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Laboratory evaluation of a rapid diagnostic test for dairy mastitis

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Research Article

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Abstract

Rapid diagnostic tests that differentiate between Gram positive, Gram negative and the absence of aerobic bacteria in milk samples from dairy cows with clinical mastitis can support antimicrobial treatment decisions and contribute to a more prudent use of antimicrobials in the dairy industry. The objective of this study was to evaluate the test characteristics of the novel rapid BACT mastitis test in discriminating causes of clinical mastitis under laboratory conditions. Test outcomes of 155 milk samples from clinical mastitis cases were incubated for 14–16 h in the BACT test and compared to results of bacteriological culture. The accuracy for detection of bacterial growth and Gram positive growth was 91 and 89%, respectively. The BACT test could provide an accurate and relatively fast decision tool for farmers to aid in antimicrobial treatment decisions in cases of clinical mastitis.

Antimicrobial stewardship in veterinary medicine is of utmost importance to combat the increasing health threat of antimicrobial resistance. This encompasses the optimal use of antimicrobial treatments (Weese *et al.*, 2013). In dairy farming, the majority of antimicrobials are routinely used in the treatment of clinical mastitis and for dry cow therapy, and usually irrespective of the causative pathogen or specific cow related (clinical) determinants. Strategies for treatment of individual mastitis cases based on cow-specific risk factors, clinical parameters and fast identification of the causative pathogen have the potential to greatly improve judicious use of antimicrobials in dairy farming without jeopardizing treatment outcomes and animal welfare (Mansion-de Vries *et al.*, 2016; Lago and Godden, 2018; Jong de *et al.*, 2022). On-farm (point-of-care) classification of mastitis causing bacteria as Gram positive, Gram negative or no growth has proven to be adequate for an evidence-based antimicrobial treatment decision, where antimicrobial therapy is not indicated in the treatment of mild-to-moderate mastitis cases caused by Gram-negative bacteria or when no causative bacteria can be cultured (Wilson *et al.*, 1999; Leininger *et al.*, 2003; Leimbach and Krömker, 2018; Jong de *et al.*, 2022).

A new lateral flow, microfluidic test device (BACT Point-of-Cow mastitis test, FluimediX, Denmark) has recently been developed for the rapid differentiation of mastitis causing bacteria in dairy cows. The objective of this study was to evaluate the test characteristics of the BACT mastitis test compared to bacteriological culture to discriminate between Gram positive, Gram negative or no growth in milk samples derived from dairy cows with clinical mastitis in a veterinary laboratory setting.

Materials and methods

Four different veterinary practices in the Netherlands with an in-house certified microbiological laboratory took part in this study. Between February and October 2021, freshly derived and aseptically taken milk samples from dairy cows with clinical mastitis were sent in by farmers as part of the regular diagnostic sample flow and were used to compare the outcomes of the BACT mastitis test to the results of classical bacteriologic culturing methods. Samples were eligible for inclusion if they were derived from dairy cows with acute clinical mastitis, not older than 72 h at arrival in the laboratory and stored at 2–8 °C.

Incoming milk samples were processed for bacteriological culturing according to NMC guidelines (Hogan *et al.*, 1999). In summary, a sample of 0.01 ml of milk was inoculated using sterile disposable loops onto 6% sheep blood agar (SBA) and MacConkey (MC) plates (Oxoid Ltd., Basingstoke, United Kingdom). Growth of bacteria on all plates was examined after overnight aerobic incubation (18–24 h) at 37 °C. The number and morphology and biochemical properties of the colonies on the different plates were assessed and recorded to further identify the colonies up to species level.

Table 1. Contingency tables for evaluation of the test characteristics of the BACT mastitis test (BACT) to detect (A) bacterial growth (BG) or (B) Gram-positive growth (GR+) compared to bacteriological culture (BC)

	BACT:		BACT:		
A	Growth		No growth		Totals
BC: Growth	110	(a)[a]	14	(b)	124
BC: No growth	3	(c)	28	(d)	31
Totals	113		42		155
	BACT:		BACT:		
B	Gram-positive growth		Gram-negative growth		
BC: Gram-positive growth	51	(a)	4	(b)	55
BC: Gram-negative growth	8	(c)	41	(d)	49
Totals	59		45		104

[a](a) = true positive; (b) = false negative; (c) = false positive; (d) = true negative.

