Evaluation of the University of Minnesota Tri-plate and 3M Petrifilm for the isolation of *Staphylococcus aureus* and *Streptococcus* species from clinically mastitic milk samples

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ABSTRACT

The primary objective was to compare microbiological results of the University of Minnesota Tri-plate and the 3M Petrifilm Staph Express (STX) Count Plate to standard culture techniques for identification of clinical mastitis caused by *Staphylococcus aureus*. The secondary objective was to evaluate the Tri-plate's ability to differentiate *Streptococcus* spp. from other gram-positive organisms. The tests were evaluated using clinically positive mastitic milk samples ($n = 282$) to determine their ability to diagnose the pathogens of interest. A Tri-plate was classified positive for *Staph. aureus* when at least 1 colony exhibiting β -hemolysis was present on the Factor media portion of the plate. When the plate was used in this manner and read by a trained laboratory technician, the sensitivity of the Tri-plate was 97.9% and the specificity was 81.8%. When the Tri-plate was evaluated by the laboratory technician for its ability to diagnose *Streptococcus* spp., both sensitivity and specificity of the test were very good (92.6 and 89.5%, respectively). Using the Petrifilm, samples were classified as positive for *Staph. aureus* if any red-violet colonies were present on the Petrifilm after an initial 24-h incubation. When used in this manner, the Petrifilm had a sensitivity of 97.4% and a specificity of 76.1%. Further evaluation of the Petrifilm was done using the STX disk, which was used to confirm the presence of *Staph. aureus*. When using the presence of 1 pink colony on the disk, the sensitivity of the Petrifilm was 92.1% and the specificity was 93.1%. Both the Tri-plate and the 3M STX Petrifilm successfully diagnosed *Staph. aureus* in clinical milk samples when used in a laboratory setting and the Tri-plate successfully differentiated *Streptococcus* spp. from other gram-positive organisms.

Key words: mastitis, culture, Tri-plate, Petrifilm Staph Express

INTRODUCTION

Mastitis is the most costly infectious disease in the dairy industry (Erskine et al., 2003) and as a result, much work has been done developing comprehensive control programs aimed at disease prevention. Even in situations where all preventive measures are carried out, there continues to be cases of clinical mastitis. The presence of these cases requires farms to incorporate diagnostic and treatment protocols into their overall mastitis control programs. These treatment protocols should be farm-specific and recognize the predominant pathogens present (LeBlanc et al., 2006). Knowing the pathogens present on a farm and in an individual cow will allow producers to make rational therapy decisions as well as implement specific control measures that would be best suited for the pathogen present. Continued surveillance will allow producers to detect the presence of new or emerging pathogens on the farm (Ruegg, 2003).

Rational use of antibiotics and appropriately targeted treatment strategies were the major foci of mastitis control programs (LeBlanc et al., 2006). Yet, therapy of clinical mastitis remains a topic of debate as no consistent approach to the early identification and treatment of clinical mastitis cases has been developed. Ideally, aseptically acquired milk samples obtained from each clinical case of mastitis would be cultured in a laboratory before making an individual cow treatment decision, but in many cases, and for various reasons, treatment decisions are made empirically. Leslie et al. (2002) identified the need for rapid, sensitive, specific, and inexpensive aids to categorize cases of IMI. Since then, various studies evaluated cow-side tests able to successfully categorize mastitis-causing pathogens into potential treatment categories (Ruegg, 2005; Lago et al., 2006; McCarron et al., 2009).

