emma qPCR – an innovative solution for detection of clinical mastitis in veterinary practices

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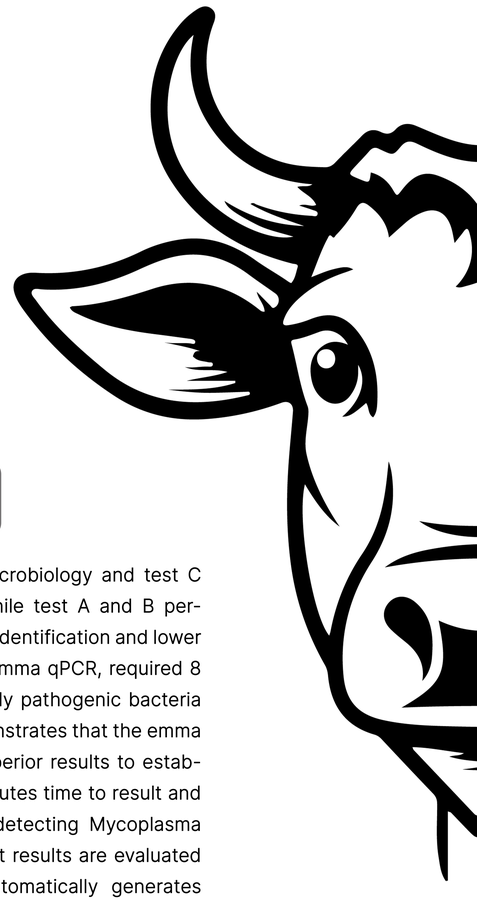

Summary

Rapid and accurate pathogen identification is crucial for targeted, evidence-based treatment of clinical mastitis. There are various tests for use in veterinary practices on the market, but there are big differences in result time and quality. This comparative study examined 95 milk samples with 5 different tests in parallel. The emma qPCR, classical microbiology and test C detected specific pathogens, while tests A and B only indicated the gram status and had much lower detection rates. With 90 minutes result time, the emma qPCR was the fastest and only suitable test for detecting Mycoplasma spp.
These results highlight the quality differences between the tests and emphasize the importance of suitable diagnostics for evidence-based mastitis treatment.

1 | Introduction

Accurate treatment of clinical mastitis in dairy cows requires not only a thorough understanding of the clinical symptoms but also a rapid and precise diagnosis of the causative pathogens (Mansion-de Vries et al. 2015). To achieve fast and evidence-based antibiotic treatment, several diagnostic tests that can be performed in veterinary practices and farms are available. The emma qPCR system (ender molecular multiplex approach) is among the most innovative and fastest diagnostic solutions. This system enables veterinarians to obtain qPCR results within 90 minutes and detects the most significant pathogens, including Mycoplasma spp. directly in their practice.

2 | Methods & materials

95 milk samples from cows with clinical mastitis were examined in parallel with the emma qPCR system, classical microbiology and three commercially available rapid tests (see Table 1).
The emma qPCR test followed the manufacturer's instructions using either the «environmental» or «contagious» panel, each covering different pathogens (see Figure 1). Classical microbiology analysis was done after IDF guidelines (International Dairy Federation 2021), while rapid diagnostic tests A, B and C followed the manufacturer's instructions.
Seven samples were excluded due to invalid qPCR results requiring follow-up testing, which was not done in this study due to time constraints.

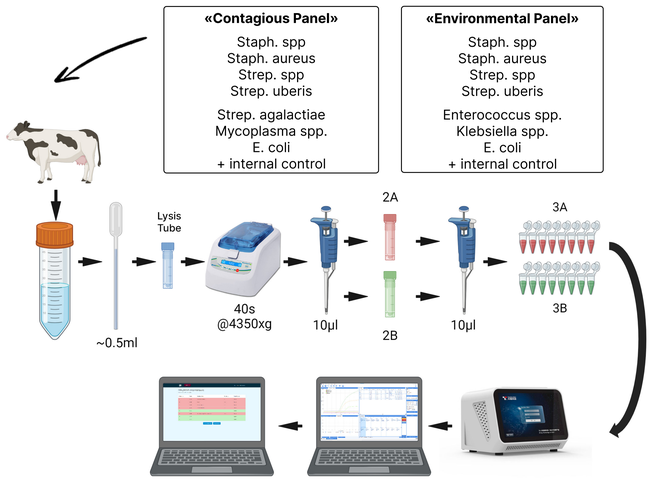

Figure 1: Diagnostic setup of the emma qPCR system. The displayed working procedure requires approximately 15 minutes hands on time for a run with 8 milk samples. After 55 minutes, pathogen identification is automatically performed via a web-based evaluation.

