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Agreement between four commercial diagnostic tests and routine bacteriological culture of milk to determine the udder infection status of dairy cows

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ABSTRACT

Mastitis is usually treated based on clinical signs or somatic cell count information rather than on results of bacteriological culture of milk. In many countries an optimal mastitis treatment is considered important from the perspective of therapy efficacy, prudent antimicrobial use and farm economics. Farmers can optimize their mastitis treatment decisions if they know whether and which mastitis pathogen is involved. Information on the mastitis pathogen involved can be acquired from diagnostic mastitis tests such as culture-based tests. This study aimed to determine the agreement of four commercial culture-based mastitis tests with routine bacteriological culture of milk to determine the intramammary infection status of a quarter or cow. The commercial culture-based tests evaluated in this study were CHROMagar Mastitis (CHROMagar, France), Hardy Diagnostics Mastitis Triplate (Hardy Diagnostics, USA), Minnesota Easy Culture System II Tri-plate (University of Minnesota, USA), and VétoRapid (Vetoquinol, the Netherlands). We used 866 prospectively collected milk samples, routinely submitted to the bacteriological laboratory of GD Animal Health for routine bacteriological culture of milk from April to June 2016. Samples were cultured on routine bacteriological culture of milk and on the commercial culture-based tests. We calculated the agreement beyond chance of each commercial culture-based test result with the result of routine bacteriological culture using 2x2 contingency tables. Furthermore, inter-reader agreement was determined for 597 samples read by two masked readers. The agreement of the four commercial culture-based mastitis tests with routine bacteriological culture of milk for Gram-positive bacteria ranged from 0.14 (95% CI 0.11-0.16) using Hardy Diagnostics Mastitis Triplate to 0.25 (95% CI 0.22-0.28) using Minnesota Easy Culture System II Tri-plate. The agreement for Gram-negative bacteria was approximately 0.70 (95% CI 0.66-0.74) for all four commercial culture-based tests. The agreement for no growth ranged from 0.22 (95% CI 0.19-0.25) using Hardy Diagnostics Mastitis Triplate to 0.34 (95% CI 0.31-0.38) using VétoRapid. This category was affected by prevalence and bias as the prevalence adjusted and bias adjusted kappa ranged from 0.63 (95% CI 0.56-0.69) using CHROMagar Mastitis to 0.68 (95% CI 0.62-0.74) using Hardy Diagnostic Mastitis Triplate. Agreement between readers was almost perfect. Although only for Gram-negative bacteria a good agreement was found between commercial culture-based tests and routine bacteriological culture of milk, and further on-farm evaluations are needed to determine the effect of these findings on udder health, commercial culture-based tests are of added value to support decisions whether and how to treat cows with mastitis.

1. Introduction

Mastitis, an inflammation of the mammary gland mainly caused by bacteria, is the indication for which most antimicrobials are used on dairy farms (Pol and Ruegg, 2007). Currently, most cows with mastitis are treated with antimicrobials (Pol and Ruegg, 2007; Santman-Berends et al., 2015), even though some may not need antimicrobials (Roberson, 2003; Barkema et al., 2006; Pinzón-Sánchez et al., 2011). From a perspective of both prudent antimicrobial use as well as of limiting unnecessary costs, antimicrobials should preferably only be

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used in cases where an intramammary infection (IMI)[1] can be confirmed, and, if possible, where probability of cure is high (Barkema et al., 2006; Kromker and Leimbach, 2017). Thus, for each mastitic cow one needs to decide whether treatment with antimicrobials is required (Royster and Wagner, 2015).

The general used parameters to decide on treatment like signs of mastitis, history of mastitis, or stage of lactation are not distinct enough to really target mastitis treatment (Pinzón-Sánchez et al., 2011). A targeted treatment can only be made if the causative pathogen is known, making diagnostic testing inevitable (Roberson, 2003). Additionally, farmers have indicated to be interested in additional testing to know the causative pathogen for udder health-related problems (Griffioen et al., 2016).

A broad range of diagnostic tests is available to determine the IMI status of a cow. However, tests like electronic conductivity measures or California Mastitis Test provide insufficient information to target treatment. Furthermore, laboratory tests are considered impractical due to the long time lag between sampling and test results and are therefore used infrequently (Griffioen et al., 2016).

Over the years commercial culture-based mastitis tests have been developed (Ganda et al., 2016; Leimbach and Kromker, 2018). Several laboratory studies evaluated the accuracy of commercial culture-based mastitis tests like Minnesota Easy Culture System II Bi- and Tri-plate, VétoRapid, and Petrifilm plates. These studies showed that commercial culture-based mastitis tests generally were able to categorize mastitis cases into treatment groups or to indicate mastitis-causing bacteria (McCarron et al., 2009a; Cameron et al., 2013; Royster et al., 2014; Viora et al., 2014; Ferreira et al., 2018). Furthermore, they inform on the causative bacterium within 24 h, and helped to decide on mastitis treatment without affecting cure rate, but with reducing the antimicrobial use (Leslie et al., 2005; Lago et al., 2011). Nevertheless, a number of commercial culture-based mastitis tests are available with undetermined diagnostic accuracy.

Four commercial culture-based mastitis tests were selected to be evaluated in this study: CHROMagar Mastitis (CHROMagar), Hardy Diagnostics Mastitis Triplate (Hardy Diagnostics), Minnesota Easy Culture System II Tri-plate (University of Minnesota), and VétoRapid (Vetoquinol). These four commercial culture-based tests were selected based on their commercial availability, their potential to be used on-farm, and the differences in utilized media. CHROMagar Mastitis and VétoRapid utilize chromogenic media substrates while Hardy Diagnostics Mastitis Triplate and Minnesota Easy Culture System II Tri-plate utilize more conventional culture media, including for example Edwards medium. The envisaged role for these tests is to diagnose an IMI on-farm. This information could help farmer or veterinarian to decide on udder health-related treatments in addition to already available information.

This study aimed to determine the agreement of four commercial culture-based mastitis tests with routine bacteriological culture of milk to determine the IMI status of a quarter or cow.

2. Materials and methods

2.1. Samples

Milk samples were prospectively collected from samples sent to the bacteriological laboratory of GD Animal Health (Deventer, the Netherlands) for routine bacteriological culture of milk between 4 April and 22 June 2016. Samples routinely sent in were considered eligible while samples sent in for research project purposes were excluded. No clinical information was available on the cows the milk was collected from since such information is not requested at submission. Samples were cultured according to the routine procedure for bacteriological culture of milk, and by using each of the four commercial culture-based mastitis tests on the same day as far as possible. Otherwise samples were frozen at −20 °C. When possible, somatic cell count (SCC)[2] was determined.

2.2. Routine bacteriological culture of milk

Routine bacteriological culture of milk was performed at the bacteriological laboratory of GD Animal Health according to NMC guidelines (NMC, 1999). In brief, from each sample, 0.01 mL was inoculated onto 6% sheep blood agar (Biotrading, Mijdrecht, the Netherlands). Presumptive growth of mastitis-causing pathogens was examined after incubation for 18 to 24 h at 37 °C (under aerobic conditions) and again after 48 h. Identification of presumptive mastitis-causing pathogens was performed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) using the MALDI Biotyper Microflex LT (Bruker Daltonics GmbH, Germany) (Barreiro et al., 2010). Milk samples that tested negative with standard culture procedure and having a SCC above 200,000 cells/mL were cultured again onto sheep blood agar following a combination of freezing and pre-incubation (Sol et al., 2002). An IMI was defined as a pure culture or predominance of one or two types of presumptive mastitis-causing pathogens with growth of at least six (in case of a pure culture) or more than ten (when more than one type was present) colonies on the plate, respectively. In case of growth of *Staphylococcus aureus*, *Streptococcus agalactiae*, or hemolytic streptococci the presence of already one colony was considered as an IMI. No growth was defined as no growth of presumptive mastitis-causing pathogens. Contamination was defined as growth of more than two phenotypically different colony types, without a dominant presumptive mastitis-causing pathogen. The SCC was determined using fluorescence flow cytometry (CombiScope 600, Delta Instruments, Drachten, the Netherlands) (ISO 13366-2|IDF 148-2:2006, 2006).

