

Comparison of rapid cultivation tests to detection mastitis in dairy cows

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Abstract: The objective of this study was to evaluate and compare two methods for rapid detection of udder pathogens: MicroMast rapid plates versus the ClearMilk Test. Both methods belong to cultivation-based laboratory methods for the detection of Gram-positive bacteria (staphylococci and streptococci) and Gram-negative bacteria directly under on-farm conditions. During the study, 520 cows were investigated on dairy farms in eastern Slovakia. Subsequently, 144 quarter milk samples from the positive cows with California mastitis test scores 1-3 were subjected to laboratory cultivation using both rapid tests. Values obtained from these tests show the MicroMast test sensitivity for positive samples at 84.7% and the ClearMilk test sensitivity at 93.1%. After biochemical and protein identification of cultured isolates, on both rapid tests MicroMast test and ClearMilk were identified as the main pathogens present *Staphylococcus* spp. (*S. aureus* and *S. chromogenes*) and *Streptococcus* spp. (*Str. bovis*). Based on the results, both tests are comparable and can therefore be used in practice for the rapid detection of udder pathogens.

Keywords: dairy cows, mastitis, MicroMast test, ClearMilkTest, udder pathogens

MicroMast Rapid Test

This test is designed for rapid 24-hour culture of samples directly under on-farm conditions. The results were evaluated after 24 hours of incubation at 37 °C for each plate, which is divided into three zones (Fig. 2).

Growth in the **first zone “A”** is used to rule out sample contamination and to confirm bacterial growth (non-selective agar).

The second **zone “B”** is intended for the identification of Gram-positive pathogens, such as *Staphylococcus aureus*, coagulase-negative staphylococci (CNS), *Streptococcus agalactiae*, *Streptococcus uberis*, *Enterococcus* spp., *Corynebacterium* spp. or *Bacillus* spp.

The **third zone “C”** is intended for the identification of Gram-negative pathogens, including *Escherichia coli*, *Klebsiella* spp., *Proteus vulgaris*, and *Pseudomonas* spp.

ClearMilk Test

This test is used for the rapid 24-hour cultivation of milk samples under on-farm conditions. Milk samples were applied to the surface of a plate containing three different selective chromogenic agars. The results were analyzed after incubation of each plate, which is divided into three zones (Fig. 2): **G- sector:** This sector supports the growth of chromogenically distinguishable bacteria belonging to the group of Gram-negative rods.

Staphylococcal sector: This sector is designed for the growth of *Staphylococcus* spp.

Streptococcal sector: This sector supports the growth of *Streptococcus* spp.

Figure 2: Cultivation of milk samples on MicroMast plates and ClearMilk teste



Conclusion

The results obtained from this study allowed us to demonstrate that although the use of the California Mastitis Test (CMT) under on-farm conditions does not enable pathogen identification, it can indicate an ongoing or early-stage inflammation of the udder. When comparing two rapid milk sample cultivation methods, the sensitivity of positive samples was observed to be 84.7% using MicroMast plates and as high as 93.1% with the ClearMilk test. The confirmed presence of *Staphylococcus* spp., *Streptococcus* spp., a significant proportion of coagulase-negative staphylococci (CNS), and *E. coli* in milk samples from mastitic cows highlights the potential health risks for end consumers. Based on the results, both tests used are comparable and can therefore be implemented in practice for the rapid detection of intramammary pathogens. One advantage of the ClearMilk test may be its more accurate detection and faster differentiation of Gram-positive pathogens due to its separate zones for *Staphylococcus* spp. and *Streptococcus* spp.

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