4. Results

Detection rate and gram status

The pathogen detection rate was highest with the emma qPCR system and test C (90.9 % and 94.3 %), followed by classical microbiology (79.5 %). Test A and B showed a significantly lower detection ability (28.4 % and 40.9 %; see Figure 2a).

The proportion of detected Gram-positive bacteria was similar for the emma qPCR system, classical microbiology and test C (77.3%, 65% and 78.4%), while test A and B yielded significantly less (28.4% and 27.3%). No statistically significant differences were found in the proportions of gram-negative bacteria (test C 15.9%; classical microbiology 14.8%, emma qPCR and test B 13.6%). No detection of gram-negative bacteria is possible with test A (see Figure 2b).

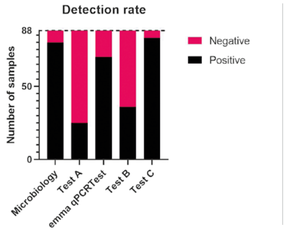


Figure 2 a) Detection rates of the different methods.
Classical microbiology, the emma qPCR system and test C had a significantly higher detection rate than tests A and B.

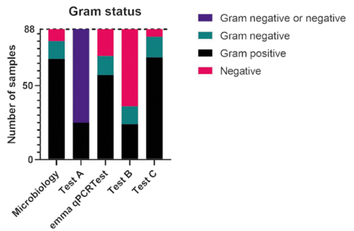


Figure 2 b) Gram status of the detected pathogens.
Test A cannot distinguish between negative and Gram-negative test results, while no significant difference in the proportion of Gram-negative test results was found with the other four test methods. Classical microbiology, the emma qPCR system and test C detected significantly more gram-positive pathogens than tests A and B.

Pathogen identification

The emma qPCR system, classical microbiology and Test C provide test results with pathogen identification. The detection of Staphylococcus aureus, Non-aureus Staphylococcus, Streptococcus spp. and coliform bacteria did not differ statistically significantly. Enterococcus spp. was detected in 4.5 % of the samples in classical microbiology and in 1.1 % with emma qPCR, whereas no detection occurred with test C. The emma qPCR system was the only test that was able to detect Mycoplasma spp. (detected in 19 of 88 samples; 21.6 %).

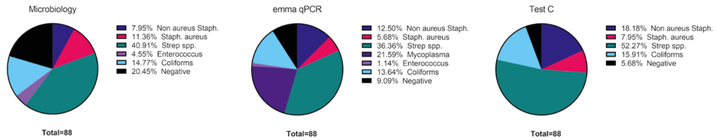


Figure 3: Distribution of pathogen species detected.
No significant differences were found in the detection of Staphylococcus aureus, non-aureus Staphylococcus, Streptococcus spp. and coliform bacteria. The emma qPCR and classical microbiology detected significantly more Enterococcus spp. than Test C. The emma qPCR system was the only diagnostic method that could detect Mycoplasma spp. infections.

5. Discussion

Five different test methods with different technologies such as qPCR, microbiology or lateral flow were compared. The detection rate of positive samples was highest using emma qPCR and test C, followed by classical microbiology. Test A and B showed a significantly lower detection rate. The detection rate of Gram-positive pathogens was highest with emma qPCR, classical microbiology and test C, while test A and B detected significantly less. No significant differences were found in the detection rate of gram-negative bacteria.

Using the emma qPCR system, classical microbiology and test C, pathogen identification at a species level is possible. No statistically significant differences were found, except Mycoplasma spp. The emma qPCR system is the only test that can detect Mycoplasma spp. as this pathogen does not grow under routine microbiology conditions. Mycoplasma spp. was detected in 19 of 88 samples (21.6 %). Enterococcus spp. was detected more frequently with classical microbiology than with emma qPCR, whereas no detection was obtained with test C. The difference between microbiology and emma qPCR is explained by the fact that the emma qPCR environmental panel, which includes Enterococcus spp., was only used in 25% of the tests.

These results indicate remarkable quality differences in the results of different mastitis tests. While tests A and B performed significantly worse due to a lack of pathogen identification and a lower detection rate of positive samples, the emma qPCR system, classical microbiology and test C yielded almost equivalent results. These results show that the emma qPCR test, which can be used in practice, achieves comparable results to the microbiological tests established in practices, but in a fraction of the time.