2.3. Commercial culture-based mastitis tests

All four commercial culture-based mastitis tests were inoculated with a new sterile cotton swab after mixing the milk sample gently and dipping the swab in the milk for eight to ten seconds to become fully saturated. The commercial culture-based mastitis tests were incubated and read after 18 h–24 h, according to the manufacturer's guidelines (Minnesota Easy Culture System User's Guide, 2013; CHROMagar Mastitis version 2, 2014; Hardy Diagnostics Instructions for use Mastitis Triplate, 2016; VétoRapid Mastitits-Schnelltest, 2014). The commercial culture-based tests were regarded positive for IMI when one or more different colony morphologies grew. All different colony morphologies were identified visually as precise as possible, without further confirmation. When the colony could not visually be indicated to bacterial species level the reader reported the bacterial group or when that was not possible either 'Gram-positive' or 'Gram-negative' was noted. If more than two different colony morphologies were present on a test, 'contamination' was added in the report. When no bacterial growth was noticed 'no growth' was reported. Additionally, readers noted whether or not they were uncertain about the result.

2.3.1. CHROMagar Mastitis

CHROMagar Mastitis (CHROMagar, Paris, France) consists of two agars in two different Petri dishes. One agar specific for Gram-positive bacteria (with peptone and yeast extract, salt, and a chromogenic mix), and one specific for Gram-negative bacteria (with peptone and yeast extract, and a chromogenic mix). Both agars were incubated at 37 °C according to the manufacturer's guidelines (CHROMagar Mastitis version 2, 2014). Results of both plates were read independent of each other and were combined into a single result for each milk sample to analyze the accuracy of CHROMagar Mastitis.

2.3.2. Hardy Diagnostics Mastitis Triplate

Hardy Diagnostic Masitis Triplate (Hardy Diagnostics, Santa Maria, CA, USA) consists of one Petri dish with three separate sections

(triplate). One section is for total bacterial growth (a Tryptic Soy Agar with sheep blood), one is specific for streptococcal growth (a modified TKT agar with esculin), and one for Gram-negative growth (a modified MacConkey agar). The Hardy Diagnostics Mastitis Triplate was incubated at 35 °C according to the manufacturer's guidelines (Hardy Diagnostics Instructions for use Mastitis Triplate, 2016).

2.3.3. Minnesota Easy Culture System II Tri-plate

Minnesota Easy Culture System II Tri-plate (University of Minnesota, St. Paul, MN, USA) consists of one Petri dish with three separate sections (triplate). One section is specific for Gram-positive growth (factor agar), one for streptococcal growth (modified TKT agar), and one for Gram-negative growth (MacConkey agar). The Minnesota Easy Culture System II Tri-plate was incubated at 37 °C according to the manufacturer's guidelines (Minnesota Easy Culture System User's Guide, 2013).

2.3.4. VétoRapid

VétoRapid (Vetoquinol, 's-Hertogenbosch, the Netherlands), consists of one Petri dish with three separate sections (triplate). One section is specific for staphylococci (modified mannitol salt agar), one for streptococci (modified Edwards agar containing crystal violet and polymyxin B), and one for Gram-negative bacteria (agar containing bile salts and vancomycin to prevent Gram-positive growth). VétoRapid was incubated at 37 °C according to the manufacturer's guidelines (VétoRapid Mastitis-Schnelltest, 2014).

2.4. Readers of commercial culture-based tests

All commercial culture-based tests were cultured and read by one of five readers. A number of samples was read by a second reader to determine the level of agreement beyond chance between two readers. Readers were blinded to the results of routine bacteriological milk culture and to each other's results. However, readers were not blinded to the results of the different commercial culture-based tests read by themselves. The commercial culture-based tests were read per test instead of per sample to diminish the influence of prior results of other commercial culture-based tests. Readers of routine bacteriological milk culture were blind for commercial culture-based test results.

One of five readers was a laboratory technician having experience reading bacteriological culture results of milk samples, three readers lacked experience reading bacteriological culture results of milk samples: two of them were laboratory technicians and one was an intern, and one was a veterinarian having limited experience reading bacteriological culture results of milk samples. None had specific experience reading commercial culture-based tests.

2.5. Statistical analysis

Results of culture-based mastitis tests were digitalized using NetQ (Collector 2015.Q2, Survalyzer, Utrecht, the Netherlands). Results of routine bacteriological milk culture as well as those of the four culture-based mastitis tests were combined into one dataset. Only samples having a result of all four culture-based tests and of routine bacteriological milk culture were used.

Results of all tests were categorized as shown in Table 1. For example, for routine bacteriological milk culture the category 'Gram-positive' comprised *Staphylococcus* spp. including *S. aureus* and coagulase negative staphylococci (CNS)[3], *Streptococcus* spp. including *Streptococcus uberis*, *S. agalactiae* and *Streptococcus dysgalactiae*, and other Gram-positive bacteria, whereas 'Gram-negative' comprised lactose-fermenting coliforms including *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp. (KEC)[4], non-lactose-fermenting Gram-negative bacteria including *Pseudomonas* spp., and other Gram-negative bacteria. For CHROMagar Mastitis the category 'Gram-positive' comprised *S. aureus*, *S. uberis*, *S. agalactiae* and other Gram-positive bacteria, the category 'Gram-negative' comprised *E. coli*, KEC, *Pseudomonas* spp. and other Gram-negative bacteria.

Three types of milk samples were examined: 1) all samples, 2) mastitis samples, a subset of one being milk samples with abnormal

Table 1
Routine bacteriological milk culture results of all milk samples used in study, of milk samples originating from quarters suspected of mastitis, and of milk samples with SCC ≤ 200.000 cells/mL.

	All milk samples[1] (n = 866)		Mastitis samples[1,2] (n = 671)		SCC ≤ 200[1] (n = 141)	
	N	%	N	%	N	%
1 different colony morphology	571	65.9%	459	68.7%	82	58.2%
2 different colony morphologies	49	5.7%	35	5.2%	7	5.0%
≥ 3 different colony morphologies	106	12.2%	73	10.9%	24	17.0%
No growth	140	16.2%	104	15.6%	28	19.9%
Gram-positive bacteria	409	47.2%	302	45.2%	81	57.4%
Staphylococcus spp.	209	24.1%	151	22.6%	46	32.6%
S. aureus	128	14.8%	97	14.5%	26	18.4%
CNS[3]	84	9.7%	56	8.4%	21	14.9%
Streptococcus spp.	149	17.2%	114	17.1%	29	20.6%
S. uberis	82	9.5%	65	9.7%	14	9.9%
S. agalactiae	2	0.2%	2	0.3%	0	0.0%
S. dysgalactiae	42	4.8%	33	4.9%	7	5.0%
Other Gram-positive bacteria	73	8.4%	54	8.1%	11	7.8%
Enterococcus spp.	16	1.8%	10	1.5%	2	1.4%
Gram-negative bacteria	227	26.2%	202	30.2%	10	7.1%
Lactose-fermenting bacteria	187	21.6%	167	25.0%	7	5.0%
E. coli	146	16.9%	131	19.6%	7	5.0%
Klebsiella spp.	41	4.7%	36	5.4%	0	0.0%
KEC[4]	70	8.1%	60	9.0%	3	2.1%
Non-lactose-fermenting bacteria	11	1.3%	11	1.6%	0	0.0%
Pseudomonas spp.	5	0.6%	5	0.7%	0	0.0%
Other Gram-negative bacteria	6	0.7%	6	0.9%	0	0.0%

[1] Results of samples with up to two different colony morphologies were included in specification to bacterial species.
[2] Mastitis samples comprise milk samples with abnormal milk appearance and those with SCC > 200.000 cells/mL.
[3] CNS = coagulase negative staphylococci.
[4] KEC = *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp.

Table 2
Determination possibilities of routine bacteriological milk culture and of four commercial culture-based mastitis tests.