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Two tests with the ability to rapidly categorize clinically mastitic milk samples into broad treatment categories are the University of Minnesota Tri-plate and the 3M Petrifilm. Because of variation in growth requirements for some bacteria, on-farm culture systems could not effectively detect all pathogens associated with mastitis (Godden et al., 2007). The University of Minnesota Tri-plate is divided into 3 types of media. Factor medium is specific for gram-positive organisms, modified thallium sulfate-crystal violet-B toxin blood (MTKT) medium is specific for *Streptococcus* spp., and MacConkey medium is specific for gram-negative organisms. The Factor medium of the plate is primarily used for the identification of *Staph. aureus* and *Staphylococcus* spp. *Staphylococcus aureus* appeared as creamy, grayish, or golden colonies with a zone of complete hemolysis around the colony (Laboratory for Udder Health, 2000). Testing for the presence and type of hemolysis on blood agar-based plates represented a first simple and rapid method for the detection of *Staph. aureus* in milk samples (Lam et al., 1995). However, previous research showed that approximately 20 to 25% of the *Staph. aureus* isolates from bovine mastitis do not show detectable β -hemolysis activity in primary cultures, making this a specific but not very sensitive criterion for the identification of *Staph. aureus* (Boerlin et al., 2003).

The 3M Petrifilm Staph Express Count Plate (STX) is designed to be selective and differential for *Staphylococcus* spp. The Petrifilm produces results within 24 h $\pm$ 2 h of incubation. A further 3 h of incubation is required when the confirmatory STX Disk is applied (3M Microbiology, 2005). Rapid results may be preferred in situations where timely decisions are required, such as in on-farm culture programs (Silva et al., 2005). Many studies noted that sensitive and rapid cow-side diagnostic kits for the identification of *Staph. aureus* in milk could play an important role in the control of contagious mastitis (Hogan et al., 1986; Watts and Owens, 1988; Boerlin et al., 2003).

The primary objective was to compare microbiological results of the University of Minnesota Tri-plate and the 3M Petrifilm STX to standard culture techniques for identification of *Staph. aureus* in clinically mastitic milk samples. The secondary objective was to evaluate the Tri-plate's ability to differentiate *Streptococcus* spp. from other gram-positive organisms.

MATERIALS AND METHODS

Details of sample collection and processing, including laboratory procedures for gold standard testing, were presented in a previous paper (McCarron et al., 2009). A brief summary of the procedures is presented below.

Samples

Samples of clinically mastitic milk ($n = 282$) were collected from 21 dairy farms in Prince Edward Island. Samples with complete records ($n = 271$) were used for Tri-plate evaluation and a subset ($n = 213$) was used for evaluation of the Petrifilm STX plate. Producers were trained to recognize abnormal (mastitic) milk and aseptically collect all milk samples. Samples were never frozen, but were kept refrigerated and transported to the Atlantic Veterinary College for culture within 24 to 36 h of collection.

Gold Standard

Gold standard bacteriological cultures were performed according to the Laboratory Handbook on Bovine Mastitis (National Mastitis Council, 1987). Samples were classified as having significant growth if the growth was considered of "probable significance" or "highly significant" based on National Mastitis Council Guidelines for significance (National Mastitis Council, 1987). Disposable plastic loops were used to streak 10 μ L of each sample onto blood agar and MacConkey plates. Plates were incubated at 35°C for 24 h. The standard laboratory plates were read by a milk laboratory technician, and colonies were identified based on growth characteristics, morphology, pattern of hemolysis, catalase reaction, and Gram-staining properties. The tube coagulase test was used to differentiate *Staph. aureus* from other CNS species. Samples that grew a yeast or mold were classified as no bacterial growth. Samples that had 2 colony types were considered mixed growth and samples with 3 or more were considered contaminated. If samples exhibited mixed growth or contamination on gold standard culture, they were not used in the evaluation of the Tri-plate and Petrifilm.

Minnesota Easy Culture System II Tri-plate

The Minnesota Easy Culture System II Tri-plate, developed by the University of Minnesota's Laboratory for Udder Health (St. Paul, MN), is a culture plate that is divided in 3 sections. One section contains a proprietary Factor medium that is selective for gram-positive bacteria, 1 section contains MacConkey medium for the identification of gram-negative bacteria, and 1 section contains MTKT medium that is selective for *Streptococcus* spp. (Laboratory for Udder Health, 2000). The media were inoculated according to the manufacturer's recommendations. Sterile cotton-tipped swabs were saturated in milk and used to inoculate each section of the plate, re-dipping the swab in the sample before inoculating each section. Plates were incubated in a 35°C incubator for 24 h before being read.