6. Usability in routine

All the examined test systems proved to be user-friendly and suitable for the practice environment, with sample preparation taking only a few minutes (e.g. 15 minutes for 8 samples in parallel with the emma qPCR system). The optical evaluation of the results of the tests examined can be prone to misinterpretation, especially if untrained or changing personnel carry out the tests. This factor is eliminated with the emma qPCR system, as the evaluation is done uniformly with a cloud-based software solution and standardized test reports are generated automatically.

All tests with the exception of the emma qPCR system require a lengthy enrichment of potentially pathogenic bacteria before a test result is available. This is not only time-consuming, but – depending on where the tests are used – also poses a risk to occupational safety or can be criticized by inspection authorities.

The biggest advantage of the emma qPCR system is the significantly shorter time to obtain the results. While the examined tests with enrichment require 8 to 24 hours, the emma qPCR delivers results in just 90 minutes. This time advantage enables rapid and targeted treatment (or non-treatment) of clinical mastitis based on pathogen identification directly in the veterinarians practice.

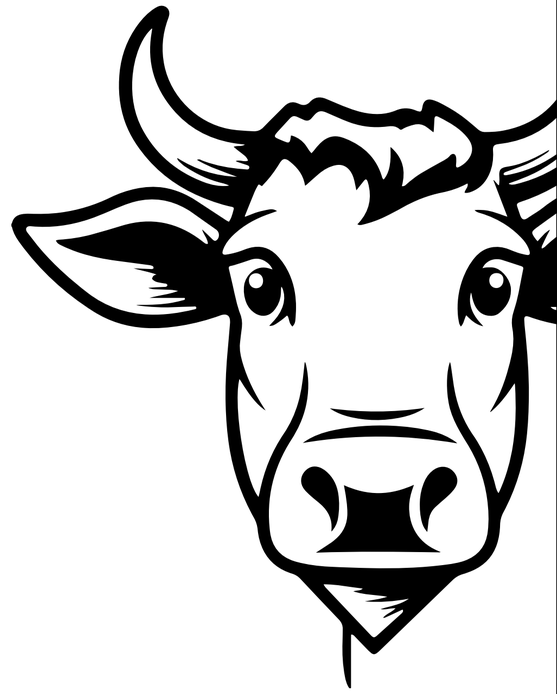
Contact

ender diagnostics AG, Freiburgstr. 251, 3018 Bern, Schweiz
Web: www.emma.vet **Mail:** emma-service@enderdiagnostics.com
Phone: +41 31 552 27 66 (Monday to Friday, 09:00 to 17:00)

emma by ender

emma qPCR – An innovative solution for the identification of clinical mastitis in the veterinary practice.

Christoph Kunas, Stephanie Walker
ender diagnostics ag, 2024



1. Summary

Rapid and accurate pathogen identification is crucial for targeted, evidence-based treatment of clinical mastitis. Several tests are currently available for use in veterinary practices or on farms, but there are major differences in terms of time and quality of results.

In this study, 5 different test methods were compared on 95 samples: emma qPCR, classical microbiology and test C provided specific pathogen detection, whereas test A and B only indicated the Gram status and had a significantly lower detection rate. At 90 minutes, emma qPCR was by far the fastest test and the only suitable method for detecting Mycoplasma spp. These results highlight the differences in quality between the tests and underline the importance of a suitable diagnostic method for the evidence-based treatment of mastitis.

2. Introduction

To treat clinical mastitis in dairy cows in a targeted manner, rapid and precise diagnosis of the pathogens is crucial in addition to the clinical picture. It is common practice to use initial antibiotic treatment at the same time as taking the sample. In order to reverse this process and base treatment on the diagnosis, several tests are available that can be carried out in the veterinary practice or, in some cases, on farms. The fastest of these tests is the innovative diagnostic solution emma (ender molecular multiplex approach). It enables veterinarians to generate a qPCR result in their own practice within 90 minutes, whereby the most important pathogens (see Figure 1) can be found comparable to established diagnostics and in addition mycoplasma can be detected. All on-site tests have the advantage that additional turnover can be generated in the practice and that the time-consuming shipping of samples to specialized testing laboratories is no longer necessary, which means that the diagnostic result and knowledge of the clinical appearance are in one and the same hand.