	Bacteriological milk culture	CHROMagar[1]	Hardy[2]	Minnesota[3]	VétoRapid[4]
No growth	X	X	X	X	X
Gram-positive bacteria	X	X	X	X	X
Staphylococcus spp.	X		X	X	X
S. aureus	X	X	X	X	X
CNS	X				X
Streptococcus spp.	X		X	X	X
S. uberis	X	X	X		X
S. agalactiae	X	X	X	X	X
S. dysgalactiae	X				X
Enterococcus spp.	X				X
Other Gram-positive bacteria	X	X			
Gram-negative bacteria	X	X	X	X	X
Lactose fermenting bacteria	X		X		
E. coli	X	X			X
KEC	X	X			X
Non-lactose fermenting bacteria	X		X		X
Pseudomonas spp.	X	X			
Other Gram-negative bacteria	X	X	X		X

[1] CHROMagar Mastitis (CHROMagar, Paris, France) consisting of two separate plates.
[2] Hardy Diagnostics Mastitis Triplate (Hardy Diagnostics, Santa Maria, CA, USA), a triplate.
[3] Minnesota Easy Culture System II Tri-plate (Minnesota University, St. Paul, MN, USA), a triplate.
[4] VétoRapid (Vetoquinol, 's-Hertogenbosch, the Netherlands), a triplate.

milk appearance or SCC > 200.000 cells/mL, and 3) low SCC samples, a subset of one being milk samples with SCC ≤ 200.000 cells/mL. Results of each commercial culture-based test were compared with results of routine bacteriological milk culture to calculate the agreement beyond change as expressed by kappa (Fleiss, 1971). When a milk sample was read by two readers within a commercial culture-based test, then one result was randomly selected. The random selection was repeated ten times for each commercial culture-based test. The obtained agreements were averaged per commercial culture-based test.

Agreement was computed for four diagnostic categories: no growth, Gram-positive bacteria, Gram-negative bacteria, and contamination as well as for each bacterial species or bacterial group that could be identified on the specific commercial culture-based mastitis test (Table 2). Additionally, kappa value was computed to determine the agreement between two commercial culture-based tests, and to determine the inter-reader agreement using the milk samples that were read by two readers. Results agreed when both tests or readers under comparison indicated the presence or absence of the diagnostic category or bacterial species or group. Results disagreed when one test or reader indicated a diagnostic category or bacterial species or group as present while the other test or reader indicated the diagnostic category or bacterial species or group as absent, or vice versa.

Kappa values were calculated with Stata 14.1 (StataCorp, 2015) and accounted for two or more readers since the identity of the readers differed (Fleiss, 1971). Confidence intervals were calculated following the formula $\kappa \pm 1.96\,\text{S. E.}$ with $\text{S. E.} = \sqrt{\kappa(1-\kappa)/n}$, with κ being kappa value and n the sample size. The prevalence adjusted and bias adjusted kappa (PABAK)[5] was calculated using the formula $2P_o - 1$, with P_o being the proportion of observed agreement calculated as $P_o = {}^{a+d}/_{n}$ (Sim and Wright, 2005). With a being the number of positive agreed results and d the number of negative agreed results for the commercial culture-based test and routine bacteriological culture of milk. For PABAK confidence intervals were calculated following the formula $PABAK \pm 1.96\sqrt{VAR}$ with $VAR = {}^{4P_o(1-P_o)}/_{n}$ (Byrt et al., 1993; Looney and Hagan, 2008). For both kappa and PABAK differences were considered significant when the 95% confidence interval (95% CI)[6] of two commercial culture-based tests were not overlapping within sample type per diagnostic category. For both kappa and PABAK the guideline according to Landis and Koch (1977) was used to determine the magnitude of agreement. Therefore, a value of > 0.80 was considered to be almost perfect, > 0.60-0.80 substantial, > 0.40-0.60 moderate, > 0.20-0.40 fair, > 0.00-0.20 slight, and ≤ 0.00 poor.

We calculated the required sample size using the following formula (Watson and Petrie, 2010): $4\frac{(1-\kappa)}{W^2}\left((1-\kappa)(1-2\kappa) + \left(\frac{\kappa(2-\kappa)}{2\pi(1-\pi)}\right)\right)1.96^2$

We assumed a prevalence (π) of 0.1 for the least prevalent diagnostic category no growth, accepted a 95% CI width (W) of 0.1, and wanted to estimate the sample size to give an almost perfect agreement between the commercial culture-based tests and routine bacteriological milk culture and therefore used an anticipated value for κ of 0.8. The required number of samples was 622.

3. Results

Between 4 April and 22 June 2016, 1472 milk samples were sent in for routine bacteriological milk culture of which 1447 were eligible to be included in the study (Fig. 1). In total 866 milk samples were cultured on all four commercial culture-based mastitis tests and routine bacteriological milk culture. Of these samples, 671 had an SCC > 200.000 cells/mL or an abnormal milk appearance and were thus considered to originate from quarters that likely had mastitis. One hundred forty-one milk samples had an SCC ≤ 200.000 cells/mL (low SCC samples). Of 54 samples no SCC was measured, with most of these because the machine was broken. A total of 597 samples was read by two readers.

Most of the samples yielded one type of colonies (66%) according to routine bacteriological milk culture (Table 2). The most prevalent bacteria cultured with routine bacteriological milk culture were Gram-positive bacteria, followed by Gram-negative bacteria. From 16% of the milk samples no relevant mastitis-causing bacteria were cultured and were thus considered to have no growth. Most frequent cultured species were *E. coli*, *S. aureus*, CNS, and *S. uberis*. *Streptococcus agalactiae* was cultured least frequent. From 106 milk samples more than two different colony morphologies were cultured and thus were considered contaminated. The cross-tabulated results of the four commercial culture-based tests with the results of routine bacteriological culture of milk are shown in Table 3.

Just above 55% of the samples were cultured on the same day both by performing the routine procedure for bacteriological milk culture and the commercial culture-based tests. The remaining samples (n = 388) were stored at - 20 °C to be cultured using the commercial culture-based mastitis tests at a later time within the study period. Reasons for storing samples at -20 °C were samples being submitted on

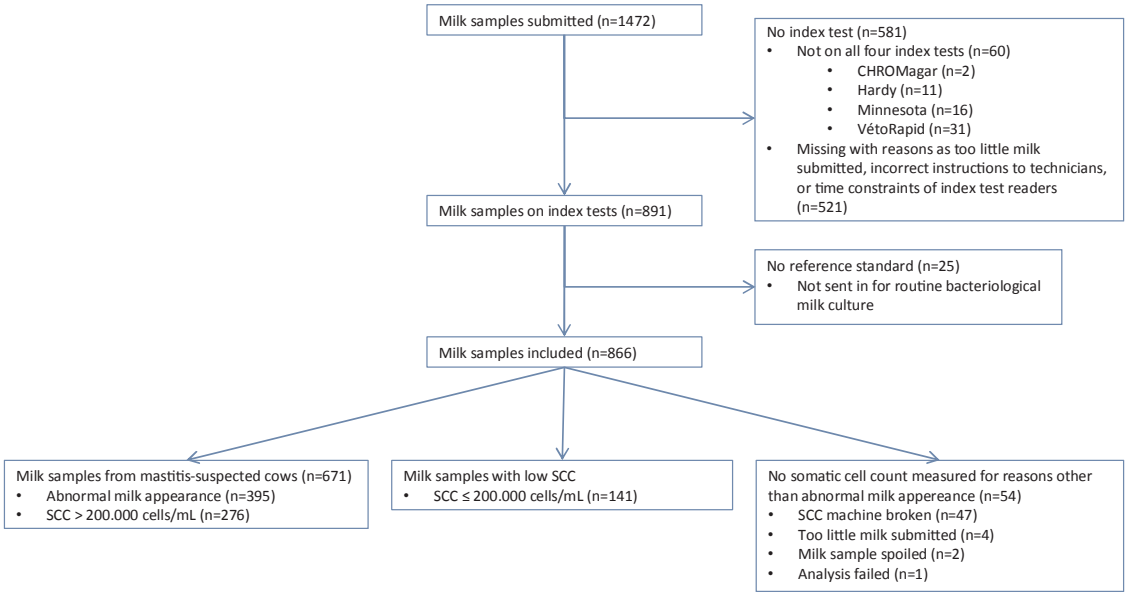

Fig. 1. Flow diagram of milk samples submitted to the GD Animal Health service laboratory and used to assess agreement between four commercial culture-based tests (index tests) and routine bacteriological milk culture.
The four culture-based tests were:
CHROMagar Mastitis (CHROMagar, Paris, France), a combination of two separate plates
Hardy Diagnostics Mastitis Triplate (Hardy Diagnostics, Santa Maria, CA, USA), a triplate
Minnesota Easy Culture System II Tri-plate (Minnesota University, St. Paul, MN, USA), a triplate
VétoRapid (Vetoquinol, 's-Hertogenbosch, the Netherlands), a triplate

days before leaves (weekend or holiday (n = 162)) or time constraints (n = 226).

