Laboratory evaluation of 3M Petrifilms and University of Minnesota Bi-plates as potential on-farm tests for clinical mastitis

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ABSTRACT

The objective was to determine test characteristics and compare 2 potential on-farm culture systems for clinical mastitis, the Minnesota Easy Culture System II Bi-plate and Petrifilm. The tests were evaluated using clinically positive mastitic milk samples ($n = 282$) to determine their ability to differentiate appropriate treatment groups; all cases that had gram-positive growth were considered treatment candidates ($n = 161$), whereas cases that grew gram-negative organisms only or yielded no bacterial growth were classified as no treatment ($n = 121$). For Petrifilm, both undiluted and 1:10 diluted milk samples were used. To create treatment categories, 2 types of Petrifilms were used, Aerobic Count (AC) and Coliform Count (CC). Both Bi-plates and Petrifilms were read after 24 h of incubation. Analysis was conducted at various colony count thresholds for the Petrifilm test system. The combination of Petrifilms that had the highest sensitivity classified a case as gram-negative if there were ≥ 20 colonies present on the CC. If there were < 20 colonies present on the CC and > 5 colonies present on the AC, a case would be classified as gram-positive. The Bi-plate had a sensitivity of 97.9% and a specificity of 68.6%. The Petrifilm test system had a sensitivity of 93.8% and a specificity of 70.1%. There was no significant difference in the sensitivities between the tests. All Bi-plates and Petrifilms were read by a laboratory technician and a group of masked readers with limited microbiology training. Kappa values for the masked readers were 0.75 for Bi-plates and 0.84 and 0.86 for AC and CC Petrifilms, respectively. The Bi-plate and Petrifilm were able to successfully categorize clinical cases of mastitis into 2 treatments based on their ability to detect the presence of a gram-positive organism. Neither method had the ability to determine if a sample was contaminated. The results of this study indicate that both tests were able to appropriately categorize cases, which could poten-

tially result in a reduction in the quantity of antibiotics used to treat clinical cases of mastitis.

Key words: mastitis, culture, Bi-plate, Petrifilm

INTRODUCTION

Mastitis remains the most costly infectious disease to the dairy industry and is the most frequent cause of antibacterial use on dairy farms (Ersikine et al., 2003). A recent comprehensive study of clinical mastitis on Canadian dairy farms found the mean incidence rate of clinical mastitis at 23.0 cases per 100 cow-years (Olde Reikerink et al., 2008).

In Canada, mastitis treatment accounts for more than half of all antibiotics used by dairy producers (Leger et al., 2003). Much of the antibiotics used to treat clinical mastitis may not be justified because of high self-cure rates and low efficacy of treatments against certain pathogens. For example, the use of intramammary antimicrobials for the treatment of *Escherichia coli* clinical cases was most often ineffective because of the short duration of infections and high spontaneous cure rates (Smith et al., 1985; Hogan and Smith, 2003). There are public concerns that the overuse of antibiotics in agriculture may lead to antimicrobial resistance in humans (Health Canada, 2003). For these reasons, the judicious use of antibiotics by veterinarians and producers continues to be emphasized throughout the dairy industry.

Clinical mastitis is caused by a wide range of bacteria. When designing a treatment regimen, having information on the causative organism to choose an antimicrobial with an appropriate spectrum of activity is important (Constable and Morin, 2003). Still, when a dairy producer encounters a clinical case of mastitis, many times a diagnosis of the causative organism is not obtained before treatment. One reason for not obtaining this information is the delay that exists between sampling an affected quarter and receiving results from a diagnostic laboratory. If producers experience delays in receiving results, they may choose to treat empirically rather than wait for a culture result (Neiser et al., 2006).

Received August 27, 2008.

Accepted December 18, 2008.

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A recent Canadian study reported that 43.9% of 3,033 milk samples submitted from cows with clinical mastitis yielded no bacterial growth (Olde Reikerink et al., 2008). These negative results may be due to spontaneous clearance of pathogens, cyclical shedding of *Staphylococcus aureus*, phagocytosis of bacteria within the milk sample, and the presence of too few bacterial colonies to yield a diagnosis (Silva et al., 2005). In these situations, the treatment with an antimicrobial may not be warranted. Additionally, even if a milk sample is positive for a pathogen, the characteristics of the pathogen must be considered before making a treatment decision (Constable and Morin, 2003).