The criterion used to classify a sample as positive for *Staph. aureus* was growth on the Factor media that exhibited β -hemolysis around at least 1 colony. One colony of *Staph. aureus* is considered significant by the National Mastitis Council, so plates were assigned to the *Staph. aureus* category if a single colony exhibiting β -hemolysis was present. If there was growth on the Factor medium that did not exhibit β -hemolysis, growth on either of the other media, or no growth on any media, the sample was assigned to the *Staph. aureus*-negative category. Each Tri-plate was read by a trained milk microbiology technician as well as 4 masked readers with limited microbiology experience to determine their ability to detect *Staph. aureus* by identifying β -hemolysis on Factor media.

Tri-plates were evaluated for their ability to diagnose *Streptococcus* spp. Plates were considered positive for *Streptococcus* spp. if there was colony growth (at least 1 colony) on the MTKT media. Again, each Tri-plate was read by a trained milk microbiology technician as well as 4 masked readers with limited microbiology experience.

3M Petrifilm Staph Express

The second media system used was the 3M Petrifilm STX. The Petrifilm STX is a sample-ready culture medium system, which contains a cold-water-soluble gelling agent. The chromogenic, modified Baird-Parker medium in the plate is selective and differential for *Staph. aureus*. Red-violet colonies on the plate are *Staph. aureus* (3M Microbiology, 2005). In cases where the color of the colonies was not easily identified or when colonies other than red-violet were present on the plate, the 3M Petrifilm STX disk may be used to identify *Staph. aureus*. The STX disk contained a dye and DNA. *Staphylococcus aureus* produces DNase that reacts with the dye to form pink zones. Milk samples were diluted 1:10 with sterile water, a 1-mL aliquot was plated on each STX Petrifilm, and plates were incubated at 35°C for 24 h. Colony growth, number of colonies present, and color of colonies were recorded by the laboratory technician. Initial test characteristics (sensitivity, specificity, and predictive values) were calculated based solely on the presence of red-violet colonies as per the manufacturer's interpretation criteria. Subsequently, all Petrifilms that were positive for growth (at least 1 colony) had the STX disk inserted into the plate and were re-incubated at 37°C for 3 h ($n = 123$). Plates that exhibited at least 1 colony with a pink zone were classified positive for *Staph. aureus*. Pink colonies were then picked and re-grown using standard methods to confirm their identity.

All Petrifilms were read by the 4 masked readers and the laboratory technician; however, after the STX disks were applied, plates were read only by the laboratory technician; therefore, no inter-reader comparisons were made.

Statistical Analysis

All results were analyzed using Intercooled Stata 9 (StataCorp, 2005). For assessment of the Tri-plate system's ability to identify *Staph. aureus* and *Streptococcus* spp., sensitivity (**Se**), specificity (**Sp**), positive predictive value (**PPV**), and negative predictive value (**NPV**) were calculated by comparing the Tri-plate classifications to the gold standard results. The Petrifilm STX was evaluated in the same manner to determine its ability to diagnose *Staph. aureus*.

RESULTS

Samples

A total number of 282 fresh milk samples were received from producers. Three samples had incomplete records and 18 samples were removed from the analysis because they exhibited mixed or contaminated growth on the gold standard results, leaving 261 samples for Tri-plate analysis. Gold standard culture results for each sample used in the analysis are in Table 1. The prevalence of *Staph. aureus* used to evaluate the Tri-plates was 18.0% and the prevalence of all *Strep.* spp. combined was 30.7%.