The agreement beyond change between the four commercial culture-based tests and routine bacteriological milk culture is shown in Table 4. The agreement for Gram-positive bacteria was fair for all tests except for Hardy Diagnostics Mastitis Triplate that consistently had a significantly lower agreement (0.14 (95% CI 0.11-0.16) using all samples) than the other three tests. The overall low agreement for Gram-positive bacteria was caused by routine bacteriological culture of milk resulting in no growth (29% of disagreed results over all four tests together (318/1,086)), Gram-negative bacteria (34% of the disagreed results (370/1,086)), or contamination (36% of the disagreed results (387/1,086)), and by the commercial culture-based tests resulting in no growth (84% of the disagreed results over all tests together (179/214)), and Gram-negative bacteria (8% of the disagreed results (18/214)).

The agreement was substantial for Gram-negative bacteria ranging from 0.69 (95% CI 0.66-0.73) to 0.71 (95% CI 0.68-0.74). In low SCC samples the agreement was moderate ranging from 0.40 (95% CI 0.31-0.48) to 0.48 (95% CI 0.39-0.56). However, PABAK ranged from 0.72 (95% CI 0.57-0.87) to 0.80 (95% CI 0.67-0.93), which was comparable with the other sample types, indicating an effect of prevalence and bias on the calculated kappa value in low SCC samples.

The low agreement for no growth in all samples was caused by routine bacteriological milk culture resulting in Gram-positive bacteria (73% of the disagreed results over all four tests together (179/249)), Gram-negative bacteria (21% of the disagreed results (53/249)), or contamination (9% of the disagreed results (23/249)), and by the commercial culture-based tests indicating Gram-positive bacteria (90% of the disagreed results over all four tests together (318/352)), and Gram-negative bacteria (15% of the disagreed results (53/352)). For both no growth and contamination PABAK was moderate to substantial, which was higher than the calculated kappa value, which was slight to

Table 3
Cross-tabulation of four commercial culture-based mastitis tests with routine bacteriological milk culture to detect mastitis-causing pathogens in milk samples submitted for routine bacteriological milk culture.

		Bacteriological culture of milk (n = 866)							
		Gram-positive		Gram-negative		No growth		Contaminated	
	Result	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
CHROMagar	Positive	333	250	205	82	48	71	39	74
	Negative	76	207	22	557	89	658	67	686
Hardy	Positive	376	314	197	77	31	33	52	130
	Negative	33	143	30	562	106	696	54	630
Minnesota	Positive	358	261	200	82	50	66	32	71
	Negative	51	196	27	557	87	663	74	689
VétoRapid	Positive	342	261	193	74	64	83	49	109
	Negative	67	196	34	565	73	646	57	651

CHROMagar Mastitis (CHROMagar, Paris, France), a combination of two separate plates.
Hardy Diagnostics Mastitis Triplate (Hardy Diagnostics, Santa Maria, CA, USA), a triplate.
Minnesota Easy Culture System II Tri-plate (Minnesota University, St. Paul, MN, USA), a triplate.
VétoRapid (Vetoquinol, 's-Hertogenbosch, the Netherlands), a triplate.

Table 4
Agreement of four commercial culture-based mastitis tests with routine bacteriological milk culture for four diagnostic categories using milk samples submitted for routine bacteriological milk culture. Three types of samples were compared: all submitted samples, samples originating from quarters suspected of having mastitis, and samples with SCC ≤ 200.000 cells/mL (low SCC samples).

	Gram-positive				Gram-negative				No growth				Contaminated			
	κ[1]	(95% CI)	PABAK[2]	(95% CI)	κ[1]	(95% CI)	PABAK[2]	(95% CI)	κ[1]	(95% CI)	PABAK[2]	(95% CI)	κ[1]	(95% CI)	PABAK[2]	(95% CI)
All samples (n = 866)																
CHROMagar	0.23[a]	(0.20,0.26)	0.25	(0.19,0.31)	0.71	(0.68,0.74)	0.76	(0.70,0.82)	0.26[a,b]	(0.23,0.29)	0.63	(0.56,0.69)	0.26	(0.23,0.29)	0.67	(0.61,0.74)
Hardy	0.14[b]	(0.11,0.16)	0.20	(0.15,0.25)	0.70	(0.67,0.73)	0.75	(0.69,0.81)	0.22[a]	(0.19,0.25)	0.68	(0.62,0.74)	0.23	(0.20,0.26)	0.57	(0.51,0.64)
Minnesota	0.25[a]	(0.22,0.28)	0.28	(0.22,0.34)	0.70	(0.67,0.73)	0.75	(0.69,0.81)	0.30[b,c]	(0.27,0.33)	0.65	(0.59,0.71)	0.21	(0.18,0.24)	0.66	(0.60,0.73)
VétoRapid	0.22[a]	(0.19,0.25)	0.24	(0.18,0.30)	0.69	(0.66,0.73)	0.75	(0.69,0.81)	0.34[c]	(0.31,0.38)	0.64	(0.58,0.70)	0.26	(0.23,0.28)	0.62	(0.55,0.68)
Mastitis samples (n = 671)																
CHROMagar	0.25[a]	(0.21,0.28)	0.25	(0.19,0.32)	0.74	(0.70,0.77)	0.77	(0.70,0.83)	0.24[a]	(0.21,0.27)	0.63	(0.55,0.70)	0.19	(0.16,0.22)	0.67	(0.60,0.74)
Hardy	0.15 [b]	(0.12,0.18)	0.19	(0.13,0.25)	0.71	(0.68,0.75)	0.75	(0.68,0.81)	0.26[a,b]	(0.23,0.30)	0.70	(0.63,0.77)	0.18	(0.15,0.21)	0.55	(0.48,0.63)
Minnesota	0.25[a]	(0.22,0.28)	0.26	(0.19,0.33)	0.73	(0.69,0.76)	0.76	(0.70,0.82)	0.30[a,b]	(0.26,0.33)	0.65	(0.58,0.72)	0.17	(0.14,0.20)	0.67	(0.60,0.74)
VétoRapid	0.22[a]	(0.19,0.26)	0.24	(0.17,0.30)	0.72	(0.69,0.76)	0.76	(0.69,0.82)	0.32[b]	(0.29,0.36)	0.65	(0.57,0.72)	0.20	(0.17,0.23)	0.61	(0.54,0.69)
Low SCC samples (n = 141)																
CHROMagar	0.14[a]	(0.08,0.20)	0.23	(0.09,0.37)	0.41	(0.33,0.50)	0.73	(0.59,0.88)	0.33[a]	(0.25,0.41)	0.59	(0.43,0.75)	0.40	(0.32,0.48)	0.67	(0.52,0.83)
Hardy	−0.03[b]	(-0.05,0.00)	0.21	(0.07,0.34)	0.40	(0.32,0.48)	0.73	(0.59,0.88)	0.11[b]	(0.06,0.16)	0.60	(0.44,0.76)	0.33	(0.26,0.41)	0.59	(0.42,0.75)
Minnesota	0.16[a]	(0.10,0.22)	0.31	(0.15,0.46)	0.40	(0.31,0.48)	0.72	(0.57,0.87)	0.28[a]	(0.20,0.35)	0.61	(0.45,0.77)	0.26	(0.19,0.34)	0.64	(0.48,0.80)
VétoRapid	0.17[a]	(0.11,0.23)	0.26	(0.11,0.40)	0.48	(0.39,0.56)	0.80	(0.67,0.93)	0.41[a]	(0.33,0.49)	0.60	(0.44,0.76)	0.35	(0.27,0.43)	0.61	(0.45,0.77)

CHROMagar Mastitis (CHROMagar, Paris, France), a combination of two separate plates.
Hardy Diagnostics Mastitis Triplate (Hardy Diagnostics, Santa Maria, CA, USA), a triplate.
Minnesota Easy Culture System II Tri-plate (Minnesota University, St. Paul, MN, USA), a triplate.
VétoRapid (Vetoquinol, 's-Hertogenbosch, the Netherlands), a triplate.
[a−c]Significant differences within columns per sample type.
[1] Fleiss kappa accounting for varying identity of two readers with 95% CI [2]PABAK = prevalence adjusted and bias adjusted kappa calculated as $2*P_o - 1$, with $P_o = \frac{a+d}{n}$ with 95% CI.

Table 5
Agreement between readers for four commercial culture-based mastitis tests read by two readers for four diagnostic categories using milk samples submitted for routine bacteriological milk culture. Three groups of samples were compared: all submitted samples, samples originating from quarters suspected of having mastitis, and samples with SCC ≤ 200.000 cells/mL (low SCC samples).