There is a need for a more targeted approach to clinical mastitis therapy (Keefe and Leslie, 1997). One method to target therapy is to use an on-farm culture system. By using an on-farm system, producers would be able to make informed mastitis treatment decisions that may lead to the reduction in the use of antimicrobials. On-farm tests that are rapid, sensitive, and have high negative predictive values (NPV) would be valuable tools for producers. Evidence that on-farm testing could be used to make clinical mastitis therapy decisions was described by Leslie et al. (2002). Selective growth media may be useful on-farm tools for determining the major pathogen categories that cause mastitis, thus enabling a producer to choose an appropriate treatment. Many dairy farms in the United States are large enough to set up and maintain milk culturing laboratories. The results generated from these on-farm laboratories could be used to develop treatment protocols and monitor long-term mastitis situations (Sears and McCarthy, 2003). Godden et al. (2007) reiterated the need for the adoption of rapid on-farm culture systems that would allow producers to make informed treatment decisions. By treating fewer cases with antibiotics and discarding less milk that contains residues, the cost per case of mastitis was reduced (Silva et al., 2004). Tests that will play a part in reducing the economic impact of mastitis, as well as promote the prudent use of antimicrobials on dairy farms, need to be validated (Godden et al., 2007).

There has been a perceived increase in the adoption of on-farm culture systems for the selective treatment of clinical mastitis on dairy farms in the United States (Lago et al., 2006). In Canada, the demographics of the dairy industry are different than in the United States. The average dairy herd size in Canada in 2007 was 68 cows (Canadian Dairy Information Centre, 2008), whereas the average US herd in 2007 had 125 cows (Miller et al., 2007). The size of a herd may play a role in the uptake and type of on-farm test system that is suitable.

The Minnesota Easy Culture System II Bi-plate (University of Minnesota Laboratory for Udder Health, St. Paul) and 3M Petrifilm (3M Microbiology, St. Paul, MN) tests both have the capability of distinguishing samples with no bacterial growth, gram-positive infections, and gram-negative infections. The aim was to evaluate, in a laboratory setting, the ability of the tests to categorize milk samples from clinical mastitis cases into potential treatment categories.

MATERIALS AND METHODS

Samples

A convenience sample of 21 farms located close to the Atlantic Veterinary College (Charlottetown, Prince Edward Island, Canada) was recruited. Milk samples ($n = 282$) were collected by trained dairy producers from cows with clinical mastitis. Clinical mastitis was defined as milk that appeared abnormal with or without other local or systemic signs. Samples were collected after routine teat preparation using the technique described by (National Mastitis Council, 1999). Samples were refrigerated on the farm, never frozen, and picked up daily by technicians. The total time elapsed between farmer sample collection and laboratory culture did not exceed 36 h and was typically <24 h.

Gold Standard

Gold standard bacteriological cultures were performed according to the Laboratory Handbook on Bovine Mastitis (National Mastitis Council, 1999). 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 additional tests were conducted to confirm the organism present in each case. Results of these cultures were then used to assign cases to 2 treatment categories. The treatment category consisted of all samples that had significant growth of a gram-positive organism only, whereas the no-treatment category consisted of all samples that resulted in no significant microbial growth or growth of a gram-negative organism only. Samples that grew a yeast or mold (no bacterial growth) were included in the no-treatment category. Samples that had 2 colony types were considered mixed growth and samples with 3 or more were considered contaminated. Mixed-growth and contaminated samples were not used

in the decision models; however, the impact of these samples was evaluated.