Table 1: Overview of the test methods.

	emma qPCR	microbiology	test A	test B	test C
test principle	RT-qPCR	microbiology	lateral flow	enrichment medium	microbiology
incubation	not needed	24 hours	7.5 hours	12 hours	24 hours
time to result	1.5 hours	up to 72 hours	8 hours	12 hours	24 hours
differentiation	pathogen or pathogen group	pathogen or pathogen group	gram positive	gram positive gram negative	pathogen or pathogen group
evaluation	automated, semiquantitative	optical, biochemical	optical (test strip)	optical (color change of medium)	optical (growth)

3 | Results

The emma qPCR system and test C had the highest pathogen detection rates, followed by classical microbiology. Test A and B had lower detection abilities (see Figure 2a).
All methods, except test A (technically unable to detect gram negatives), had similar gram negative detection rates, while the emma qPCR system, classical microbiology and test C identified more gram positive pathogens than test A and B (see Figure 2b).
In species-level pathogen identification, the emma qPCR system, classical microbiology and test C showed no difference in detection of Staphylococcus aureus, non-aureus Staphylococcus, Streptococcus spp. and coliform bacteria, but test C had a lower detection rate for Enterococcus spp. (see Figure 3). The emma qPCR system was the only test that detected Mycoplasma spp., found in 19 of 88 samples (21.6%).

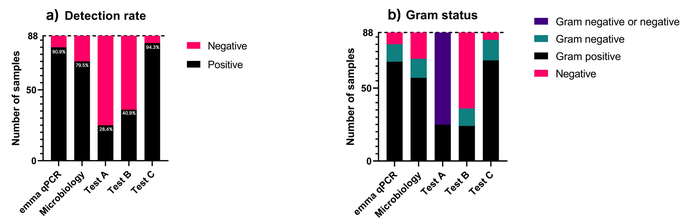

Figure 2a: Pathogen detection rates of the different methods. The pathogen detection rates with the emma qPCR, classical microbiology and test C were higher than with test A and B.
2b: Gram status of the detected pathogens. No difference in proportion of gram negative pathogens was found, except for test A, which was unable to differentiate negative and gram negative test results. The emma qPCR system, microbiology and test C detected more gram positive pathogens than test A and B.

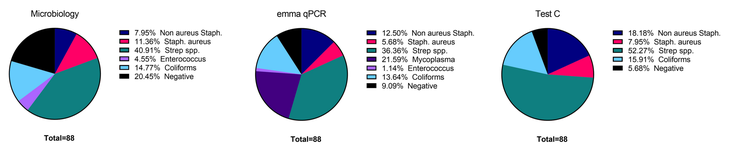

Figure 3: Distribution of detected pathogen species. No difference in detection of Staphylococcus aureus, non-aureus Staphylococcus, Streptococcus spp. and coliform bacteria was found, but test C had a lower detection rate for Enterococcus spp. The emma qPCR system was the only diagnostic method that was technically able to detect Mycoplasma spp.

4 | Discussion

The emma qPCR system, classical microbiology and test C provided nearly equivalent results, while test A and B performed worse due to lacking pathogen identification and lower detection rates. All tests, except the emma qPCR, required 8 to 72 hours of enrichment of potentially pathogenic bacteria prior to getting a test result. This demonstrates that the emma qPCR test provides comparable or superior results to established rapid tests, but with only 90 minutes time to result and the additional advantage of reliably detecting Mycoplasma spp. Furthermore, the emma qPCR test results are evaluated with a web-based software that automatically generates standardized test reports, reducing the risk of misinterpretation compared to other methods. These advantages enable rapid and targeted treatment (or non-treatment) of clinical mastitis based on pathogen identification directly in the practice.

Take home points

- emma is an innovative qPCR system for mastitis diagnostics, that enables veterinarians to identify mastitis pathogens from raw milk within 90 minutes, without enrichment of potentially pathogenic bacteria.
- The emma qPCR test achieves comparable or superior results to established rapid tests with the advantage of reliably detecting Mycoplasma spp.
- The standardized web-based evaluation of the emma qPCR system automatically generates test reports, reducing the risk of misinterpretation compared to other methods (e.g. visual interpretation).

References

International Dairy Federation (IDF), 2021. Guidance on the application of conversion equations for determination of microbiological quality of raw milk, Bulletin of the IDF 511; International Dairy Federation (IDF), Brussels, Belgium.

Mansion-de Vries, E. M., Hoedemaker, M., Krömker, V., 2015. Evidence-based aspects of clinical mastitis treatment. Tierärztliche Praxis Ausgabe G, Grosstiere/Nutztiere, 43(5), 287-295.

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