Research paper

Evaluation of a culture-based pathogen identification kit for bacterial causes of bovine mastitis

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Abstract

Accurate identification of mastitis-causing bacteria supports effective management and can be used to implement selective use of antimicrobials for treatment. The objectives of this study were to compare the results from a culture-based mastitis pathogen detection test kit (*'VetoRapid'*, Vétoquinol) with standard laboratory culture and to evaluate the potential suitability of the test kit to inform a selective treatment programme. Overall 231 quarter milk samples from five UK dairy farms were collected. The sensitivity and specificity of the test kit for the identification of *Escherichia coli*, *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus uberis* and *Enterococcus* spp., ranged from 17% to 84% and 92% to 98% respectively. Twenty-three of 68 clinical samples were assigned as meeting the requirement for antimicrobial treatment (Gram-positive organism cultured) according to standard culture results, with the test kit results having sensitivity and specificity of 91% and 78% respectively. Several occurrences of misidentification are reported, including *Staphylococcus aureus* being misidentified as coagulase-negative staphylococci and vice-versa. The test kit provides rapid preliminary identification of five common causes of bovine mastitis under UK field conditions and is likely to be suitable for informing selective treatment of clinical mastitis caused by Gram-positive organisms.

Introduction

Intra-mammary infection (IMI), or mastitis, is estimated to be the most expensive disease in the dairy industry and is the most frequent reason for antimicrobial use (Mitchell and others 1998; More and others 2010). In a US study, 80% of all antimicrobial drugs administered to dairy cattle were used for treatment or prevention of mastitis (Pol and Ruegg 2007). Effective control of mastitis can reduce problems attributed to the extensive use of antimicrobials in food animals such as antimicrobial residues and the potential for antimicrobial resistance (Erskine and others 2003; Makovec and Ruegg 2003; Pol and Ruegg 2007; UK-government 2013).

Identification of mastitis-causing organisms on individual farms has been a key component of prevention planning for many years (Ruegg 2003). Once the causative agents of clinical and sub-clinical cases are established at the herd level, control measures can be targeted to reduce the source and spread of bacteria. A more recent development has been the use of pathogen identification to inform individual cow treatment protocols for clinical mastitis during lactation, with the potential to further reduce antimicrobial use (Cameron and others 2013; Lago and others 2011a). Several authors have reported that mild and moderate clinical mastitis cases caused by *E. coli* species do not benefit from antimicrobial therapy (Roberson 2012; Suojala and others 2013).

Targeted treatment requires rapid and accurate pathogen identification tests. Standard laboratory culture and modern molecular diagnostics are the preferred methods, but they cannot be performed outside of professional laboratories. The delay associated with submitting samples to a laboratory means that there is normally more than a 24 hour delay between detecting the mastitis and receiving the result, making them difficult to use in targeted treatment protocols currently. The limited number of

pathogen species associated with most mastitis cases (Bradley and others 2007; Koivula and others 2007; Makovec and Ruegg 2003; Tenhagen and others 2006; Watts and Yancey 1994) facilitates the design of simplified culture-based detection kits, where a single milk sample can be cultured concurrently on selective multiple media plates. With appropriate interpretative guides, these kits allow crude, culture-based differentiation of major pathogen groups to be performed by a suitably trained person (non-microbiologist), with preliminary results available within 18-24 hours of sampling.

A recent US trial that used selective treatment protocols for clinical mastitis demonstrated no difference in cure rate, recurrence rate or long term production output when withholding antimicrobial treatment from cases where Gram-negative bacteria, no bacteria or yeasts were cultured using an 'on-farm' test kit (Lago and others 2011a, b). The proportion of such clinical cases will vary depending on the farm. Reduced antimicrobial use has also been reported from US farms using on-farm pathogen culture kits at the end of lactation (on cows with low somatic cell counts) to help decide whether to administer antimicrobials at dry off (Cameron and others 2013).