3M Petrifilm

The second media system used was the 3M Petrifilm Aerobic Count (**AC**) and the 3M Petrifilm Coliform Count (**CC**). The Petrifilm AC plate is a ready-made culture medium that is used for counting aerobic bacteria. It contains standard methods nutrients, a cold-water gelling agent, and an indicator dye that facilitates colony counting (3M Microbiology, 2005). The Petrifilm CC plate contains violet red bile nutrients, a gelling agent, and an indicator dye that facilitates colony counting. The top film traps gas produced by lactose-fermenting coliforms; gas trapped around red colonies indicates confirmed coliforms (3M Microbiology, 2005). A 1-mL aliquot of each sample was plated on the Petrifilm AC and CC plates. In addition, milk samples were diluted 1:10 with sterile water and plated on 2 more Petrifilm AC and CC plates. All plates were incubated at 35°C for 24 h. Petrifilms were used in combination to create treatment categories of no growth, gram-positive growth only, and gram-negative growth only. Each Petrifilm was read by the technician and categorized as positive if there were 20 or more colonies present. Colony growth on both the AC and CC Petrifilms was classified as gram-negative and assigned to the no-treatment category. Colony growth on only the AC Petrifilm was classified as a gram-positive and assigned to the treatment category. If there were <20 colonies on both Petrifilms, the sample was categorized as no treatment.

Further evaluation of the Petrifilms was carried out to determine the effect of different colony count threshold values on the Petrifilm test system's sensitivity (**Se**) and specificity (**Sp**). This was done using the results from only the diluted sample Petrifilms. To evaluate the effects of different cut-off values, the technician's results were first compared with those of the automated 3M Petrifilm reader to determine the agreement between the 2 reading methods. Because the level of agreement for each type of Petrifilm was very high, the results of the automated reader were used to determine test **Se** and **Sp** at various colony-forming unit thresholds.

Minnesota Easy Culture System II Bi-Plate

The Minnesota Easy Culture System II Bi-plate, developed by the University of Minnesota Laboratory for Udder Health (St. Paul, MN) is a culture plate that is divided in half. One half contains a proprietary Factor medium that is selective for gram-positive bacteria and the other half contains MacConkey medium for the

identification of gram-negative bacteria (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 swab one half of the plate, then re-dipped in the sample to swab the other half. Plates were incubated in a 35°C incubator for 24 h before being read. Depending on which side of the Bi-plate was positive for growth, the Bi-plates were assigned to a treatment category. Growth (1 or more colonies) on the Factor medium only was considered gram-positive growth and assigned to the treatment category. Growth (1 or more colonies) on the MacConkey medium only was considered gram-negative and assigned to the no-treatment category. If there was growth on both media of the Bi-plate, the plate was considered contaminated, but due to the presence of gram-positive organisms these plates were assigned to the treatment category. Finally, if there was no growth on either media, the sample was assigned to the no-treatment category.

Interreader Agreement

All Petrifilms were read by a milk laboratory technician in addition to 3 masked readers with limited microbiology training to determine the interreader agreement beyond chance (κ). Readers were asked to identify the presence of colony growth and if positive, to record the colony count as greater or fewer than 20 colonies. Bi-plates were read by the same laboratory technician and 4 masked readers. All readers were asked to identify the presence of bacterial colonies (1 or more) and which side of the plate the growth was on. Interreader agreement was assessed by calculating pooled κ statistics for both test systems.

Statistical Analysis

All results were analyzed using Intercooled Stata 9 (StataCorp, 2005). The Petrifilm test system (AC and CC combined) was first evaluated to determine the performance of the test on undiluted versus diluted milk samples. The Petrifilm treatment decision results were compared with those of the gold standard to determine the **Se**, **Sp**, positive predictive value (**PPV**), and NPV of the Petrifilm test system.

Data obtained by the technician and the automated reader were assessed using McNemar's test for bias before calculating κ statistics for both the AC and CC Petrifilms. Because the level of agreement for each type of Petrifilm was very high, the results of the automated reader were used to create **Se** versus **Sp** plots (also called 2 graph receiver operating characteristics) to evaluate test **Se** and **Sp** at various colony-forming unit thresholds.