	Gram-positive				Gram-negative				No growth				Contaminated			
	κ[1]	95%CI	PABAK[2]	95%CI	κ[1]	95%CI	PABAK[2]	95%CI	κ[1]	95%CI	PABAK[2]	95%CI	κ[1]	95%CI	PABAK[2]	95%CI
All samples (n = 597)																
CHROMagar	0.74[a]	(0.71,0.78)	0.78[a]	(0.72,0.83)	0.91	(0.89,0.94)	0.93	(0.90,0.96)	0.87[a,b]	(0.84,0.90)	0.94[a,b]	(0.91,0.97)	0.61[a]	(0.57,0.65)	0.83[a,c]	(0.78,0.87)
Hardy	0.72[a]	(0.68,0.75)	0.83[a,b]	(0.78,0.87)	0.87	(0.84,0.90)	0.89	(0.85,0.93)	0.86[a]	(0.83,0.88)	0.97[a]	(0.95,0.99)	0.40[b]	(0.36,0.43)	0.61[b]	(0.55,0.67)
Minnesota	0.83[b]	(0.80,0.86)	0.87[b]	(0.83,0.91)	0.89	(0.86,0.91)	0.91	(0.87,0.94)	0.79[b]	(0.76,0.83)	0.90[b]	(0.87,0.94)	0.31[c]	(0.27,0.35)	0.73[b,c]	(0.67,0.78)
VétoRapid	0.86[b]	(0.83,0.89)	0.88[b]	(0.85,0.92)	0.89	(0.87,0.92)	0.91	(0.88,0.94)	0.89[a,b]	(0.87,0.92)	0.94[a,b]	(0.91,0.97)	0.67[a]	(0.63,0.71)	0.80[c]	(0.75,0.85)
Mastitis samples (n = 454)																
CHROMagar	0.73[a,b]	(0.69,0.77)	0.76	(0.70,0.82)	0.92	(0.89,0.94)	0.93	(0.89,0.96)	0.88[a]	(0.85,0.91)	0.95[a,b]	(0.92,0.98)	0.64[a]	(0.59,0.68)	0.84[a]	(0.79,0.89)
Hardy	0.68[a]	(0.64,0.72)	0.79	(0.73,0.85)	0.88	(0.85,0.91)	0.89	(0.85,0.93)	0.83[a,b]	(0.80,0.87)	0.96[a]	(0.93,0.99)	0.36[a,b]	(0.31,0.40)	0.58[b]	(0.50,0.65)
Minnesota	0.81[b,c]	(0.77,0.84)	0.84	(0.79,0.89)	0.89	(0.87,0.92)	0.91	(0.87,0.95)	0.77[b]	(0.73,0.81)	0.89[b]	(0.85,0.93)	0.33[a,b]	(0.29,0.38)	0.73[a]	(0.67,0.79)
VétoRapid	0.84[c]	(0.80,0.87)	0.86	(0.82,0.91)	0.89	(0.86,0.92)	0.90	(0.86,0.94)	0.87[a]	(0.84,0.90)	0.93[a,b]	(0.90,0.96)	0.67[a]	(0.63,0.71)	0.80[a]	(0.75,0.86)
Low SCC samples (n = 106)																
CHROMagar	0.76[a]	(0.68,0.84)	0.81[a]	(0.70,0.92)	0.82	(0.75,0.89)	0.91	(0.82,0.99)	0.88[a]	(0.82,0.94)	0.92	(0.85,1.00)	0.53[a]	(0.44,0.63)	0.75	(0.63,0.88)
Hardy	1.00[b]	(1.00,1.00)	1.00[b]	(1.00,1.00)	0.77	(0.69,0.85)	0.87	(0.77,0.96)	1.00[b]	(1.00,1.00)	1.00	(1.00,1.00)	0.58[a]	(0.49,0.68)	0.74	(0.61,0.86)
Minnesota	0.93[c]	(0.88,0.98)	0.96[a,b]	(0.91,1.00)	0.82	(0.74,0.89)	0.89	(0.80,0.97)	0.91[a,c]	(0.86,0.97)	0.96	(0.91,1.00)	0.12[b]	(0.06,0.18)	0.70	(0.56,0.83)
VétoRapid	0.95[c]	(0.91,0.99)	0.96[a,b]	(0.91,1.00)	0.86	(0.80,0.93)	0.94	(0.88,1.00)	0.97[b,c]	(0.94,1.00)	0.98	(0.94,1.00)	0.67[a]	(0.58,0.76)	0.79	(0.68,0.91)

CHROMagar Mastitis (CHROMagar, Paris, France), a combination of two separate plates.
Hardy Diagnostics Mastitis Triplate (Hardy Diagnostics, Santa Maria, CA, USA), a triplate.
Minnesota Easy Culture System II Tri-plate (Minnesota University, St. Paul, MN, USA), a triplate.
VétoRapid (Vetoquinol, 's-Hertogenbosch, the Netherlands), a triplate.
[a−c]Significant differences within columns per sample type.
[1] Fleiss kappa accounting for varying identity of two readers with 95% CI [2]PABAK = prevalence adjusted and bias adjusted kappa calculated as $2*P_o - 1$, with $P_o = \frac{a+d}{n}$ with 95% CI.

Table 6
Agreement of four commercial culture-based mastitis tests when compared to each other for four diagnostic categories using milk samples submitted for routine bacteriological milk culture. Three types of samples were compared: all submitted samples, samples originating from quarters suspected of having mastitis, and samples with SCC ≤ 200.000 cells/mL (low SCC samples).