In Europe, it remains common practice to treat all cases of clinical mastitis with antimicrobial therapy, despite culture-based pathogen identification kits being available. To our knowledge, there are no reports in the literature comparing such kits to standard laboratory culture for pathogen identification in samples from UK dairy herds. The aim of this study was to compare the results from a culture-based mastitis pathogen detection test kit (*'VetoRapid'*, Vétoquinol), with standard laboratory culture using milk samples collected from commercial UK dairy farms, and to evaluate the

suitability of the test kit to inform a selective treatment programme for clinical mastitis.

Materials and methods

Farm and animal selection

Samples were collected between May 2012 and January 2013 from five herds located in Scotland (Table 1). Three herds (1, 2 and 3), were enrolled in a year-round routine monitoring service provided by the Scottish Centre for Production Animal Health and Food Safety (SCPAHFS, School of Veterinary Medicine, University of Glasgow) with weekly visits. The remaining herds (4 and 5) were visited once by SCPAHFS veterinary surgeons to conduct an individual herd mastitis investigation. Background information from the five farms is shown in Table 1.

Quarters with clinical and sub-clinical bacterial infections were identified for sampling. Clinical mastitis was detected if a quarter was swollen and / or painful, or if there were physical changes in the milk (flakes, clots or a watery appearance). Sub-clinical mastitis was detected when the cow had a SCC greater than 200,000 cells/mL at the most recent milk recording of cow milk (composite of all milking quarters). On those animals, a California Mastitis Test (CMT) was carried out to detect the affected quarter(s) for sampling (Deb and others 2013).

Sample collection

Milk samples were collected aseptically by a single veterinary surgeon from the SCPAHFS (LV) or by an individual, trained member of the on-farm staff, following guidelines recommended by the National Mastitis Council (2004). Briefly, these require wearing disposable gloves, thoroughly washing and drying the teat,

discarding several streams of milk, dipping and wiping the teat with a pre-milking teat disinfectant, scrubbing the teat end with cotton soaked in 70% alcohol, collection of two or more streams of milk into a sterile container, and securing the cap immediately.

After collection, samples were moved within four hours to -20ºC storage either at the University of Glasgow or temporarily to an on-farm freezer until transfer while frozen to the University of Glasgow (National Mastitis Council 2004). All samples were cultured within four weeks.

Microbiological analysis

Milk samples were removed from the freezer and allowed to reach room temperature before being agitated for five seconds. Samples were processed concurrently by a laboratory technician in the Infectious Diseases Diagnostic Unit, Veterinary Diagnostic Services, School of Veterinary Medicine, University of Glasgow (IDU) using standard laboratory methods, and by LV using the test kit.

Standard laboratory culture (standard culture)

The samples were processed by standard laboratory methods for the microbiological analysis of milk (National Mastitis Council 1999). Briefly, a sterile cotton swab was saturated with the milk, and lawn cultures were prepared in the corners of MacConkey and 5% sheep blood agar plates (E & O Laboratories) before streaking with a sterile loop. The plates were incubated aerobically at 37ºC for 24-48 hours before being read. All organisms were identified by standard laboratory methods (including colony morphology, Gram stain, pattern of haemolysis and

biochemical profile). An appropriate API test (bioMérieux) was performed for speciation when required (National Mastitis Council 1999).

Culture-based pathogen detection test kit

Samples were plated concurrently onto the test kit. The test kit comprises a single plate containing three distinct sectors of selective medium: a customised agar targeting coliforms that contains bile salts and vancomycin that inhibit the growth of Gram-positive bacteria (sector 1; proprietary); a modified mannitol-salt agar (MSA) which supports the growth of organisms that tolerate high salt concentrations such as *Staphylococcus* spp., and can aid the differentiation of mannitol fermenters such as *S. aureus* from non-mannitol fermenters such as the coagulase-negative staphylococci (CNS) (sector 2; proprietary); and a modified Edwards agar that contains antimicrobials crystal violet and Polymyxin B that inhibit the growth of staphylococci and Gram-negative bacteria respectively (sector 3; proprietary).