In total, 213 samples had complete records on the Petrifilm STX. After 6 wk of sample collection, the laboratory technician noted that 1 mL of undiluted milk plated on the Petrifilms resulted in some plates that were difficult to interpret (milk clots and cases of heavy bacterial growth resulted in difficulty identifying individual colonies). At that point, a second series of Petrifilms with a 1 in 10 dilution (equivalent to 100 μ L of milk and 900 μ L of sterile diluent on the film) was included. There were 64 samples received at the beginning of the collection period that were not diluted, leaving 213 samples with complete records. Sixteen were not included in the final analysis, because they were contaminated on the gold standard culture. Previous research evaluating Petrifilm Aerobic Count and Coliform Count performance using diluted versus undiluted milk showed no difference in Se and NPV, but statistically higher Sp and PPV when diluted milk was used (McCarron et al., 2009). Wallace et al. (2008) found test characteristics of the STX were the highest for diluted (1 in 10) fresh samples from clinically mastitic

Table 1. Gold standard microbiology results of samples used to evaluate the University of Minnesota Tri-plate and the 3M Petrifilm Staph Express

Gold standard result	Samples used for Tri-plate evaluation	Percentage	Samples used for Petrifilm evaluation	Percentage
No bacterial growth	85	32.6	61	30.1
<i>Staphylococcus aureus</i>	47	18.0	38	19.3
<i>Streptococcus uberis</i>	43	16.5	36	18.3
<i>Escherichia coli</i>	27	10.3	18	9.1
<i>Streptococcus</i> spp.	18	6.9	11	5.6
<i>Streptococcus dysgalactiae</i>	18	6.9	13	6.6
CNS spp.	6	2.3	5	2.5
<i>Staphylococcus</i> spp.	4	1.5	4	2.0
<i>Pasteurella multocida</i>	3	1.2	3	1.5
<i>Staphylococcus hyicus</i>	3	1.2	3	1.5
<i>Klebsiella</i> spp.	2	0.8	2	1.0
<i>Streptococcus bovis</i>	1	0.4	0	0
<i>Bacillus</i> spp.	1	0.4	0	0
<i>Corynebacterium</i> spp.	1	0.4	1	0.5
<i>Serratia</i> spp.	1	0.4	1	0.5
<i>Enterobacter</i> spp.	1	0.4	1	0.5
Total	261	100	197	100

cows. For these reasons, only diluted milk samples were included in the analysis. Table 1 illustrates the gold standard culture results for each of the samples used to determine test characteristics of the STX Petrifilm. The prevalence of *Staph. aureus* in the samples used to evaluate the Petrifilm was 19.3%.

Minnesota Easy Culture System II Tri-plate

Table 2 illustrates the test characteristics of the Tri-plate when read by the laboratory technician. The Tri-plate was highly sensitive because only 1 sample that was positive for *Staph. aureus* on the gold standard was not diagnosed using the Tri-plate. The reduced Sp was the result of 39 samples being classified as *Staph. aureus* by the Tri-plate that were not diagnosed as *Staph. aureus* by the gold standard methods. Most (12) of these samples had no bacterial growth on the gold standard, the remaining samples included 9 *Streptococcus uberis*, 7 *Streptococcus* spp., 5 *Streptococcus dysgalactiae*, 5 *Escherichia coli*, and 1 *Staphylococcus* spp.

Table 3 shows the test characteristics of the Tri-plates when read by the 4 masked readers who used a threshold of 1 colony that exhibited β -hemolysis on Factor media to consider a plate positive for *Staph. aureus*. The Se of the Tri-plate was lower when read by the readers (43.2 to 59.1%) with limited microbiology training than when read by the technician. The level of agreement among the readers was moderate, with the calculated kappa value (actual agreement beyond chance) being 0.51. The Sp of the test was higher with the inexperienced readers, ranging from 93.8 to 95.9%. All test characteristics calculated were similar between the readers with no significant differences observed.

Table 4 shows the test characteristics of the Tri-plate when evaluated by the laboratory technician for

its ability to diagnose a *Streptococcus* sp. Sensitivity and Sp of the test were 92.6 and 89.5%, respectively. Six samples that were diagnosed as *Streptococcus* spp. using the gold standard did not grow on the MTKT media. Nineteen samples that exhibited growth on the MTKT medium were not diagnosed as *Streptococcus* spp. using the gold standard. On the gold standard, 9 of the 19 were diagnosed no growth, 5 *Staph. aureus*, 3 *E. coli*, 1 *Corynebacterium* spp., and 1 *Staphylococcus* spp. The same masked readers evaluated the Tri-plate for the presence of *Streptococcus* spp. The sensitivities of the Tri-plate, when read by these readers, ranged from 77.8 to 88.9% and specificities from 83.7 to 92.7% with a combined kappa value of 0.81.