		Gram-positive				Gram-negative				No growth				Contaminated			
		κ[1]	95%CI	PABAK[2]	95%CI	κ[1]	95%CI	PABAK[2]	95%CI	κ[1]	95%CI	PABAK[2]	95%CI	κ[1]	95%CI	PABAK[2]	95%CI
All samples (n = 866)																	
Hardy	CHROMagar	0.42[a,c]	(0.39,0.45)	0.55	(0.48,0.61)	0.82	(0.79,0.84)	0.84	(0.79,0.89)	0.49	(0.45,0.52)	0.81	(0.75,0.86)	0.30[a]	(0.27,0.33)	0.60	(0.54,0.67)
	Minnesota	0.43[a,c]	(0.40,0.47)	0.58	(0.52,0.65)	0.78	(0.75,0.81)	0.81	(0.75,0.86)	0.43	(0.40,0.47)	0.79	(0.73,0.84)	0.27[a]	(0.24,0.30)	0.60	(0.53,0.67)
	VétoRapid	0.38[a]	(0.35,0.41)	0.53	(0.47,0.60)	0.77	(0.74,0.80)	0.80	(0.75,0.85)	0.46	(0.43,0.50)	0.75	(0.70,0.81)	0.33[a]	(0.29,0.36)	0.58	(0.51,0.64)
Minnesota	CHROMagar	0.51[b]	(0.48,0.55)	0.59	(0.52,0.65)	0.79	(0.77,0.82)	0.82	(0.77,0.87)	0.50	(0.46,0.53)	0.76	(0.71,0.82)	0.29[a]	(0.26,0.32)	0.69	(0.63,0.75)
	VétoRapid	0.49[b,c]	(0.45,0.52)	0.57	(0.51,0.64)	0.77	(0.74,0.80)	0.80	(0.75,0.86)	0.44	(0.40,0.47)	0.71	(0.65,0.77)	0.32[a]	(0.29,0.35)	0.65	(0.59,0.71)
CHROMagar	VétoRapid	0.49[b,c]	(0.45,0.52)	0.56	(0.49,0.62)	0.77	(0.74,0.80)	0.80	(0.75,0.85)	0.50	(0.47,0.53)	0.74	(0.68,0.80)	0.40[b]	(0.37,0.43)	0.68	(0.62,0.75)
Mastitis samples (n = 671)																	
Hardy	CHROMagar	0.41[a]	(0.37,0.45)	0.52	(0.44,0.59)	0.81	(0.78,0.84)	0.83	(0.77,0.89)	0.52	(0.49,0.56)	0.82	(0.76,0.88)	0.30[a]	(0.26,0.33)	0.60	(0.53,0.67)
	Minnesota	0.42[a,c]	(0.39,0.46)	0.54	(0.47,0.62)	0.78	(0.75,0.81)	0.80	(0.74,0.86)	0.46	(0.43,0.50)	0.79	(0.73,0.85)	0.24[a]	(0.21,0.28)	0.58	(0.51,0.66)
	VétoRapid	0.37[a]	(0.34,0.41)	0.51	(0.43,0.58)	0.78	(0.75,0.81)	0.80	(0.74,0.86)	0.51	(0.47,0.54)	0.77	(0.70,0.83)	0.31[a,b]	(0.28,0.35)	0.57	(0.49,0.64)
Minnesota	CHROMagar	0.51[b]	(0.47,0.55)	0.56	(0.49,0.64)	0.80	(0.77,0.83)	0.82	(0.76,0.87)	0.49	(0.45,0.53)	0.76	(0.69,0.82)	0.26[a]	(0.23,0.30)	0.69	(0.62,0.76)
	VétoRapid	0.49[b,c]	(0.45,0.53)	0.56	(0.49,0.64)	0.78	(0.75,0.81)	0.80	(0.74,0.86)	0.45	(0.42,0.49)	0.72	(0.65,0.79)	0.32[a,c]	(0.28,0.35)	0.67	(0.60,0.74)
CHROMagar	VétoRapid	0.50[b,c]	(0.46,0.53)	0.55	(0.48,0.63)	0.79	(0.76,0.82)	0.81	(0.75,0.87)	0.51	(0.47,0.55)	0.75	(0.69,0.82)	0.38[b]	(0.34,0.42)	0.69	(0.62,0.76)
Low SCC samples (n = 141)																	
Hardy	CHROMagar	0.31[a]	(0.23,0.38)	0.60	(0.44,0.76)	0.71	(0.63,0.78)	0.82	(0.69,0.95)	0.28[a]	(0.21,0.35)	0.70	(0.55,0.85)	0.35[a,c]	(0.27,0.42)	0.60	(0.44,0.76)
	Minnesota	0.30[a]	(0.22,0.37)	0.68	(0.53,0.84)	0.71	(0.63,0.78)	0.82	(0.69,0.95)	0.24[a]	(0.17,0.32)	0.75	(0.61,0.89)	0.18[b]	(0.12,0.25)	0.55	(0.38,0.71)
	VétoRapid	0.32[a]	(0.24,0.39)	0.60	(0.44,0.76)	0.65	(0.58,0.73)	0.81	(0.68,0.94)	0.29[a]	(0.21,0.36)	0.65	(0.49,0.81)	0.35[a,c]	(0.27,0.43)	0.58	(0.41,0.74)
Minnesota	CHROMagar	0.40[a,b]	(0.32,0.48)	0.61	(0.45,0.77)	0.73	(0.66,0.81)	0.83	(0.71,0.96)	0.51[b]	(0.43,0.60)	0.75	(0.61,0.89)	0.36[a,c]	(0.28,0.44)	0.69	(0.54,0.85)
	VétoRapid	0.41[a,b]	(0.32,0.49)	0.61	(0.45,0.77)	0.67	(0.59,0.75)	0.81	(0.69,0.94)	0.39[a,b]	(0.31,0.47)	0.64	(0.48,0.80)	0.25[a,b]	(0.18,0.32)	0.61	(0.44,0.77)
CHROMagar	VétoRapid	0.50[b]	(0.42,0.58)	0.62	(0.46,0.78)	0.65	(0.57,0.73)	0.81	(0.68,0.94)	0.48[b]	(0.40,0.56)	0.66	(0.50,0.82)	0.48[c]	(0.40,0.56)	0.70	(0.54,0.85)

CHROMagar Mastitis (CHROMagar, Paris, France), a combination of two separate plates.
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Minnesota Easy Culture System II Tri-plate (Minnesota University, St. Paul, MN, USA), a triplate.
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[a−c]Significant differences within columns per sample type.
[1] Fleiss kappa accounting for varying identity of two readers with 95% CI [2]PABAK = prevalence adjusted and bias adjusted kappa calculated as $2*P_o - 1$, with $P_o = \frac{a+d}{n}$ with 95% CI.

Table 7
Agreement of four commercial culture-based mastitis tests with routine bacteriological milk culture for mastitis-causing pathogens using milk samples submitted for routine bacteriological milk culture. Three types of samples were compared: all submitted samples, samples originating from quarters suspected of having mastitis, and samples with SCC ≤ 200.000 cells/mL (low SCC samples).