The remaining milk sample was stored between 2 and 8 °C until the end of the day when the BACT mastitis test device was performed according to the protocol provided by the manufacturer. After filling with aseptically taken milk, and being incubated at 37 °C for 14–16 h, the colour change (either blue or yellow) of the two test chambers indicates whether there had been bacterial growth (BG) or no growth (NG) detected in the milk and whether this growth was Gram positive (GR+) or Gram negative (GR−). The BACT result was examined and recorded by a laboratory technician according to the manufacturer's instructions. A picture of the result of the BACT mastitis test was made for confirmation and stored in a database.

All data were recorded in a database using MS Excel (Microsoft Corp., Redmond, USA). A lab technician (HCV) checked all the results in the database for missing or ambiguous data and confirmed them using the pictures taken of the result of each BACT mastitis test. All cultures on the classical growth media with ≥2 colonies were regarded as positive test results (BG). When more than one species was found in bacteriological culture, this was regarded as a mixed or contaminated culture. These samples were excluded from the analysis differentiating GR+ and GR−.

A 2 × 2 contingency table was drawn for the outcomes BG and GR+ separately to calculate the sensitivity (Se), specificity (Sp), prevalence, positive predictive value (PPV), negative predictive value (NPV) and accuracy of the BACT mastitis test using bacteriological culture as the gold standard. The McNemar test was used to determine whether there were differences in outcomes between the BACT mastitis test and the result of bacteriological culture after 24 h of incubation for the outcomes BG and GR+ respectively. The inter-test agreement between BACT mastitis test and bacteriological culture was assessed using the Kappa statistic. Statistical calculations have been made within MS Excel and MedCalc Software Ltd. diagnostic test evaluation calculator (https://www.medcalc.org/calc/diagnostic_test.php).

Results

A total of 167 fresh milk samples were analysed using the BACT mastitis test. After exclusion of non-eligible data, 155 samples were included in the study (online Supplementary Table S1). The BACT mastitis tests resulted in NG after overnight incubation in 42 of these 155 samples, 105 tests had a clear BG test result (blue colour change) and additionally eight tests resulted in no clear colour change (blue/yellow) in the read out chamber but were interpreted as BG. Sixty of the BG test outcomes in the BACT mastitis test resulted in GR+, 39 in GR− and 14 were blue/yellow (which were regarded as GR+ in further analyses).

The 2 × 2 contingency tables for the outcomes BG and GR+ are presented in Table 1. Kappa values for agreement between the BACT mastitis test and bacteriological culture for BG and GR+ were 0.70 and 0.77, respectively. A larger frequency of disagreements between the BACT mastitis test result and the result of bacteriological culture was found for the NG compared to BG (14 and 3 respectively, $P = 0.02$ McNemar's test). For detecting GR+, no significant disagreement between the BACT mastitis test and bacteriological culture was found ($P = 0.39$ McNemar's test). The test characteristics of the BACT test compared to bacteriological culture are represented in Table 2. The accuracy of the BACT mastitis test for BG and GR+ are 89% and 91%, respectively. If the BACT test would have been used as sole indicator for antimicrobial treatment (only BG and GR+ outcomes in the BACT mastitis test receiving antimicrobials), 81 cows (52.3%)

Table 2. Calculated sensitivity (Se)[a], specificity (Sp)[b], disease prevalence (P)[c], positive predictive value (PPV)[d], negative predictive value (NPV)[e] and accuracy[f] of the BACT mastitis test for the outcomes bacterial growth and Gram-positive growth using bacteriological culture as the standard