Table 1. Gold standard microbiology results of samples submitted to be evaluated by Bi-plates and Petrifilms

Gold standard result	Samples submitted for Bi-plate evaluation ¹		Undiluted samples submitted for Petrifilm evaluation ²		Diluted samples submitted for Petrifilm evaluation ³	
	n	%	n	%	n	%
No bacterial growth	86	30.7	86	31.3	61	28.6
<i>Staphylococcus aureus</i>	47	16.8	47	17.1	38	17.8
<i>Streptococcus uberis</i>	42	15.0	41	14.9	36	16.9
<i>Escherichia coli</i>	27	9.6	26	9.5	18	8.5
<i>Streptococcus</i> spp.	19	6.8	18	6.5	13	6.1
<i>Streptococcus dysgalactiae</i>	18	6.4	17	6.2	11	5.2
Contaminated or mixed growth	18	6.4	17	6.2	16	7.5
<i>Staphylococcus</i> spp.	10	3.6	10	3.6	9	4.2
<i>Pasteurella multocida</i>	3	1.1	3	1.1	3	1.4
<i>Staphylococcus hyicus</i>	3	1.1	3	1.1	3	1.4
<i>Klebsiella</i> spp.	2	0.7	2	0.7	2	0.9
<i>Streptococcus bovis</i>	1	0.4	1	0.4	0	0
<i>Bacillus</i> spp.	1	0.4	1	0.4	0	0
<i>Corynebacterium</i> spp.	1	0.4	1	0.4	1	0.5
<i>Serratia</i> spp.	1	0.4	1	0.4	1	0.5
<i>Enterobacter</i> spp.	1	0.4	1	0.4	1	0.5
Total	280	100	275	100	213	100

¹Gold standard results of the 280 samples that were offered to the Bi-plate for evaluation.

²Gold standard results of the 275 undiluted samples that were offered to the Petrifilm for evaluation.

³Gold standard results of the 213 diluted samples that were offered to the Petrifilm for evaluation.

For assessment of the Bi-plate system, only results recorded by the laboratory technician were used. Sensitivity, Sp, PPV, and NPV were calculated by comparing the gold standard treatment and no-treatment classifications to the Bi-plate treatment and no-treatment classifications.

RESULTS

Samples

A total number of 282 fresh milk samples were received. Table 1 contains a summary of the results of gold standard cultures. There were 280 samples that had complete Bi-plate records (gold standard culture and read by the laboratory technician). The prevalence of treatment candidates was 54.6% in the samples that were used to calculate test characteristics for the Bi-plates. There were 275 samples with complete records for undiluted milk samples cultured on Petrifilms. After 6 wk of sample collection, readers 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 1:10 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; therefore, the total number of diluted samples cultured on Petrifilms and read by the technician was 213. The

prevalence of treatment candidates was 56.5% in the samples that were used to calculate test characteristics for the diluted Petrifilms.

3M Petrifilm: Undiluted Versus Diluted Samples

In total, there were 275 undiluted clinical milk samples that were cultured on the Petrifilm system. Seventeen samples were removed from the analysis because they were diagnosed as mixed growth or contaminated on the gold standard culture. Of these 17 samples, 12 would have been characterized as gram-positive growth using the Petrifilms and treated. There were an additional 4 samples that did not have complete records that were not included in the analysis.

There were 213 samples read by the technician that were diluted and plated on both the AC and CC Petrifilms. Of these, 16 were removed from the interpretation due to mixed growth or contamination of the gold standard plates. If these samples had been included, 11 would have been classified as gram-positive based on the Petrifilms and placed in the treatment category.

When the Petrifilms were used with diluted milk samples, the Se and NPV were not different from undiluted samples. There were differences ($P < 0.05$) in Sp (72.6 vs. 92.9%) and PPV (77.7 vs. 93.8%), both of which favored using the diluted milk samples. For this reason, further analysis to choose appropriate colony count thresholds for the Petrifilms was done using only the diluted milk samples.

Table 2. Agreement between technician and automated Petrifilm reader for Aerobic Count (AC) and Coliform Count (CC) Petrifilms using diluted milk samples

Automated plate reader	Technician AC Petrifilm			Technician CC Petrifilm		
	Negative: <20 colonies	Positive: $\geq$ 20 colonies	Total	Negative: <20 colonies	Positive: $\geq$ 20 colonies	Total
Negative: <20 colonies	75	1	76	188	2	190
Positive: $\geq$ 20 colonies	6	129	135	1	20	21
Total	81	130	211	189	22	211

Agreement Between Manual and Automated Reading of Petrifilms

Table 2 illustrates the agreement between the technician and automated Petrifilm reader for AC and CC Petrifilms using diluted milk samples. Before calculating the level of agreement between the 3M automated reader and the technician, McNemar's tests for significance were performed. The exact McNemar significance probabilities were 0.13 and 1.0 for the AC and CC Petrifilms, respectively. The calculated agreement between the technician and the automated reader was 96.7% with a kappa value of 0.93 for the AC Petrifilm. For CC Petrifilm, the calculated agreement was 98.6% with a kappa value of 0.92. Because these levels of agreement were high, the colony count values obtained from the automated reader were used to evaluate the effect that various thresholds would have on test characteristics.