The test kit was prepared and read by a single farm animal clinician from the SCPAHFS (LV) according to the manufacturer's instructions, but without any specific training or experience in microbiological techniques. A sterile cotton swab was saturated with the milk and a lawn culture was prepared in the corner of each sector before streaking with a sterile loop. The test kits were incubated aerobically at 37^{o}C for 24-48 hours.

Utilising the accompanying interpretative guidelines, a crude identification of eight of the most common mastitis-causing bacteria or bacterial groups was made as follows: *E. coli* (dark blue colonies growing on sector 1), *Klebsiella* spp. (red-purple colonies growing on sector 1), *S. aureus* (yellow, golden colonies and agar on sector 2), CNS (clear colonies and no change in the agar colour on sector 2), *S. uberis* (black

colonies and agar on sector 3), *Enterococcus* spp. (black colonies and agar on sector 3 together with pinhead yellow colonies and discoloration of sector 2), *S. dysgalactiae* (clear colonies on sector 3 with green coloured corona at 48 hours and red-brown agar coloration) or *S. agalactiae* (clear colonies on sector 3 with clear-bright corona at 48 hours and red-brown agar coloration). All organisms growing on the test kit were classified into one of these 8 groups.

Initial data analysis

The results from standard culture were considered definitive. Data were stored and analysed using Microsoft Excel 2007. Sensitivity and specificity (+/- 95% Confidence Interval (using the formula: $\bar{x} \pm 1.96 * \sigma/\sqrt{(n)}$, where $\bar{x}$ represents the mean, σ the standard deviation, and n the sample size) of the test kit compared to standard culture were calculated. To estimate positive predictive values and negative predictive values, the proportion of organisms of the appropriate type identified in all samples was taken as prevalence.

Use of the test kit for selective treatment

Two hypothetical treatments (antimicrobial treatment or no antimicrobial treatment) were assigned to each clinical sample based on results from standard culture and the test kit using the model described by Lago and others (2011a). Based on this model, antimicrobials are assigned only when a Gram-positive organism is isolated. The suitability of the resultant treatment choices were compared, with the sensitivity (95% Confidence Interval), specificity (95% Confidence Interval), positive predictive values and negative predictive values calculated for the test kit compared to standard culture.

Qualification of incorrectly identified pathogens

Two methods were used to investigate the potential for misidentification of pathogens by the test kit. First, a subset of results was created where, for a given sample, a pathogen was isolated in pure culture by both standard culture and the test kit but where there was disagreement on the identity of the pathogen. In these cases standard culture was assumed to have correctly identified the only organism present, and the test kit assumed to have misidentified the organism.

In addition, nine mastitis isolates previously identified by the IDU laboratory as *E. coli, Klebsiella* spp., *S. aureus, Staphylococcus epidermidis, S. uberis, Enterococcus faecalis* (*E. faecalis*), *Aerococcus viridians (A. viridans), S. dysgalactiae* and *S. agalactiae* were obtained from storage at the IDU and cultured in parallel using standard culture and the test kit by an IDU laboratory technician. *Aerococcus viridans* was included because of its morphological and biochemical similarity to *E. faecalis*. The IDU laboratory technician read all plates after 24-48 hours and compared the results to the original, known isolate.

Results

Microbiological Analysis

A total of 231 samples were included in the study. Sixty-eight were collected from clinical cases and 163 from subclinical cases (Table 1).

Of all standard culture results, no bacteria were recovered from 98 samples (42.4%), a pure bacterial culture was recovered from 116 samples (50.2%), and two different organisms were recovered from 17 samples (7.4%). No samples had three or more bacteria isolated.

A total of 150 individual organisms were isolated by standard culture from 133 samples, of which 46 (31%) could not be identified by the test kit as they were not one of the eight identifiable groups (25 *Bacillus* spp., 10 *A. viridans*, 3 unidentified Gram-positive rods, 3 yeast organisms, 2 *Serratia* spp., 1 *Lactococcus lactis*, 1 *Proteus* sp., 1 *Pseudomonas* sp.).