3M Petrifilm Staph Express

Samples were classified as positive for *Staph. aureus* if any red-violet colonies were present on the Petrifilm after the initial 24 h incubation. Test characteristics and predictive values are presented in Table 5. When used in this manner the Petrifilm was highly sensitive (97.4%) but not very specific (76.1%). Only 1 sample that was diagnosed *Staph. aureus* using the gold standard did not show growth of red-violet colonies. There were 38 samples that grew red-violet colonies, but were

Table 2. Test characteristics of the University of Minnesota Tri-plate when read by an experienced technician based on its ability to correctly classify samples as *Staphylococcus aureus*

Test characteristic	Value (95% confidence interval)
Sensitivity, %	97.9 (96.1–99.6)
Specificity, %	81.8 (77.1–86.5)
Positive predictive value, %	54.1 (48.1–60.2)
Negative predictive value, %	99.4 (98.5–100)

Table 3. Test characteristics of the University of Minnesota Tri-plate based on its ability to correctly classify samples as *Staphylococcus aureus* when read by masked readers with limited microbiology training

Test characteristic	Value (95% confidence interval)			
	Reader A	Reader B	Reader C	Reader D
Sensitivity, %	51.1 (45.0–57.3)	43.2 (37.1–49.3)	54.6 (48.4–60.7)	59.1 (52.9–65.3)
Specificity, %	93.8 (90.9–96.8)	95.2 (92.6–97.9)	94.8 (92.0–97.5)	95.9 (93.4–98.4)
Positive predictive value, %	63.9 (58.0–69.8)	65.5 (59.7–71.4)	68.6 (62.9–74.3)	76.5 (71.1–81.8)
Negative predictive value, %	90.0 (86.3–93.6)	88.8 (85.0–92.3)	90.9 (87.3–94.4)	91.3 (87.7–94.8)

not classified *Staph. aureus* using the gold standard culture. Eight of these samples showed no growth on the gold standard culture, 16 were *Streptococcus* spp., 7 were *Staphylococcus* spp., 6 were gram-negative coliforms, and 1 was *Corynebacterium bovis*.

Table 5 illustrates the test characteristics of the Petrifilm STX after the application of the STX disk and reincubation for 3 h. All plates were read only by the laboratory technician, and the presence of 1 pink colony was used classify a sample as positive for *Staph. aureus*. The Petrifilm STX with disk applied was highly sensitive (92.1%) because only 3 of the 38 samples that were positive for *Staph. aureus* on the gold standard were not detected using the STX Petrifilm. Two of the 3 samples did not show any visible growth on the Petrifilm and the remaining sample did show colony growth, but was not confirmed pink using the disk. The Sp of the test was significantly increased when the STX disk was used (from 76.1 to 93.1%). This was the result of only 11 samples being classified as *Staph. aureus* by the Petrifilm STX with disk applied that were not diagnosed as *Staph. aureus* using the gold standard. Each of the 11 samples had the pink colonies picked from the plate and recultured using standard methods. Five were confirmed *Staph. aureus*, 4 were *Staphylococcus* spp., 1 *Staph. hyicus*, and 1 yielded no growth. Had the 5 samples that were diagnosed as *Staph. aureus* on subsequent standard culture methods been diagnosed by the original gold standard culture the Se of the Petrifilm would be increased to 93.0% and the PPV would increase to 87.0%.

When based on results of the STX Petrifilm using the presence of red-violet colonies to diagnose *Staph. aureus*, the apparent prevalence was 38.1% and when the diagnosis was based on confirmation by the STX disk, the apparent prevalence of *Staph. aureus* was 23.4%. The application of the disk did not result in any new samples being identified as *Staph. aureus* positive.