Evaluation of Colony Count Thresholds

As with the previous analysis, all samples that were contaminated on the gold standard were removed from the data set, leaving 197 AC and CC Petrifilms with complete records from the automated reader. Sensitivity versus Sp plots were created for various combinations of colony count thresholds ranging from 1 to 30, in increments of 5. Figure 1 contains a truncated graph of the test characteristics (Se and Sp) for each combination of colony count thresholds.

The combination of Petrifilms that resulted in the optimum Se used a threshold of $\geq$ 20 colonies on the CC Petrifilm and $\geq$ 5 colonies on the AC Petrifilm to consider the test positive (Figure 1). Values above 20 (25 and 30) for the CC were evaluated, but there was virtually no gain in Se using a threshold of 25 and only a slight gain at 30 (data not shown). At every level of CC threshold evaluated, the greatest decrease in the Petrifilm test system's Se was observed when thresholds >5 were used on the AC. The test characteristics of the Petrifilm system that uses a threshold of $\geq$ 20 colonies on the CC plate and $\geq$ 5 colonies on the AC plate to classify a sample as gram-positive (treatment) are shown in Table 3.

There were only 7 samples out of the 200 that were diagnosed as treatment on the gold standard culture that the Petrifilm test system diagnosed as no treatment. Six of the 7 samples grew <5 colonies on the AC Petrifilm and 1 gram-positive sample was misclassified as a coliform. Twenty-six samples were classified as treatment based on Petrifilms, while according to the gold standard, they should not have been treated. All of the cases that fit this description yielded no bacterial growth on the gold standard, except for 4. Of the 4, 1 was positive for *Serratia* and the remaining 3 were positive for *Pasteurella multocida*. These 2 gram-negative pathogens were not coliforms and would not be identified by the CC Petrifilm.

Minnesota Easy Culture System II Bi-Plate

Eighteen of the 280 samples that were cultured on the gold standard exhibited mixed growth or contamination and were not used in the evaluation of the Bi-plates. Seventeen of the 18 samples would have been classified as treatment based on the Bi-plate results. Table 4 illustrates the test characteristics of the Bi-plate when read by the laboratory technician who used a threshold of 1 colony to consider the plate positive.

The Bi-plate was highly sensitive because there were only 3 samples that should have been included in the treatment category that the Bi-plate classified as no treatment. The gold standard results of these 3 samples were: 1 CNS, 1 *Streptococcus uberis* and 1 *Streptococcus dysgalactiae*. The reduced Sp was the result of 39 samples being classified as treatment by the Bi-plate that did not require treatment according to the gold standard. Most (28) of these samples had no bacterial growth on the gold standard, the remaining samples included 7 *E. coli*, 2 *P. multocida*, and 2 yeast.

Interreader Agreement

Agreement between readers was assessed by calculating kappa values for each test system. All readers were masked regarding the results from the gold standard and the other participants. For Bi-plates, the kappa value was calculated based on the ability to diagnose

Table 3. Test characteristics of the Petrifilm test system that uses a threshold of ≥ 20 colonies on the Coliform Count plate and ≥ 5 colonies on the Aerobic Count plate based on its ability to correctly classify a sample as gram-positive

Test characteristic	Result (95% confidence interval)
Sensitivity, %	93.8 (90.5–97.1)
Specificity, %	70.1 (63.8–76.5)
Positive predictive value, %	80.3 (74.8–85.8)
Negative predictive value, %	89.7 (85.5–93.9)

no growth, gram-positive growth only, gram-negative growth only or contamination (growth on both sides of the Bi-plate). The Bi-plates were read by 5 individuals and the combined kappa value was 0.76. Only 4 of the 5 individuals evaluated all of the Petrifilms. The kappa value for the AC Petrifilms using diluted milk samples was 0.84, based on the ability to detect colony growth with >20 colonies present. The kappa value for the CC Petrifilms was 0.86, based on their ability to diagnose a CC Petrifilm positive with >20 colonies present.