In this study, 5 test methods used for the diagnosis of clinical mastitis in veterinary practices were compared by parallel analysis of 95 milk samples.

3. Material and methods

95 milk samples from cows with clinical mastitis symptoms were tested in parallel using the emma qPCR system, classical microbiology and three commercially available rapid tests. Table 1 provides an overview of these methods.

Table 1: Overview of the test methods used

	Mikrobiology	Test A	emma PCR	Test B	Test C
Test principle	Microbiology	Lateral Flow	RT-PCR	Culture medium	Microbiology
Incubation	24 hours	7,5 hours	Not required	12 hours	24 hours
Time to result	Up to 72 hours	8 hours	1,5 hours	12 hours	24 hours
Result	Pathogen / pathogen group	Gram positives	Pathogen / pathogen group	Gram positives / negatives	Pathogen / pathogen group
Interpretation	Optical, biochemical	Optical (test strip)	Automated, semiquantitative	Optical (color change)	Optical (growth)

Emma qPCR

The milk sample was lysed with a homogenizer, followed by a qPCR with the panel “environmental” or “contagious”, both covering different groups of pathogens. The testing was done according to the manufacturer’s instructions (IFU). These panels are selected by the veterinarian prior to the test based on the cow’s clinical appearane and the prevailing pathogen situation (see Figure 1). After 55 minutes, the pathogen identification was performed by an automated, cloud-based evaluation.

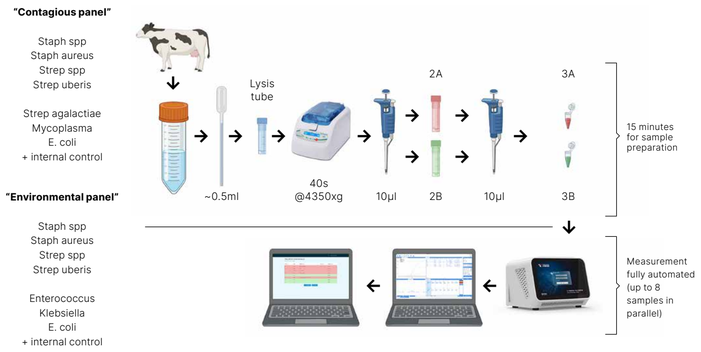


Figure 1: Elements of the emma qPCR test system. The emma qPCR setup includes laboratory equipment for sample preparation and qPCR analysis (“emma lab kit” Art. No. 100004) with the assays “contagious” (Art. No. 100000) and “environmental” (Art. No. 100001). The schematically illustrated workflow corresponds to approximately 15 minutes of hand-on time, while the following automated molecular analysis takes approximately 55 minutes.

Microbiological testing

The milk sample was plated on an agar plate and incubated at 37°C for 24 hours. The pathogen was identified by morphology, gram staining, sub cultivation on selective agar and additional biochemical tests such as the catalase, coagulase and oxidase test.

Test A

The milk sample was incubated in an enrichment medium at 37°C for 7.5 hours. Subsequently, gram-positive pathogens were detected by a color change of the test strip. The test was carried out according to the manufacturer’s instructions.

Test B

The milk sample was incubated in two different media at 37°C for 12 hours. Gram-positive and Gram-negative pathogens could be detected by a color change of the media. The test was carried out according to the manufacturer’s instructions.

Test C

The milk sample was plated on an agar plate with three different (selective) culture media and incubated at 37°C for 24 hours. The pathogens were identified based on their growth and morphology on the different sectors. The test was carried out according to the manufacturer’s instructions.

Data analysis and comparisons

Seven samples were excluded from the analysis due to invalid results with the emma qPCR test. According to the instructions in the work instructions, this result leads to a follow-up test, which was not carried out here due to time constraints.

In the evaluation of the results, the detection rate of positive samples, the Gram status of the pathogens and the pathogen identification of the various tests were compared.