The most common bacterial group isolated was CNS (19% of all pathogens). *Bacillus* spp. (single or sparse colonies) was isolated as the only organism in 17 samples (six from farm 1, seven from farm 2 and four from farm 4) and in eight samples together with another organism (twice with *E. coli* and CNS, and once each with *S. aureus*, *Enterococcus* spp., *Lactococcus lactis* and *Pseudomonas* sp.).

There were very few isolates of *S. dysgalactiae* (5 isolates), *S. agalactiae* (1 isolate) and *Klebsiella* spp. (never isolated) in the final data-set and no test characteristics were estimated for these pathogens. The sensitivity, specificity, positive predictive values and negative predictive values of the five other pathogens (*E. coli*, *S. aureus*, CNS, *S. uberis*, *Enterococcus* spp.) are shown in Table 2.

Use of the plate for selective treatment

Twenty-three of the 68 clinical samples were assigned as meeting the requirement for antimicrobial treatment according to the protocol described by Lago and others (2011a). The comparison of assignment of antimicrobials based on standard culture and the test kit results are shown in Table 3.

Qualification of incorrectly identified pathogens

Twenty-four samples were identified where a pathogen was isolated in pure culture by both standard culture and the test kit but with disagreement on pathogen

identity. CNS was incorrectly identified as *S. aureus* in four samples and *S. aureus* was incorrectly identified as CNS in two samples. *Enterococcus* spp. was incorrectly identified as *S. uberis* in two samples, *Bacillus* spp. was incorrectly identified as *Enterococcus* spp. in three samples and *A. viridans* was incorrectly identified as *S. uberis* in two samples. The remaining 11 misidentifications occurred only once each (*E. coli*, *S. agalactiae* and *Bacillus* spp. were each misidentified as *S. uberis*; CNS, *S. aureus*, *S. uberis*, *S. dysgalactiae* and *A. viridians* were each misidentified as *Enterococcus* spp; *Enterococcus* spp. and *Bacillus* spp. were each misidentified as CNS; and *A. viridians* was misidentified as *S. aureus*).

All nine stored mastitis isolates were correctly identified by standard culture on repeat culture. The test kit correctly identified *E. coli*, *Klebsiella*, *S. agalactiae* and *S. dysgalactiae* isolates. *S. aureus* was incorrectly identified as CNS (Figure 1), and *S. epidermidis* was incorrectly identified as *S. aureus*. Both *S. uberis* and *A. viridans* were incorrectly identified as *E. faecalis*.

Discussion

The first aim of this study was to compare the results from the test kit with standard culture using milk samples collected from commercial UK dairy farms. The size of the dataset was limited and the results for only five of the eight pathogens identifiable by the test kit are reported. For each of the five pathogen species assessed, the individual specificity of the test kit is above 90%. The low prevalence of each individual pathogen in this study has allowed the accurate estimate of these specificity values, reflected in the narrow confidence intervals reported. The sensitivity reported for the five pathogens assessed is much more variable (17-84%) with wider confidence intervals reflecting the low numbers of positive samples in the dataset. The

mean sensitivity estimate of the test kit was higher for all pathogens in clinical samples compared to sub-clinical samples (with the exception of *Enterococcus spp.* which was never isolated from a clinical sample). This may be due to a higher number of bacteria being present in these samples, increasing the likelihood of growth on selective media (Persson and others 2011). The results reported here may therefore be an under-estimate of the kit's performance if only used on clinical samples, and an over-estimate of the kit's performance if only used on sub-clinical samples. The accuracy of test characteristic estimates could have been improved by expanding the dataset to increase the number of positive samples.

The second aim of the study was to evaluate the suitability of using the test kit to implement a selective antimicrobial treatment regimen described previously (Lago and others 2011a), in which antimicrobial therapy is only used when Gram-positive bacteria are isolated. The test kit was found to be reasonably sensitive for identifying Gram-positive organisms from clinical cases, though the specificity is lower. This suggests that it would be possible to use the test kit to reduce antimicrobial therapy (compared to non-selective treatment) without significant risk of missing cases where Gram-positive bacteria are present. Further work is required to determine whether short and long-term outcomes (including cure rate, recurrence rate and milk yield) would be affected in cows from which antimicrobials were withheld following implementation of such a protocol under UK field conditions.