DISCUSSION

The most important part of an effective mastitis control program is the use of prevention strategies; however, appropriate therapy of individual clinical cases remains important (Erskine et al., 2003). Dairy producers frequently experience cases where the empirical treatment of mastitis with intramammary products is ineffective, due in part to inappropriate therapeutic choices. Fifty-eight percent of submitted clinical mastitis cases were culture negative or had gram-negative pathogens (Olde Reikerink et al., 2008). Others reported that pathogen profiles did not justify the use of labeled intramammary antibiotics for 50 to 80% of clinical mastitis cases (Roberson, 2003).

Having a diagnostic tool suitable for on-farm use would eliminate the empirical treatment of clinical cases and allow producers to make informed decisions, thereby decreasing the amount of antibiotics used unnecessarily. This study performed a thorough laboratory evaluation comparing available technologies, which is the first step required before such tools can be recommended in the field. Specifically, the abilities of the University of Minnesota Bi-plate and the 3M Petrifilm to correctly place milk samples into appropriate treatment categories were evaluated.

Samples

The distribution of culture results for samples used was similar to that of a recent study of the incidence rate of clinical mastitis on Canadian dairy farms (Olde

Reikerink et al., 2008). Here, 30.7% of the samples cultured resulted in no bacterial growth, whereas in the nationwide study, 43.9% of samples submitted were culture negative. *Staphylococcus aureus* was the most frequently isolated pathogen isolated in both studies, but comprised a greater percentage of the samples (16.8%) in our study compared with the national study (10.3%). Gram-negative pathogens (*E. coli*, *Pasteurella*, *Klebsiella*, *Serratia*, and *Enterobacter* spp.) accounted for 12.4% of the samples received in our study, whereas 13.9% of the samples in the Olde Reikerink et al. (2008) study were positive for gram-negative pathogens.

3M Petrifilm

The Petrifilm system combined both the AC and CC plates to determine if a sample was positive for gram-positive growth and a candidate for treatment. A previous trial conducted by Silva et al. (2004) used the Petrifilm culture system on a 600-cow dairy that was experiencing a herd problem with clinical mastitis, excessive use of antibiotics, and extended days of milk discard. When using the Petrifilm on-farm culturing as part of a treatment protocol, the farm was able to decrease days that milk was kept out of the tank, number of mastitis tubes used per case, and the percentage of cases receiving intramammary antibiotics.

There were no differences in the Se or NPV between diluted and undiluted milk samples on the Petrifilm system, even though the undiluted system used 10 times the volume of milk. Dilution of the milk samples resulted in significantly higher Sp and PPV. The increased Sp and PPV were the result of less misclassification of gold standard negative samples as positive on Petrifilm when diluted samples were used. A potential explanation for this difference is that the individuals looking at the undiluted Petrifilms were confusing debris from the whole clinical milk samples with actual colony-forming units, resulting in a false-positive diagnosis. For this reason, a more in-depth analysis into colony-forming unit thresholds was done using only the results of the diluted milk samples.

There were 26 tests (13% of samples) that the Petrifilm classified as treatment positive that were diagnosed as no treatment by the gold standard. This can

Table 4. Test characteristics of the University of Minnesota Bi-plate based on its ability to correctly classify samples as gram-positive

Test characteristic	Result (95% confidence interval)
Sensitivity, %	97.89 (96.1–99.6)
Specificity, %	67.50 (61.8–73.2)
Positive predictive value, %	78.09 (73.1–83.0)
Negative predictive value, %	96.43 (94.2–98.7)

Figure 1. Comparison of sensitivity and specificity for various combinations of colony count thresholds (grouped as 5, 10, and 20 cfu, respectively) for the diluted Aerobic Count (AC) and Coliform Count (CC) Petrifilms.

be explained by the increased volume of milk plated on each Petrifilm: 100 μ L of milk on the Petrifilm, as opposed to 10 μ L on the gold standard. There are gram-negative pathogens that cause mastitis that are not coliform bacteria. These organisms will always be classified as treatment candidates using the Petrifilm system in this manner.