	CHROMagar				Hardy				Minnesota				VétoRapid			
	κ[1]	95%CI	PABAK[2]	95%CI	κ[1]	95%CI	PABAK[2]	95%CI	κ[1]	95%CI	PABAK[2]	95%CI	κ[1]	95%CI	PABAK[2]	95%CI
All samples (n = 866)																
Staphylococcus spp.	–	–	–	–	0.22[a]	(0.19, 0.25)	0.30	(0.24,0.36)	0.30[b]	(0.27,0.33)	0.39	(0.32,0.45)	0.24[a]	(0.21,0.26)	0.29	(0.23,0.35)
S. aureus	0.33[a]	(0.30, 0.36)	0.64	(0.57,0.70)	0.34[a]	(0.30, 0.37)	0.66	(0.59,0.72)	0.47[b]	(0.43,0.50)	0.69	(0.63,0.76)	0.40[b]	(0.37,0.44)	0.62	(0.55,0.68)
CNS	–	–	–	–	–	–	–	–	–	–	–	–	0.05	(0.04,0.07)	0.35	(0.29,0.42)
Streptococcus spp.	−0.24[a]	(-0.27,-0.20)	0.32[A]	(0.25,0.38)	0.12[b]	(0.10, 0.14)	0.19[B]	(0.13,0.24)	0.28[c]	(0.25,0.31)	0.40[A]	(0.33,0.46)	0.28[c]	(0.25,0.31)	0.39[A]	(0.32,0.45)
S. uberis	0.09[a]	(0.07, 0.11)	0.42	(0.35,0.49)	–	–	–	–	–	–	–	–	0.22[b]	(0.19,0.25)	0.52	(0.45,0.58)
S. agalactiae	−0.12[a]	(-0.15,-0.10)	0.56[A]	(0.49,0.63)	−0.06[b]	(-0.08,-0.05)	0.77[B]	(0.71,0.82)	0.00[c]	(-0.01,0.00)	0.99[C]	(0.98,1.00)	−0.01[c]	(-0.01,0.00)	0.98[C]	(0.96,1.00)
S. dysgalactiae	–	–	–	–	–	–	–	–	–	–	–	–	0.28	(0.25,0.31)	0.84	(0.80,0.89)
Lactose-fermenting bacteria	–	–	–	–	0.29	(0.26, 0.32)	0.62	(0.56,0.68)	–	–	–	–	–	–	–	–
E. coli	0.75	(0.72, 0.78)	0.84	(0.79,0.89)	–	–	–	–	–	–	–	–	0.72	(0.69,0.75)	0.84	(0.79,0.89)
KEC	0.56	(0.53, 0.59)	0.84	(0.79,0.89)	–	–	–	–	–	–	–	–	0.53	(0.50,0.57)	0.83	(0.78,0.88)
Non-lactose-fermenting bacteria	–	–	–	–	0.10	(0.08, 0.12)	0.88	(0.84,0.93)	–	–	–	–	0.13	(0.11,0.16)	0.92	(0.77,1.00)
Pseudomonas spp.	0.04	(0.03, 0.05)	0.95	(0.92,0.98)	–	–	–	–	–	–	–	–	–	–	–	–
Mastitis samples (n = 671)																
Staphylococcus spp.	–	–	–	–	0.20[a]	(0.17, 0.23)	0.29	(0.23,0.36)	0.27[b]	(0.23,0.30)	0.37	(0.30,0.45)	0.25[a,b]	(0.21,0.28)	0.31	(0.24,0.38)
S. aureus	0.36[a,b]	(0.33, 0.40)	0.67	(0.60,0.74)	0.33[a]	(0.30, 0.37)	0.67	(0.60,0.74)	0.44[b]	(0.40,0.47)	0.69	(0.62,0.76)	0.42[b]	(0.38,0.45)	0.63	(0.56,0.71)
CNS	–	–	–	–	–	–	–	–	–	–	–	–	0.05	(0.03,0.06)	0.39	(0.31,0.46)
Streptococcus spp.	−0.24[a]	(-0.28,-0.20)	0.33[A,B]	(0.26,0.40)	0.15[b]	(0.13, 0.18)	0.23[A]	(0.16,0.29)	0.30[c]	(0.27,0.34)	0.42[B]	(0.35,0.50)	0.29[c]	(0.26,0.32)	0.40[B]	(0.32,0.47)
S. uberis	0.12[a]	(0.09, 0.14)	0.43	(0.35,0.50)	–	–	–	–	–	–	–	–	0.25[b]	(0.22,0.28)	0.54	(0.47,0.62)
S. agalactiae	−0.11[a]	(-0.14,-0.09)	0.59[A]	(0.52,0.67)	−0.06[b]	(-0.08,-0.04)	0.78[B]	(0.72,0.84)	0.00[c]	(-0.01,0.00)	0.99[C]	(0.98,1.00)	−0.01[c]	(-0.01,0.00)	0.98[C]	(0.96,1.00)
S. dysgalactiae	–	–	–	–	–	–	–	–	–	–	–	–	0.27	(0.24,0.31)	0.85	(0.79,0.90)
Lactose-fermenting bacteria	–	–	–	–	0.26	(0.23, 0.30)	0.57	(0.50,0.65)	–	–	–	–	–	–	–	–
E. coli	0.76	(0.73, 0.80)	0.84	(0.78,0.89)	–	–	–	–	–	–	–	–	0.73	(0.70,0.77)	0.83	(0.77,0.89)
KEC	0.58	(0.54, 0.62)	0.84	(0.78,0.89)	–	–	–	–	–	–	–	–	0.58	(0.54,0.61)	0.83	(0.77,0.88)
Non-lactose-fermenting bacteria	–	–	–	–	0.11[a]	(0.09, 0.14)	0.88	(0.83,0.93)	–	–	–	–	0.17[b]	(0.14,0.20)	0.92	(0.88,0.96)
Pseudomonas spp.	0.07	(0.05, 0.09)	0.95	(0.92,0.98)	–	–	–	–	–	–	–	–	–	–	–	–
Low SCC samples (n = 141)																
Staphylococcus spp.	–	–	–	–	0.17[a]	(0.11, 0.24)	0.19	(0.06,0.32)	0.31[b]	(0.24,0.39)	0.33	(0.18,0.49)	0.21[a,b]	(0.14,0.28)	0.22	(0.08,0.35)
S. aureus	0.24[a]	(0.17, 0.31)	0.52	(0.36,0.69)	0.44[b,c]	(0.36, 0.52)	0.64	(0.48,0.80)	0.58[b]	(0.50,0.66)	0.72	(0.57,0.87)	0.38[a,c]	(0.30,0.46)	0.55	(0.38,0.71)
CNS	–	–	–	–	–	–	–	–	–	–	–	–	0.10	(0.05,0.15)	0.27	(0.12,0.41)
Streptococcus spp.	−0.21[a]	(-0.28,-0.14)	0.26[A,B]	(0.12,0.41)	0.06[b]	(0.02, 0.10)	0.10[A]	(0.00,0.19)	0.28[c]	(0.20,0.35)	0.35[B]	(0.20,0.51)	0.32[c]	(0.25,0.40)	0.42[B]	(0.26,0.58)
S. uberis	0.04[a]	(0.01, 0.07)	0.32	(0.17,0.48)	–	–	–	–	–	–	–	–	0.20[b]	(0.14,0.27)	0.49	(0.32,0.65)
S. agalactiae	−0.12[a]	(-0.18,-0.06)	0.56[A]	(0.40,0.72)	−0.07[a,b]	(-0.12,-0.03)	0.70[A]	(0.55,0.85)	0.00[b]	(-0.01,0.01)	0.96[B]	(0.89,1.00)	0.00[b]	(-0.01,0.01)	0.96[B]	(0.89,1.00)
S. dysgalactiae	–	–	–	–	–	–	–	–	–	–	–	–	0.32	(0.24,0.40)	0.89	(0.79,0.99)
Lactose-fermenting bacteria	–	–	–	–	0.38	(0.30, 0.46)	0.86	(0.74,0.97)	–	–	–	–	–	–	–	–
E. coli	0.45	(0.36, 0.53)	0.84	(0.72,0.96)	–	–	–	–	–	–	–	–	0.58	(0.50,0.66)	0.88	(0.77,0.99)
KEC	0.27	(0.19, 0.34)	0.87	(0.76,0.98)	–	–	–	–	–	–	–	–	0.17	(0.11,0.24)	0.89	(0.78,0.99)
Non-lactose-fermenting bacteria	–	–	–	–	−0.03	(-0.05, 0.00)	0.80	(0.67,0.94)	–	–	–	–	−0.01	(-0.03,0.01)	0.96	(0.90,1.00)
Pseudomonas spp.	−0.02	(-0.04,0.01)	0.88	(0.78,0.99)	–	–	–	–	–	–	–	–	–	–	–	–

CHROMagar Mastitis (CHROMagar, Paris, France), a combination of two separate plates.
Hardy Diagnostics Mastitis Triplate (Hardy Diagnostics, Santa Maria, CA, USA), a triplate.
Minnesota Easy Culture System II Tri-plate (Minnesota University, St. Paul, MN, USA), a triplate.
VétoRapid (Vetoquinol, 's-Hertogenbosch, the Netherlands), a triplate.
[a−c]Significant differences within rows per sample type for kappa.
[A−C]Significant difference within rows per sample type for PABAK.
[1] Fleiss kappa accounting for varying identity of two readers with 95% CI [2]PABAK = prevalence adjusted and bias adjusted kappa calculated as $2*P_o-1$, with $P_o = \frac{a+d}{n}$ with 95% CI.

fair.

The agreement beyond chance between two readers was 0.72 or higher for each commercial culture-based test for all diagnostic categories (Table 5). Contamination was the exception. For contamination in all samples and mastitis samples readers agreed most using CHROMagar Mastitis or VétoRapid (kappa ranging 0.61 (95% CI 0.57-0.65) to 0.67 (95% CI 0.63-0.71)) while the agreement was significantly lower using Hardy Diagnostics Mastitis Triplate or Minnesota Easy Culture System II Tri-plate (kappa ranging 0.31 (95% CI 0.27-0.35) to 0.40 (95% CI 0.36-0.43)). The agreement between readers was significantly lower for no growth in all samples using Minnesota Easy Culture System II Tri-plate, although kappa still was substantial (0.79 (95% CI 0.76-0.83). The same was seen in mastitis samples where Minnesota Easy Culture System II Tri-plate also had the lowest kappa (0.77 (95% CI 0.73-0.81). In low SCC samples all readers agreed almost perfectly regardless the commercial culture-based test used. Also for Gram-positive bacteria readers agreed almost perfectly using Minnesota Easy Culture System II Tri-plate or VétoRapid regardless sample type.

When the results of two commercial culture-based tests were compared, Minnesota Easy Culture System II Tri-plate, VétoRapid, CHROMagar Mastitis had all a significant higher agreement with each other for Gram-positive bacteria in all samples than CHROMagar Mastitis, Minnesota Easy Culture System II Tri-plate, or VétoRapid had with Hardy Diagnostics Mastitis Triplate (Table 6). All commercial culture-based tests had the same level of agreement with each other for Gram-negative bacteria, regardless sample type. The agreement of the commercial culture-based tests with each other was lower than the agreement between two readers reading the same commercial culture-based tests, but generally higher than the agreement of the commercial culture-based tests with routine bacteriological culture of milk.

Minnesota Easy Culture System II Tri-plate had a significantly higher agreement with routine bacteriological culture of milk for *Staphylococus* spp. (0.30 (95% CI 0.27-0.33)) than Hardy Diagnostics Mastitis Triplate (0.22 (95% CI 0.19-0.25)) and VétoRapid (0.24 (95% CI 0.21-0.26)) (Table 7). Also for *S. aureus* the agreement of Minnesota Easy Culture System II Tri-plate with routine bacteriological culture of milk was higher, as well as the agreement of VétoRapid with routine bacteriological culture of milk, than CHROMagar Mastitis and Hardy Diagnostics Mastitis Triplate. For Gram-negative bacteria as *E. coli* and the group KEC, the agreement of CHROMagar Mastitis and VétoRapid with routine bacteriological culture of milk were moderate to substantial, while PABAK was almost perfect. The effect of prevalence and bias on the kappa value became higher in low SCC samples.

3.1. Uncertainty reading results of commercial culture-based tests

The number of results readers were uncertain about was highest using CHROMagar Mastitis or Hardy Diagnostics Mastitis Triplate, both 30% of the results. These were followed by VétoRapid with 18% of the results leading to uncertainty, and Minnesota Easy Culture System II Tri-plate with 14%. When the reader noted to be uncertain, most often a Gram-positive result (> 90%) was noted by the reader, regardless the commercial culture-based test used.

