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A NEW AUTOMATED TOOL FOR MILK BACTERIOLOGY: MASTATEST

Study to evaluate agreement of results obtained from Mastatest and a conventional agar technique.

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ABSTRACT

Mastatest is an automated device for the bacteriological analysis and antibiotic susceptibility testing of milk from cows with clinical mastitis.

It requires a stable Internet connection and is very easy to use. A cartridge is filled with milk from an infected quarter, placed in the device, and the analysis is run. **The bacteriological analysis results and the MIC values for 3 antibiotics used conventionally for mastitis are available in less than 24 hours.** 199 milk samples were analysed twice using Mastatest and the simplified bacteriological technique used at Haute Auvergne Veterinary Clinic.

The results revealed a high concordance in determining Gram type and good concordance in precise bacterial identification. Mastatest is a particularly interesting tool for milk bacteriology. It is very easy to use and gives rapid results so the appropriate therapy can be chosen. It is therefore ideal for use by inexperienced operators. **As with any analysis result, it must be interpretated in conjunction with the essential case history and advice from an expert: the veterinarian.**

Photo 1: The testing device

Photo 2: Filling cartridges with milk sample

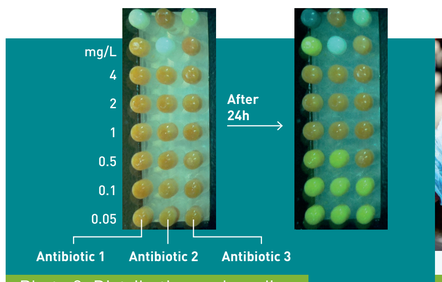
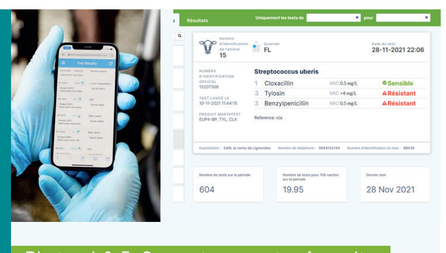


Photo 3: Distribution and reading of wells



Photos 4 & 5: Computer reports of results obtained in less than 24 hours

EQUIPMENT DESCRIPTION

This analyser was developed in New Zealand. It consists of a device connected to a computer platform via the Internet (photo 1) and 24-well cartridges containing different reagents providing specific colorimetric information.

The milk to be analysed is poured into the cartridge (photo 2).

Six wells are used for bacterial identification and, if only 1 germ is identified, the other 18 are used to determine MIC values for 3 antibiotics (penicillin, cloxacillin and tylosin for Gram positive bacteria) (photo 3).

The main purpose of the device is to take repeated photographs of the contents of the wells which contain indicators (most are coloured). The images are interpreted remotely by an algorithm, which determines the presence and nature of a bacterium in less than 24 hours.

Differentiated growth according to the antibiotic concentration within the wells also provides an MIC value and susceptibility results for each of the 3 antibiotics present (photos 4 & 5).

References
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Jones G., Bork O., Ferguson S.A., Bates A. (2019) Comparison of an on-farm point-of-care diagnostic with conventional culture in analysing bovine mastitis samples. J. Dairy Res, 86: 222–225.
Lago A and Godden SM (2018) Use of rapid culture systems to guide clinical mastitis treatment decisions. The Veterinary Clinics of North America, Food Animal Practice 34, 389–412.
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COMPARATIVE STUDY

This study was carried out among the Haute Auvergne Veterinary Clinic (CVHA, 15100 Saint-Flour, France) dairy farmers and only included milk taken from quarters with clinical mastitis.Each milk sample analysed with Mastatest also underwent bacteriological culture using the CVHA "3-agar method" (detailed description in Salat *et al.*, 2016). **The Mastatest antibiotic susceptibility results were compared with those obtained by the clinic using the broth method in accordance with standard NF 47-107.**

RESULTS

199 samples were analysed, and results are presented in Table 3. Results in Tables 1 and 2 only concern pure cultures (111 samples). In terms of major pathogen identification (37 samples), concordance was 86.5% for coliforms (*E. coli, Klebsiella, Enterobacter*, etc.). Furthermore, concordance was 70% for *Staphylococcus aureus* (10 samples) and 73% for *Streptococcus uberis* (33 samples).

Concordance between he Mastatest antibiotic susceptibility results and those from the broath method used at the clinic is presented in Table 4.

Gram type comparison	Results
Concordance	91%
No concordance	9%

Table 1: Comparison Gram type results obtained from Mastatest and the CVHA technique

Bacterial identification	MASTATEST results
Exact concordance between genus and species	55%
Genus concordance	12%
No concordance	33%

Table 2: Comparison of bacteria identification results obtained using Mastatest and the CVHA technique

		MASTATEST				
		Sterile	1 germ	2 germs	Contaminated	Total
CVHA	Sterile	17	10	0	0	27
	1 germ	11	113	16	1	141
	2 germs	0	12	3	0	15
	Contaminated	0	7	9	0	16
	Total	28	142	28	1	199

Table 3: Comparison of overall bacteriological analysis results obtained using Mastatest and the CVHA technique

		MASTATEST					
		Penicillin		Cloxacillin		Tylosin	
		Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
CVHA	Susceptible	27	2	24	5	22	3
	Resistant	0	0	0	0	1	2

Table 4: Comparison of antibiotic susceptibility results obtained using Mastatest and the CVHA technique

DISCUSSION

Gram type result concordance is high (>90%). Lago and Godden (2018) showed a Gram positive detection accuracy of 78% for Tri-plate or Bi-plate multi-compartment agars. Bacterial identification accuracy is acceptable with 70% and 73% of results being concordant with the agar-based identification technique used in the clinic for *Staphylococcus aureus* and *Streptococcus uberis*. Jones (2019) demonstrated non-inferiority between Mastatest results and laboratory agar method (compliant with NMC standards). Coliform concordance between the two methods was higher at 86.5%. In the same study, Jones (2019) concluded that Mastatest was more sensitive (*p* 0.032). On 17 occasions, neither the device nor the bacterial culture was able to determine the presence of bacteria.

200 µl of milk were cultured with Mastatest per well, a higher quantity than that conventionally used for bacterial culture (10 to 60 µl): this increases the chances of detecting an infectious agent. However, the device was largely unable to detect samples contaminated with multiple bacteria (1 out of 17). This is hardly surprising given the analysis methodology. The sterile milk sample must be of a high quality for all bacteriological diagnostic methods, operator training is therefore essential to ensure relevant results. **Despite the small numbers, classification concordance was considered high (80 to 92%) and close to the ISO 20776-2 threshold of 90%.**

Comparison with the conventional laboratory antibiotic susceptibility test showed a tendency for Mastatest to overestimate resistance, however the MIC values are determined in milk, which is closer to reality. It may be recommended to confirm a resistant result with a conventional antibiotic susceptibility test, pending validation in a larger population.

PRACTICAL APPLICATIONS

This device has 3 fundamental qualities:

it is very easy to use, provides a result in less than 24 hours, and does not require an experienced operator to obtain a result.

Widespread of bacteriological analysis of milk is a prerequisite for appropriate and prudent use of antibiotics. Like conventional bacteriology, Mastatest can be used in cases of severe, recurrent mastitis or treatment failure, **and also as part of a selective treatment strategy for mild and moderate clinical mastitis.** It can therefore reduce antibiotic use (by 24% according to Bates, 2020).

It can be used in veterinary practices, particularly those with low potential of milk bacteriology, on farm managing sufficiently large numbers of animals to support regular use, or by centralising milk samples from neighbouring farms.