For application in the field, the usefulness of this test system can be best evaluated by looking at its predictive values. Of particular interest to a dairy producer is the NPV of a test. This is the probability that, given a negative test, the cow does not need to be treated. The clinical consequences of not treating an animal that should have been treated outweigh the consequences of treating a case that did not require antimicrobials. In a scenario in which an animal with clinical mastitis tests negative and the decision is made not to treat the cow, a producer wants to be confident that the correct decision has been made. In the set of 197 diluted milk samples that were used in this study, the NPV was 89.7% and the PPV was 80.3%. These numbers were influenced by the prevalence (56.5%) of treatment-positive cases in the group of samples that were diluted and cultured on the Petrifilm.

Predictive values of a test change with different populations of animals tested because they are driven by the true prevalence of disease in the study population as well as the test characteristics Se and Sp (Dohoo et al., 2003). A more accurate estimate of predictive values can be obtained by using proportions of disease positive and disease negative cases from a larger, more representative study population. When using the calculated Se and Sp from our study and the proportions of disease positive [$p(D+)$] for gram-positive and proportion of disease negative [$p(D-)$] for gram-negative or no growth, from Olde Reikerink et al. (2008), predictive values were calculated using the formulas

$$PPV = \frac{p(D+) \times Se}{p(D+) \times Se + p(D-) \times (1 - Sp)} \text{ and}$$

$$NPV = \frac{p(D-) \times Sp}{p(D-) \times Sp + p(D+) \times (1 - Se)}.$$

The PPV calculated for Petrifilm was 60.4% and the NPV was 95.9%. This very high NPV indicated that a

producer could be very confident in a negative test result, as the probability that a negative culture actually needed treatment was 4.1%. The lower PPV indicated that using the Petrifilm will continue to result in the unnecessary treatment of cases that do not require antimicrobial treatment. Nonetheless, a reduction in the overall percentage of cases that are treated can still be realized. Using the Petrifilm system in this set of samples would have resulted in 66.0% of cases being treated and a potential 34.0% reduction in the amount of antimicrobials used needlessly.

The Petrifilm system was easy to use, but required some training; colony growth was easy for the individuals to interpret, especially when diluted samples were used. The agreement between readers (kappa) was used to evaluate this ease of interpretation for both the AC and CC Petrifilms. The high combined kappa statistic indicated that agreement between individuals was very good.

Minnesota Easy Culture System II Bi-Plate

The University of Minnesota Bi-plate is highly sensitive in its ability to determine treatment categories for clinical cases of mastitis. Previous laboratory work on the validation of the Bi-plate determined Se of 83.2%, Sp of 74.5%, PPV of 77.4%, and NPV of 80.9% using 101 frozen-thawed milk samples from clinical cases (Hochhalter et al., 2006). A field trial using the Bi-plate determined Se of 96.8% and Sp of 81.8% (Godden et al., 2007) based on 101 fresh quarter milk samples that were cultured on-farm.

In this study, there were 37 samples (14%) that the Bi-plate classified as treatment candidates that were diagnosed as no treatment by the gold standard. Of these samples, 28 were negative for growth on the gold standard yet grew at least 1 colony on the Factor media of the Bi-plates. This could be because of the difference in the volume of milk plated on the standard blood agar plates (10 μ L per half plate) versus a saturated cotton swab of milk per half plate on the Bi-plate. The other 9 samples that the gold standard diagnosed as do not treat were all gram-negative organisms on the gold standard culture (7 *E. coli* and 2 *Pasteurella* spp.). The 2 *Pasteurella* spp. that were diagnosed by the gold standard were not expected to be classified as gram-negative organisms on the Bi-plate as they do not grow on MacConkey media. All of the *E. coli* samples had growth on both sides of the Bi-plate; therefore, due to the presence of a gram-positive they were placed in the treatment category. Withholding treatment may represent a paradigm shift for many dairy producers. As a result, a strategy that minimized under-treatment was adopted. Consequently, all plates with growth on both sides of the Bi-plate were considered treatment cases.