DISCUSSION

A complete mastitis control program should include the use of microbiologic analysis of individual milk

samples to determine which mastitis pathogens are present on the farm (Ruegg, 2003). Having on-farm access to accurate, rapid microbiological tests that are capable of diagnosing some of the most prevalent mastitis pathogens may facilitate the incorporation of this valuable data into mastitis control programs.

When implementing preventative strategies for mastitis, information about the prevalence of specific pathogens is useful (Makovec and Ruegg, 2003). The National Mastitis Council Recommended Mastitis Control Program suggests milking cows with confirmed contagious IMI last and marketing or permanently segregating cows that are persistently infected with *Staph. aureus* or other nonresponsive microbial agents. Confirming the presence of *Staph. aureus* quickly and accurately may allow better execution of segregation strategies. For producers making decisions on treatment protocols (routine, extended therapy, withholding treatment, and culling), identification of *Staph. aureus* is of value (Sol et al., 2000).

Samples

The 2 mastitis-causing pathogens that are the focus here are *Staph. aureus* and *Streptococcus* spp. Both of these pathogens are considered significant mastitis-causing organisms by the National Mastitis Council (National Mastitis Council, 1987) and are 2 of the most prevalent pathogens encountered. For epidemiologic monitoring and investigation of risk factors for prevention of cases, identification of the type of gram-positive organism (*Streptococcus* spp. or *Staph. aureus*) is important. *Streptococcus* spp. were isolated from 12.2%

Table 4. Test characteristics of the University of Minnesota Tri-plate when read by an experienced technician based on its ability to correctly classify samples as *Streptococcus* spp.

Test characteristic	Value (95% confidence interval)
Sensitivity, %	92.6 (89.4–95.8)
Specificity, %	89.5 (85.8–93.2)
Positive predictive value, %	79.8 (74.9–84.7)
Negative predictive value, %	96.4 (94.2–98.7)

Table 5. Test characteristics of the 3M Staph Express Petrifilm when read by an experienced technician using the presence of red-violet colonies on initial culture to classify samples as *Staphylococcus aureus* versus using the confirmatory disk

Test characteristic	Value (95% confidence interval)	
	Red-violet colonies	Staph Express disk
Sensitivity, %	97.4 (95.1–99.6)	92.1 (88.3–95.9)
Specificity, %	76.1 (70.2–82.1)	93.1 (89.5–96.6)
Positive predictive value, %	49.3 (42.4–56.3)	76.1 (70.1–82.0)
Negative predictive value, %	99.2 (97.9–100)	98.0 (96.1–100)

of milk samples submitted to the Wisconsin Veterinary Diagnostic Laboratory and *Staph. aureus* were isolated from 9.7% of samples (Makovec and Ruegg, 2003). More recently, a Canadian prevalence study of clinical mastitis found 12.5% of samples submitted were positive for *Streptococcus* spp. and 10.3% positive for *Staph. aureus* (Olde Riekerink et al., 2008). Here, *Streptococcus* spp. were the most prevalent species cultured. Of the 261 samples used to evaluate the Tri-plate, 30.7% grew *Streptococcus* spp. on the gold standard culture. *Staphylococcus aureus* was the second most prevalent organism cultured on the gold standard, with a prevalence of 18.0%. In the 197 samples used to evaluate the Petrifilm, 30.5% were diagnosed as *Streptococcus* spp. on the gold standard and 19.3% were diagnosed as *Staph. aureus*.

