 1. | 70 | 0 | Negative | Negative | Healthy |
| 2. | 80 | 0 | Negative | Negative | Healthy |
| 3. | 70 | 0 | Negative | Negative | Healthy |
| 4. | 70 | 0 | Negative | Negative | Healthy |
| 5. | 337 | 2 | Distinct positive | Positive | Mastitis |
| 6. | 405 | 2 | Distinct positive | Positive | Mastitis |
| 7. | 70 | 0 | Negative | Negative | Healthy |
| 8. | 70 | 0 | Negative | Negative | Healthy |
| 9. | 70 | 0 | Negative | Negative | Healthy |
| 10. | 70 | 0 | Negative | Negative | Healthy |
| 11. | 87 | 0 | Negative | Negative | Healthy |
| 12. | 240 | 2 | Distinct positive | Positive | Mastitis |
| 13. | 112 | 1 | Weak positive | Negative | Mastitis |
| 14. | 75 | 0 | Negative | Negative | Healthy |
| 15. | 74 | 0 | Negative | Negative | Healthy |
| 16. | 70 | 0 | Negative | Negative | Healthy |
| 17. | 295 | 2 | Distinct positive | Positive | Mastitis |
| 18. | 345 | 2 | Distinct positive | Positive | Mastitis |
| 19. | 84 | 0 | Negative | Negative | Healthy |
| 20. | 90 | 0 | Negative | Negative | Healthy |
| 21. | 86 | 0 | Negative | Negative | Healthy |
| 22. | 117 | 1 | Weak positive | Negative | Mastitis |
| 23. | 506 | 2 | Distinct positive | Positive | Mastitis |
| 24 | 479 | 2 | Distinct positive | Positive | Mastitis |

*CMT score/interpretation details are provided in Table 1.

**Bacteriological Quality** The bacteriological quality assessment revealed a significant increase in bacterial counts during storage at 37°C. Initial standard plate counts were measured at 5.664±0.287 log10 cfu/ml and coliform counts at 3.885±0.093 log10 cfu/ml. After one hour of storage, there was a substantial increase in bacterial growth, with standard plate counts rising to 8.526±0.090 log10 cfu/ml and coliform counts to 6.495±0.301 log10 cfu/ml over the six-hour period. This growth pattern highlights the rapid deterioration of milk quality at elevated temperatures and is consistent with findings by Weaver and McDaniel (2018); Hashmi & Saleem (2015).

The present study's findings align with research conducted by Deddefo *et al.* (2023), which highlights significant concerns regarding the microbiological quality of bulk milk in dairy farms. The study conducted in Asella, Ethiopia, reported that 66%, 88%, and 32% of farms had Total Bacterial Count (TBC), Coliform Count (CC), and Coagulase-Positive Staphylococci (CPS) counts, respectively, exceeding the standard international limits for raw cow's milk intended for direct human consumption. The geometric means of TBC, CC, and CPS were 5.25 log cfu/ml, 3.1 log cfu/ml, and 2.97 log cfu/ml, respectively, indicating a high level of contamination in the bulk milk.

Several factors were identified as contributing to the poor microbiological quality of bulk milk, including dirty barns, dirty cows, and soiled udders and teats. The study also found that TBC levels were higher during the rainy season, suggesting that environmental conditions play a significant role in milk contamination (Deddefo *et al.* 2023).

## DISCUSSION

The results confirm the effectiveness of SCC, CMT, and Hotis tests in detecting mastitis and assessing milk quality. The CMT and Hotis tests were particularly valuable in identifying mastitis and specific pathogens. The visible clots observed in the CMT provided a direct measure of somatic cell concentration, while the Hotis test's color change offered a specific indication of Streptococcus agalactiae infection. These methods are effective for early detection and diagnosis, allowing for timely intervention (Shuster *et al*., 2004; Galdhar *et al*., 2005).

The significant increase in bacterial counts after just one hour of storage at 37°C underscores the rapid growth of microorganisms under suboptimal conditions. This finding aligns with previous studies that have highlighted the importance of maintaining proper storage temperatures to prevent bacterial proliferation and ensure milk safety (Hashmi & Saleem 2015; Singh *et al.,* 2012). The deterioration in milk quality at elevated temperatures further emphasizes the need for

prompt refrigeration to preserve milk and prevent potential health risks (Weaver & McDaniel 2018).

Overall, the study illustrates that routine use of SCC, CMT, and Hotis tests can significantly improve the management of mastitis and ensure high-quality milk production. By integrating these tests into regular dairy management practices, farmers can better monitor animal health and milk quality, ultimately enhancing the safety and efficacy of dairy operations (Halasa *et al*., 2009; Vargova *et al.,* 2011).

## CONCLUSIONS

This study demonstrates the utility of SCC, CMT, and Hotis tests in identifying healthy milch animals and emphasizes the importance of timely refrigeration to preserve milk quality. Additionally, the practice of washing teats with warm water was found to significantly decrease bacterial count levels, highlighting the importance of proper milking hygiene. Future research should focus on exploring additional methods for improving milk safety and quality.

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