In our study, the PPV of the Bi-plate was 78.1%. Again, this was influenced by the prevalence (54.6%) of treatment-positive cases in the group of samples that were cultured on the Bi-plate. As in the evaluation of the Petrifilms, our calculated Se and Sp were applied to the larger, more representative population that had a slightly lower prevalence of gram-positive cases. The resulting PPV was 60.2% and the NPV was 98.5%. Based on this NPV, the probability that cow with a negative Bi-plate culture actually needed treatment was only 1.5%.

In total, the University of Minnesota Bi-plate diagnosed 63.8% of the samples received as gram-positive organisms. Using a Bi-plate culture to determine which cases of clinical mastitis receive antimicrobial therapy could potentially result in a 36.2% reduction in treatment if the current practice of a producer was to treat all clinical cases. This reduction in treatment assumes that all gram-positive cases should be treated. There will continue to be unnecessary treatment of some cases that are caused by bacterial species that are not susceptible to intramammary therapy. Still, when compared with the practice of treating all cows with abnormal milk, the use of Bi-plates combined with treatment protocols based on their outcomes has the potential to reduce the amount of antibiotics used on the farm.

The Bi-plate was easy to use and with some training, colony growth was easy for the individuals to interpret. The agreement between readers was used to evaluate this ease of interpretation and the high combined kappa statistic indicated that agreement between the readers was very good.

When comparing these 2 test systems, the Bi-plate did not outperform the Petrifilm in Se and NPV, whereas the Petrifilm system had no differences in Sp and PPV. Neither method had the ability to reliably determine if a sample was contaminated. Should this technology be used on farm, duplicate samples should be submitted to a diagnostic laboratory for quality control purposes. The Bi-plate is able to show mixed growth and contamination if there is a combination of gram-positive and gram-negative organisms present; however, samples that consist of mixed gram-positive organisms cannot be identified. Therefore, proper training in collection and handling of samples is an important aspect of on-farm culture. Both systems provided accurate results within 24 h of culturing. The effect of delaying treatment for 24 h has not been well studied and has the potential to result in reduced treatment outcomes (Neaser et al., 2006). An on-farm evaluation is required to evaluate this statement as well as the overall impact that on-farm culture may have on cow health, recurrence rates of mastitis, and economic feasibility.

CONCLUSIONS

The University of Minnesota Bi-plate and the 3M Petrifilm tests were able to successfully categorize clinical cases of mastitis into 2 treatment categories based on their ability to detect the presence of a gram-positive organism. Neither test had the ability to reliably determine if a sample was contaminated. Both the Bi-plate and 3M Petrifilm system were highly sensitive tests, 97.9 and 93.8%, respectively. There was no significant difference in the sensitivities between the 2 tests. The very high NPV (98.5% for Bi-plate and 95.9% for Petrifilm) were important attributes of each of these tests that will minimize the number of cases requiring therapy that go untreated. The results indicate that both tests have the potential to appropriately categorize cases, which could result a reduction in the amount of antibiotics used to treat clinical cases of mastitis.

ACKNOWLEDGMENTS

The authors thank Theresa Andrews, Lloyd Dalziel, Katie MacIntosh, Michael Trenholm, Cynthia Mitchell, and Shana Richard of the Department of Health Management (University of Prince Edward Island, Charlottetown, PEI, Canada) for their technical help with this project. This research was financed by Natural Sciences and Engineering Research Council of Canada (Ottawa, Ontario, Canada); Alberta Milk (Edmonton, AB, Canada); Dairy Farmers of New Brunswick (Sussex, NB, Canada), Nova Scotia (Truro, NS, Canada), Ontario (Mississauga, ON, Canada), and Prince Edward Island (Charlottetown, PEI, Canada); Novalait Inc. (Sainte-Foy, PQ, Canada); Dairy Farmers of Canada (Ottawa, Ontario, Canada); Canadian Dairy Network (Guelph, Ontario, Canada); Agriculture and Agri-Food Canada (Ottawa, Ontario); Public Health Agency of Canada (Ottawa, Ontario); Technology PEI Inc. (Charlottetown, PEI, Canada); Université de Montréal (St. Hyacinthe, PQ, Canada); and University of Prince Edward Island (Charlottetown, PEI, Canada) through the Canadian Bovine Mastitis Research Network (St. Hyacinthe, PQ, Canada).

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