Test characteristics BACT	Growth	Gram-positive growth
Sensitivity % (CI)	88.7 (81.7–93.7)	95.3 (88.4–98.7)
Specificity % (CI)	90.3 (74.3–98.0)	83.7 (70.3–92.7)
Disease prevalence % (CI)	80.0 (72.8–86.0)	63.4 (51.7–71.6)
PPV % (CI)	97.4 (92.6–99.1)	91.0 (84.3–95.0)
NPV % (CI)	66.7 (54.6–76.9)	91.1 (79.6–96.4)
Accuracy % (CI)	89.0 (83.0–93.5)	91.0 (84.9–95.3)

The Clopper–Pearson exact test has been used to calculate confidence intervals.
[a]Se Calculated from Table 1: a/(a + b).
[b]Sp Calculated from Table 1: d/(c + d).
[c]P Calculated from Table 1: (a + b)/(a + b + c + d).
[d]PPV (Se × p)/(Se × p + (1−Sp) × (1−p).
[e]NPV Sp × (1−p)/((1−Se) × p + Sp × (1−p)).
[f]Accuracy Se × p + Sp × (1−p).

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would not have received antimicrobials of which 12 (7.8%) would have falsely not been treated with antimicrobials, using bacteriological culture as the gold standard.

Discussion

We tested the characteristics of the BACT mastitis test on 155 fresh clinical mastitis milk samples from dairy cows compared to bacteriological culture considered as the gold standard. The calculated accuracies for BG and GR+ of the BACT test were 89% and 91%, respectively. This is comparable to the reported test characteristics of other commercially available on-farm rapid test devices for clinical mastitis in dairy cows like the Accumast (85% accuracy)- (Ganda *et al*., 2016), the MastDecide (79% accuracy) (Leimbach and Krömker, 2018), VétoSlide (74% accuracy) and the VétoRapid (81% accuracy) (Malcata *et al*., 2021). It should be noted, however, that comparisons between reported test characteristics are complicated because study design and populations differ and test characteristics are also influenced by the distribution of mastitis pathogens (Malcata *et al*., 2021). A further limitation is that bacteriological culture is not the perfect test to detect mastitis pathogens, but it is still the standard in clinical practise and serves as the gold standard in most comparisons for on-farm mastitis tests (Jong de *et al*., 2022).

The most important characteristic of the rapid on-farm culture systems should be that they accurately detect mastitis cases caused by Gram-positive bacteria for which antimicrobial therapy is indicated. Based on the results of our study, 7.8% of the cows (12 out of 155) would have falsely been allocated to a no-antimicrobial treatment group based on the results of bacteriological culture if the outcome of the BACT mastitis test was used as a sole consideration for antimicrobial treatment, although this will be dependent on the distribution of mastitis pathogens. In other clinical studies using on-farm diagnostic tests to allocate cows to antimicrobial treatment or no-antimicrobial treatment it appeared that between 12 and 32% of the cows were falsely allocated to the no-antimicrobial treatment group (Lago *et al*., 2011; Lago and Godden, 2018; McDougall *et al*., 2018).

A few tests ($n = 12$) were excluded for analysis because the milk did not properly flow into the test chambers. This can be a result of using very clotted milk which obstructs the microtubes filling the test chambers or when not enough care is taken when filling the milk inlet with enough milk. The BACT test is, therefore, less useful for testing very clotted milk. In a few other cases, it was not easy to exactly define the colour of the read-out chamber (blue or yellow) which is necessary for proper interpretation of the result. In these cases, the test result was interpreted as the worst possible outcome (e.g. BG and GR+) which would in practise indicate antimicrobial treatment.

Finally, a limitation to our study is that all the tests were performed and interpreted by experienced lab technicians under laboratory conditions. Its applicability and reliability in the field where it should be used by farmers or farm workers and the clinical outcome of the resulting therapy needs to be determined in a well-designed field trial. The BACT mastitis test is, however, relatively convenient and easy to use after basic instruction. In theory, this can easily be performed by a farmer under field conditions.

In conclusion, the BACT mastitis test has proven to be a reliable and relatively fast test under laboratory conditions to discriminate clinical mastitis cases caused by Gram-positive bacteria, Gram-negative bacteria and those where no bacteria could be cultured.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029924000104

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