Minnesota Easy Culture System II Tri-plate

The Tri-plate culture system was designed to identify some of the most common pathogens infecting the bovine udder (Bey and Farnsworth, 2000). Godden et al. (2007) evaluated the system in laboratory and field studies for its ability to differentiate growth from no growth, gram-positive from gram-negative and growth of *Staphylococcus* versus *Streptococcus* spp. They found the system accurate and attained a high level of agreement (kappa values between 0.80 and 0.93) when used for those 3 purposes. However, when users tried to differentiate pathogen groups further or to identify specific pathogens (*Staph. aureus* vs. CNS spp.) much lower agreement was observed. When the University of Minnesota Bi-plate (which uses Factor and MacConkey media) was evaluated for its ability to differentiate growth from no growth and gram-positive from gram-negative organisms, the test was highly sensitive (97.9%; McCarron et al., 2009). Similarly, agreement among readers was high (kappa = 0.76) when asked to differentiate the above categories. In the current research, when readers were asked to identify *Staph. aureus* on the Tri-plate, in concurrence with Godden et al. (1997), the agreement among readers was low (kappa = 0.51).

β -Hemolysis represents an important criterion for rapid presumptive identification of *Staph. aureus* in primary cultures (Boerlin et al., 2003). Of the 3 coagulase-positive *Staphylococcus* spp. regularly encountered in clinical milk samples, *Staph. aureus* was the only one with hemolytic activity (Lam et al., 1995). Yet, 20 to 25% of the *Staph. aureus* isolates from bovine mastitis did not present detectable β -hemolysis in primary cultures. The proportion of β -hemolytic *Staph. aureus* found in bovine mastitis varies from region to region (Larsen et al., 2002). Therefore, the performance of a test that uses hemolysis as part of the diagnostic criteria may vary with regards to the population under investigation (Boerlin et al., 2003). In this study, the Tri-plate was highly sensitive (97.9%) with moderate Sp (81.8%) when used by a laboratory technician to diagnose the presence of *Staph. aureus*. But, when read by masked readers, the sensitivities were much lower (ranging from 43.2 to 59.1%). In contrast, the Sp of the test was higher for the inexperienced readers, ranging from 93.8 to 95.9%, possibly because these readers were more reluctant to call a colony positive, resulting in fewer false positives. Similar work by Lam et al. (1995) used the presence or absence of β -hemolysin production as a method to diagnose *Staph. aureus*. Plates were read by an experienced observer and 45 out of 54 samples were correctly identified, resulting in a Se of 83% and a Sp of 98%. Predictive values of the test were calculated at various levels of prevalence. At a prevalence of 21% (the median prevalence among the herds in their study) the PPV was 91% and the NPV was 96%.

When done by the inexperienced readers, observation of hemolysis resulted in a highly specific test with very high negative predictive values. Farm-based users of the Tri-plate in this manner could be confident in detecting animals that are negative for *Staph. aureus* and, depending on the prevalence in the herd, could be very confident in a negative test result. Unfortunately, if this test were used to detect positive cows for segregation from the herd, a user without extensive microbiological experience would miss several positive animals. The very high Se obtained by the laboratory technician indicates that with thorough training, the

Tri-plate can be used to identify animals truly infected with *Staph. aureus* in a farm situation.

Tri-plates were evaluated for their ability to diagnose *Streptococcus* spp. based on the presence of colony growth on the MTKT media. When read by the technician, both the Se and Sp of the test were high (92.6 and 89.5%, respectively). The lower Sp was the result of 19 samples showing growth on the MTKT media that were not diagnosed as *Streptococcus* spp. on the gold standard. Almost half (9) of the samples showed no growth on the gold standard. This may be due to a larger inoculum of milk used on the Tri-plate (one saturated cotton swab for one-third of the plate versus the 10- μ L loop used for the gold standard). Five samples that were diagnosed as *Staph. aureus* on the gold standard exhibited colony growth on MTKT and were therefore considered *Streptococcus* spp.-positive. The presence of 1 colony of *Staph. aureus* in the gold standard, regardless of the presence of other colony types, resulted in a diagnosis of *Staph. aureus*. The MTKT-positive samples may have been mixed *Streptococcus* spp. and *Staph. aureus* infections.