Results from the test kit were compared to results from standard laboratory culture. Pathogen identification by both of these methods relies on phenotypic characteristics that are known to occur frequently in the particular pathogen group or species. Identification by standard laboratory culture is likely to be more accurate as it considers many more phenotypic and biochemical characteristics than the test kit.

297 However, results from standard culture are known to be imperfect and subject to inter-
298 laboratory variation (Pitkälä and others 2005). Modern molecular techniques can
299 improve sensitivity and reduce identification errors that result from phenotypic
300 variation within species (Bautista-Trujillo and others 2013; Becker and others 2004;
301 Keane and others 2013). This study could have been improved by identifying all
302 isolates (from laboratory culture and test kits) by molecular methods, though this was
303 cost prohibitive. Comparison of 'on-farm' culture kits to standard laboratory culture is
304 common in the recent scientific literature (Lago and others 2011a, b; Royster and
305 others 2014), but the imperfect nature of organism identification by phenotypic traits,
306 even in professional laboratories using standard methods, must always be considered
307 when interpreting results. Several multiplex real-time PCR kits (that are not affected
308 by phenotypic variation) are commercially available for mastitis pathogen
309 identification, but they cannot yet be performed outside of professional laboratories,
310 making them unsuitable for on-farm pathogen identification.

311 The results reported here compare favourably to several previous reports of
312 test characteristics for similar on-farm culture test kits using non-diluted, frozen
313 clinical case samples. The sensitivity and specificity of the test kit for clinical cases
314 caused by *E. coli* reported here (67% and 92% respectively) are similar to those
315 reported for a different test kit in a Canadian study (71% and 89%, respectively)
316 (Wallace and others 2011). Positive coliform results must always be interpreted
317 carefully (particularly from sub-clinical samples where they are rarely isolated) as
318 there is always a risk that the bacteria have originated from contamination of the
319 sample rather than from a genuine IMI. The test characteristics of the test kit for
320 clinical cases caused by *S. aureus* (sensitivity: 83%, specificity: 94%) are also similar
321 to those reported previously using an alternative test kit (sensitivity: 69.6%,

specificity: 96%) (Wallace and others 2011). The sensitivity and specificity of the test kit for identification of *S. uberis* reported here (84% and 92% respectively) are better than those reported for identifying streptococci using an alternative test kit (specificity: 54%, specificity: 22%) (Wallace and others 2011). To the authors' knowledge, this is the first study that compares the results from a culture-based mastitis pathogen detection test kit with standard culture for CNS and *Enterococcus* spp.

For two pathogens (*S. agalactiae* and *S. dysgalactiae*) there were few positive samples (1 and 5 respectively), and *Klebsiella* spp. was never isolated by standard culture during the study. Therefore, it was not possible to assess the performance of the test kit for these pathogens. Stored, frozen milk samples (in which the pathogen is already known) could have been used to assess the test kit, and therefore pre-determine the number of each pathogen type assessed. However, this would not be representative of the prevalence on commercial UK farms, and would have introduced the risk of sample contamination during storage and handling and loss of viability of pathogens during long-term storage, making any results less reliable. Storing frozen milk samples for four weeks is unlikely to have any detrimental effect on pathogen viability, even without the use of preservative (Murdough and others 1996; Sol and others 2002).

Previous studies comparing on-farm culture-based test kits with standard culture have reported that test characteristics can change depending on the experience of the operator (McCarron and others 2009; Royster and others 2014). This effect was not assessed in the current study. Pathogen identification using the test kit is subjective, and therefore the characteristics are likely to vary depending on the experience of the operator.