4. Discussion

To minimize unnecessary antimicrobial use in the dairy industry, there is a need for tests to determine the IMI status of a cow. Bacteriological milk culture could be used, although the definition of the IMI status based on bacteriological milk culture results has been debated for many years (Bradley et al., 2005; Lam et al., 2009; Andersen et al., 2010; Dohoo et al., 2011a). Currently, the most common test used to determine an IMI is bacteriological culture of milk. However, the sensitivity and specificity of bacteriological culture of milk to determine an IMI is low (Dohoo et al., 2011b). Nevertheless, a commercial culture-based test might be a practical tool to facilitate prudent use of antimicrobials on dairy farms (Ruegg et al., 2009; Wallace, 2011; Keefe et al., 2013).

Earlier work showed that commercial culture-based tests are able to categorize mastitis cases into treatment groups (McCarron et al., 2009a; Lago et al., 2011; Cameron et al., 2013; Royster et al., 2014; Viora et al., 2014; Ganda et al., 2016). For Gram-negative bacteria we found an average agreement between commercial culture-based tests and routine bacteriological culture of milk of 0.70 (95% CI ranged 0.66-0.74), which was slightly lower than using Accumast (0.84 (95% CI 0.77-0.91)) (Ganda et al., 2016), but comparable with what was found using Minnesota Easy Culture System II Tri-plate (ranging 0.63-0.75 (95% CI ranging 0.57-0.81)) (Royster et al., 2014). However, the other diagnostic categories showed a lower agreement between commercial culture-based tests and routine bacteriological culture of milk than earlier found in literature. Especially for Gram-positive bacteria (kappa on average 0.21 (95% CI ranging 0.11-0.28)), and for no growth (kappa on average 0.28 (95% CI ranging 0.19-0.38)) kappa was lower than the earlier found 0.6 (Royster et al., 2014). Also the proportion of observed agreement ((total of agreed positive results + total of agreed negative results)/total results, also referred to as accuracy) for Gram-positive bacteria of 0.62 (95% CI 0.59-0.65) found in our study (data not shown) was lower than found using the Minnesota Easy Culture System II Tri-plate (0.81) (Ferreira et al., 2018), or using Petrifilm plates (0.85) (Mansion-de Vries et al., 2014). Explanations for this low agreement might be that routine bacteriological milk culture presented only relevant growth and assigned samples with irrelevant growth to the no growth or contamination category, while readers of commercial culture-based tests noted all growth present regardless the type of growth. Also, readers might have missed contamination, which was also noticed by Royster et al. (2014). However, when these commercial culture-based tests will be used on farms, both will result in cows being treated with the potential that treating is unnecessary (Ruegg et al., 2009; Lago et al., 2011). Of bigger concern might be that approximately 5% of all samples cultured will be left untreated based on the no growth result of the commercial culture-based test while routine bacteriological milk culture resulted in Gram-positive bacteria, as Gram-positives should be treated (Ruegg et al., 2009). However, farmers probably tend to include more information to decide on treatment than solely a test result (Scherpenzeel et al., 2016) as 33% of the farmers decided to treat cows with a no growth result on commercial culture-based tests (Neeser et al., 2006). Therefore, the number of untreated cows that ideally ought to be treated likely will be limited.

The proportions of observed agreement we found for Gram-negative bacteria of on average 0.88 (95% CI 0.80-0.95), for no growth of on average 0.82 (95% CI 0.69-0.96), and for contamination of on average 0.82 (95% CI 0.73-0.90) (data not shown) were comparable with literature. For example to detect mastitis-associated bacteria using Accumast the proportion of observed agreement was 0.85 (Ganda et al., 2016) or 0.90, using Minnesota Easy Culture System II Tri-plate the observed agreement was 0.73, using SSGN it was 0.79, or using SSGNC it was 0.75 (Ferreira et al., 2018). These were also in line with the observed agreements found using Minnesota Easy Culture System II Bi-plate and Tri-plate (Royster et al., 2014).

A kappa paradox was quite explicit present for the categories no growth, and contamination as the observed agreement was high in these categories with many samples being negative for no growth or contamination according to both commercial culture-based tests and routine bacteriological milk culture. However, the kappa statistic was low. Interpreting solely the kappa statistic might lead to a paradoxical underestimation of the true agreement as prevalence and bias may have affected kappa estimates (Byrt et al., 1993). Calculating PABAK could give an estimation of that effect. In our study the kappa for no growth was on average 0.28 (95% CI ranged from 0.19-0.38) while PABAK was on average 0.65 (95% CI ranged from 0.56-0.74), and for contamination kappa was 0.24 (95% CI ranged from 0.18-0.29) while PABAK was on average 0.63 (95% CI ranged from 0.51-0.74). These differences in

prevalence and bias among studies make direct comparison of kappa values difficult, especially when no contingency tables or additional measures like observed agreement or PABAK are given, and differences among studies should therefore be interpret with caution.

Different readers had a high agreement for Gram-positive bacteria, and even higher for Gram-negative bacteria, or no growth when reading the same commercial culture-based test, indicating that commerical culture-based tests were read consistently. For CHROMagar Mastitis and Hardy Diagnostics Mastitis Triplate the agreement for Gram-positive bacteria was lower than for Minnesota Easy Culture System II Triplate or VétoRapid which could be explained by readers being uncertain about their results more frequent using CHROMagar Mastitis and Hardy Diagnostics Mastitis Triplate. The same uncertainty likely also caused the lower agreement for contamination. Probably some readers noted Gram-positive bacteria but were uncertain about their decisions, while others noted contamination. That the agreement between readers was higher than between commercial culture-based tests and routine bacteriological milk culture was also seen in an earlier evaluation of the commercial culture-based test Minnesota Easy Culture System II Tri-plate (Royster et al., 2014).

Within udder health, three types of cows might be considered for treatment: cows with clinical mastitis, with subclinical mastitis, and cows before drying off (Sears and McCarthy, 2003; van den Borne et al., 2010; Cameron et al., 2014; Kromker and Leimbach, 2017) and were considered the target populations for the commercial culture-based tests. Unfortunately, clinical information on the cows the milk samples were collected from lacked in our study. Therefore, we divided the samples in mastitis suspected samples and low SCC samples based on the SCC and milk appearance to examine the performance of the commercial culture-based tests among the different target populations. This differentiation could also be made by farmers using the Californian Mastitis Test and milk appearance (Dingwell et al., 2003; Bhutto et al., 2012). The performance of commercial culture-based tests among low SCC is for example of interest at drying off and previously commercial culture-based tests were able to detect IMI's within this group (Cameron et al., 2013). The most striking difference between the sample types was the agreement for Gram-negative bacteria that dropped from almost perfect using all samples or mastitis samples to moderate using low SCC samples. However, as also shown by PABAK, this agreement seemed to be underestimated as PABAK was comparable with the other sample types, likely explained by Gram-negative bacteria being less prevalent in low SCC samples. The study results might be biased as the source population likely was a subset of the target population, with an over-representation of more severe cases. Farmers might have submitted samples of, for example, more severe mastitis cases while with commercial culture-based tests available they might use the test for all cases where a treatment decision need to be made with respect to udder health. However, as the PABAK hardly differ among the different sample types, we consider it reasonable to extrapolate the results to the target population.

Veterinarians generally advise dairy farmers to culture milk samples on a regular basis to be able to manage udder health, either by using a culture-based test to decide on treatment, or by regularly submitting milk samples to a laboratory to be informed on the most important type of mastitis pathogens and antimicrobial susceptibility on farms (Barkema et al., 2006; Lam et al., 2009; Ruegg et al., 2009). Currently, roughly 72% of the cows with clinical mastitis is treated with antimicrobials in the Netherlands (Santman-Berends et al., 2015). Apparently, farmers decide on selective treatment using parameters other than test results since these test results are infrequently used. However, farmers do have a need to substantiate their decisions by additional testing (Griffioen et al., 2016). Culture-based tests might be suitable to support these decisions. Although we found only for Gram-negative bacteria a good agreement between culture-based tests and routine bacteriological culture of milk, and further on-farm evaluations are needed to determine the effect of these findings on udder health, culture-based tests are of added value to support decisions whether and how to treat cows with intramammary infections.

Conflicts of interest

none

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