3M Petrifilm Staph Express

The Petrifilm STX plate was designed to provide rapid results for the diagnosis of *Staph. aureus* after 24 ± 2 h of incubation when red-violet colonies are present. The Petrifilm STX Disk should be applied to the plate and reincubated for 3 h whenever colonies other than red-violet are present on the plate; for example, black or blue-green colonies (3M Microbiology, 2005). The manufacturer's interpretive criteria suggest that the appearance of red-violet colonies on the initial incubation is presumptive evidence for the diagnosis of *Staph. aureus*. Results from Silva et al. (2005) did not support this recommendation and found it necessary to use the Staph Express Disk for confirmation even when red-violet colonies were the only ones present. They found that the apparent prevalence of *Staph. aureus* in milk samples processed using Petrifilm was significantly greater than the prevalence in milk samples processed using standard microbiological techniques. The results of the current study show that the STX Petrifilm had a higher Se when using only the presence of red-violet colonies than when the presence of pink colonies on the Staph Express Disk was used, 97.4 versus 92.1%. In the population of samples that were used in this study, only 38 were diagnosed as *Staph. aureus* on the gold standard. The difference in sensitivities was due to the difference in classification of only 2 samples; therefore, this difference may be of little biological significance. The high sensitivities obtained in both methods of evaluation

indicate that using the STX Petrifilm resulted in very few false-negative classifications. This is an important attribute for a test to have if it were being used as part of a mastitis control program because the occurrence of false-negative results could result in the maintenance of infected animals within a herd (Silva et al., 2005). The Sp of the STX Petrifilm was greatly improved by the use of the STX disk (from 76.1 to 93.1%). Results from Silva et al. (2005) showed that the STX Petrifilm was highly specific (98.5%) when the disk was used. In the current study, the Sp of using the presence of red-violet colonies was decreased because 38 samples that were diagnosed as *Staph. aureus* using the Petrifilm were not *Staph. aureus* on the gold standard. These results show that a variety of organisms other than *Staph. aureus* will grow red-violet on the STX Petrifilm; therefore, caution should be used when using this test without the confirmatory disk, especially if segregation or culling decisions are made based on the results. The application of the STX disk did not improve the Se of the test as the manufacturer suggests, rather the Sp of the test was greatly improved.

In the case of β -hemolysis on the Tri-plate, where previous experience of the reader would potentially affect the outcome of the test, readers with extensive experience and inexperienced readers were used. Our findings, along with results of other studies that have evaluated potential on-farm tests for mastitis, support the need for thorough training of individuals reading the tests. Previously published work also recommends that periodic assessment of accuracy of on-farm methods by submission of duplicate samples to a microbiology laboratory be carried out (Silva et al., 2005).

When the 2 tests were read by the laboratory technician, there was no significant difference in the detection of *Staph. aureus* (Se). The specificities of the 2 tests were similar when the presence of red-violet colonies only was used to assess the Petrifilm, but with the application of the STX disk, the Petrifilm was a more specific test than the Tri-plate.

CONCLUSIONS

Both the University of Minnesota Tri-plate and the 3M STX Petrifilm were able to successfully detect *Staph. aureus* in clinical milk samples when used in a laboratory setting. The presence of β -hemolysis on the Tri-plate was a highly sensitive method to diagnose *Staph. aureus* when read by a trained laboratory technician. However, when read by individuals with limited microbiology experience, the Se of the test was much lower. Specificity of the Tri-plate system was higher when read by the inexperienced readers than by the

laboratory technician. The Tri-plate was able to successfully differentiate *Streptococcus* spp. from other gram-positive organisms.

The 3M Petrifilm was very sensitive when used to diagnose the presence of *Staph. aureus*. When using the presence of red-violet colonies only to diagnose a sample positive with *Staph. aureus*, the Se and Sp of the Petrifilm were similar to those of the Tri-plate. The Sp of the Petrifilm was increased significantly by using the STX confirmatory disk.

To determine their suitability as on-farm tests for specific mastitis-causing pathogens, each of these tests should be evaluated in field situations. Training of individuals performing and reading the tests will be key to their success on the farm. For quality control purposes, samples taken on the farm should be saved and submitted to a diagnostic laboratory periodically to assess the ongoing accuracy of any on-farm culture system.

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