We reported six instances of misidentification between *S. aureus* and CNS in the subset of data where a single pathogen was isolated in pure culture by both standard culture and the test kit but with disagreement on pathogen identity. The two *S. aureus* cultures misidentified as CNS were presumptively identified by standard culture on the basis of their DNAse and haemolytic activity (2/2), and biochemical reactivity (1/1; ID32 Staph, BioMérieux). Four staphylococcal isolates misidentified as *S. aureus* were presumptively identified by standard culture as CNS due to their lack of haemolytic (3/4) and DNAse activity (4/4). DNAse enzyme activity correlates well with coagulase activity (Boerlin and others 2003), and is used as an alternative to coagulase testing in the IDU. The test kit differentiates *S. aureus* from CNS by recording MSA activity alone. However, this single phenotypic test may be unreliable in differentiating *S. aureus* from CNS since rare CNS strains are MSA-positive. Furthermore, *S. aureus* strains are not consistently haemolytic, and rare *S. aureus* strains can test negative for DNAse, coagulase or MSA activity (Akineden and others 2011; Boerlin and others 2003; Kateete and others 2010). Such phenotypic variations may account for some of the discrepancies reported here.

Two enterococcal isolates (*E. faecalis* and *E. faecium*, identified by the laboratory using API 20 Strep (bioMérieux) were mistakenly identified as *S. uberis* when using the test kit. Growth on MSA and colony size at 24 hours are the phenotypic traits used by the test kit but these are not consistent across strains. Standard culture can more readily distinguish enterococci based on their morphology, growth on MacConkey agar, and biochemical properties. Enterococci are rarely a primary cause of IMI and are often causes of sample contamination.

A monoculture of *Bacillus* spp. was recovered from five milk samples using standard culture. Using the test kit, these five samples were reported as positive for

CNS (1), *Enterococcus* spp. (3) and *S. uberis* (1) monocultures. Given the pure cultures obtained, it is likely that *Bacillus* grew on Sectors 2 and 3 of the test kit. *Bacillus* spores are ubiquitous in the environment, and the few colonies recovered suggest that these are sample contaminants, stressing the importance of sterile sample collection technique if using the test kit system.

A. viridans is a Gram-positive catalase-negative coccus, morphologically and biochemically similar to *Enterococcus*, but an infrequent mastitis pathogen only occasionally identified in routine diagnostics (Pitkala and others 2004). In this study, monocultures of *A. viridans* were recovered from four samples using standard culture. Since *A. viridians* grows on Sectors 2 and 3 of the test kit (data not shown), the organism was misidentified as *S. aureus* (1), *S. uberis* (2) and *Enterococcus* spp. (1).

The significance of discrepant results depends on the decision made based on the result. For implementing a treatment programme as described above, identifying Gram-positive organisms as Gram-negative will lead to inappropriate withholding of antimicrobial therapy, though this occurred infrequently. In standard culture, routine Gram staining would eliminate this. Important management decisions (such as drying off a cow or quarter, or culling an animal from the herd) are often made when an animal is deemed to be chronically infected with contagious organisms that are difficult to treat, such as *S. aureus*. The results from this study suggest that standard culture (or a validated PCR test) remain the preferred pathogen identification tool for informing these decisions. Additional animal information (including SCC and clinical mastitis history, milk production, lactation number, etc.) should also always be considered in these cases.

In conclusion, use of the test kit is suitable for implementing targeted selective treatment of Gram-positive organisms in bovine mastitis under UK field conditions. The test reliability varies for each causative pathogen.

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Table 1

Farm background information from five herds that contributed clinical and sub-clinical mastitis milk samples for evaluation of a culture-based mastitis pathogen detection test kit.

Farm	Number milking cows	Average Days in Milk[a]	Average 305d milk yield[a]	3-month average bulk tank SCC[a]	Incidence of clinical mastitis[b]	Number clinical samples	Number subclinical samples
1	535	173	10730	156	44 cases	33	39
2	545	194	9020	237	52 cases	4	54
3	70	213	7985	290	72 cases	1	17
4	218	178	10392	320	64 cases	21	32
5	89	191	8603	315	75 cases	9	21

[a] At date of enrolment into the study

[b] The incidence of mastitis clinical was calculated for a 1-year period based on on-farm data records and expressed as cases/100 cows calving/year

509 **Table 2**

510 Sensitivity (Se), specificity (Sp), positive predictive values (PPV) and negative predictive values (NPV) of a culture-based mastitis pathogen detection

511 kit (‘*VetoRapid’*, Vétoquinol, Buckinghamshire, UK) for five common bacterial pathogens / pathogen groups, compared to standard laboratory culture.

Pathogen	**Sample type**	**Positive**	**Negative**	**TP**	**FN**	**TN**	**FP**	**Se** (95%CI)	**Sp** (95%CI)	**PPV**	**NPV**
Escherichia coli	Clinical	15	53	10	5	49	4	67 (43-91)	92 (85-99)	70%	91%
	SC	4	159	1	3	158	1	25 (0-72)	99 (97-100)	34%	98%
	Total	**19**	**212**	**11**	**8**	**207**	**5**	**58 (35-81)**	**98 (96-100)**	**72%**	**96%**
Coagulase-negative Staphylococci	Clinical	4	64	2	2	58	6	50 (1-99)	91 (84-98)	26%	97%
	SC	25	138	6	19	130	8	24 (7-41)	94 (90-98)	41%	88%
	Total	**29**	**202**	**8**	**21**	**188**	**14**	**28 (12-44)**	**93 (89-97)**	**37%**	**90%**
Staphylococcus aureus	Clinical	6	62	5	1	58	4	83 (53-100)	94 (88-100)	58%	98%
	SC	14	149	8	6	141	8	57 (32-82)	95 (91-99)	53%	96%
	Total	**20**	**211**	**13**	**7**	**199**	**12**	**65 (44-86)**	**94 (91-97)**	**52%**	**96%**
Streptococcus uberis	Clinical	13	55	12	1	52	3	92 (77-100)	95 (89-100)	81%	98%
	SC	6	157	4	2	142	15	67 (31-100)	90 (85-95)	22%	98%
	Total	**19**	**212**	**16**	**3**	**194**	**18**	**84 (67-100)**	**92 (88-96)**	**48%**	**99%**
Enterococcus spp.	Clinical	0	68	0	0	64	4	NA	94 (88-100)	NA	NA
	SC	12	151	2	10	140	11	17 (0-39)	93 (89-97)	15%	94%
	Total	**12**	**219**	**2**	**10**	**204**	**15**	**17 (0-39)**	**93 (90-96)**	**11%**	**96%**

512 SC: sub-clinical; TP: true positive; FN: false negative; TN: true negative; FP: false positive; CI: confidence interval

Table 3

Comparison of hypothetical selective treatment choices for 68 cases of clinical mastitis based on results from milk samples cultured by standard laboratory culture and by *'VetoRapid'* (Vétoquinol, Buckinghamshire, UK), a culture-based mastitis pathogen detection test kit. Antimicrobial therapy was assigned only when a Gram-positive bacterium was isolated.

Treatment outcome based on *'Vetorapid'* test kit	Treatment outcome based on standard laboratory culture		
	Antimicrobials	No antimicrobials	Total
Antimicrobials	21	10**	31
No antimicrobials	2*	35	37
Total	23	45	68

* plate result false negative for *Streptococcus uberis* (*S. uberis*) (1) and *S. dysgalactiae* (1).
** plate result false positive for *Enterococcus* spp. (4), CNS (2), *S. uberis* (2), *Staphylococcus aureus* (*S. aureus*) and *S. uberis* (1), *S. dysgalactiae* (1).

520 **Figures legends**

521

522 Figure 1. Two different *Staphylococcus aureus* isolates from mastitic milk samples
523 cultured on a pathogen detection kit (*'VetoRapid'*, Vétoquinol, Buckinghamshire,
524 UK). Both isolates are growing in Sector 2, a modified Mannitol-Salt agar which
525 supports the growth of organisms that tolerate high salt concentrations A: White
526 colonies with yellow discoloration of the agar correctly identified as *Staphylococcus*
527 *aureus.* B: White colonies with no discolouration of the agar incorrectly identified as
528 coagulase-negative staphylococci. The pathogens came from stored mastitis isolates
529 previously identified by the IDU laboratory as *Staphylococcus aureus* and were
530 cultured in parallel using standard laboratory culture and the test kit.