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Evaluation of Petrifilm for the Isolation of *Staphylococcus aureus* from Milk Samples

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ABSTRACT

Four experiments were conducted to evaluate the Petrifilm Staph Express Count plate (3M, Minneapolis, MN) for diagnosis of mastitis caused by *Staphylococcus aureus*. The objective of experiment 1 was to determine the sensitivity of Petrifilm compared with results of standard and augmented microbiological techniques, and the objective of experiment 2 was to compare microbiological results of composite and quarter milk samples processed using Petrifilm. Experiment 3 was conducted to determine the specificity of the Petrifilm method based on different interpretation parameters, and the objective of experiment 4 was to determine the repeatability of reading Petrifilm Staph Express plates. Results of standard microbiological techniques used for experiments 1 and 2 were compared with results of samples preprocessed using centrifugation or preincubation. The prevalence of recovery of *Staph. aureus* from milk samples processed using Petrifilm was significantly greater than the prevalence of milk samples processed using standard microbiological techniques. The sensitivity of isolation of *Staph. aureus* was 65.6, 75.0, 84.4, and 87.5% for standard, centrifugation, incubation, and Petrifilm methods, respectively. The occurrence of a distinct pink zone surrounding a colony was highly specific for *Staph. aureus*, and the specificity was 98.5 and 96.0% for experiments 3 and 4, respectively. The use of a weak pink zone to diagnose *Staph. aureus* resulted in a high rate of false-positive results. The interpretation of results of Petrifilm Staph Express was associated with the person that read the plates. Results from all 4 experiments indicate potential for the Petrifilm products as a diagnostic tool in some herd situations when *Staph. aureus* is the pathogen of interest. Results also indicate the need for standardization of interpretive criteria for personnel working with the products.

(**Key words:** mastitis, culture, Petrifilm, microbiology)

INTRODUCTION

The isolation and identification of pathogens obtained from milk samples is a fundamental aspect of mastitis control programs. An effective milk-culturing program requires considerable commitment of farm resources, and farmers expect to receive useful information. However, up to 50% of milk samples submitted from cows with subclinical or clinical mastitis may not yield pathogens, and farmers may perceive such negative culture results as wasted resources (Makovec and Ruegg, 2003).

Negative results of bacteriological culture of mastitis cases can result from spontaneous clearance of pathogens (Eberhart et al., 1979; Smith et al., 1985), cyclical shedding of chronic *Staphylococcus aureus* infections (Sears et al., 1990), or the presence of few bacterial colonies in milk (Sears et al., 1990). Milk samples from infected quarters may also be negative if bacteria have been engulfed by phagocytes (Newbould and Neave, 1965; Hill et al., 1978). The occurrence of false negative results of milk samples submitted for culture can result in maintenance of infected animals within a herd and may contribute to the failure of programs designed to control contagious mastitis. It is important to balance the use of sensitive and costly laboratory procedures against the cost of other interventions. The choice to use a more rigorous diagnostic method should be determined for each herd based on the herd goals and the manager's ability to use the resulting data.

Several methods have been used to enhance recovery of pathogens from milk samples. The use of larger inocula increased the relative sensitivity for *Staph. aureus* from 78 (0.01 mL) to 90% (0.1 mL) (Lam et al., 1996). Incubation of milk samples for 4 to 18 h before culture increased the recovery rate of bacterial pathogens compared with the use of standard microbiological methods (Dinsmore et al., 1992). Freezing and subsequent thawing of milk samples before inoculation increased the recovery by 2.5 and 1.5 times for *Streptococcus agalactiae* and *Staph. aureus* respectively (Vil-

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lanueva et al., 1991). Centrifugation of milk samples and subsequent culture of sediment increased the recovery of *Staph. aureus* by 86% (Zecconi et al., 1997). The use of various combinations of these methods has also been reported (Dinsmore et al., 1992; Sol et al., 2002).

Petrifilm plates (3M, Minneapolis, MN) are sample-ready selective culture systems that are marketed for rapid bacteriological isolation and enumeration of bacteria from food products. The Petrifilm Staph Express Count plate contains chromogenic, modified Baird-Parker medium that is selective and differential for *Staphylococcus* spp. Confirmation of *Staph. aureus* is performed using a disk that contains deoxyribonuclease and a dye that reacts to produce a distinct pink zone around *Staph. aureus* colonies.

A series of 4 trials were performed to evaluate the use of Petriflim Staph Express Count plate used for isolation of *Staph. aureus* from milk samples. The objective of experiment 1 was to compare microbiological results of Petrifilm to standard and augmented culture techniques for diagnosis of mastitis caused by *Staph. aureus*. The objective of experiment 2 was to compare results of Petrifilm using composite and quarter milk samples. The objective of experiment 3 was to determine the specificity of Petrifilm based on different interpretative criteria. The objective of experiment 4 was to determine the repeatability of Petrifilm among several individuals that read the plates.

MATERIALS AND METHODS

Experiment 1

Quarter milk samples (n = 362) were obtained from cows (n = 145) located on a single commercial dairy herd (Farm A) in Wisconsin. Farm owners were trained to collect quarter milk samples from selected lactating cows after premilking cow preparation and before attachment of the milking unit. Collection of milk samples was performed as described by the National Mastitis Council (1999), and samples were frozen for up to 1 wk. Cows were selected for sampling based on the following criteria: high SCC (>400,000 cells/mL; n = 25 cows, 97 milk samples), postparturient (2 to 4 d postpartum; n = 41 cows, 163 milk samples), and cases of clinical mastitis (abnormal milk as identified by farm personnel; n = 79 cows, 102 milk samples). To increase the prevalence of *Staph. aureus* in the sample population, frozen composite milk samples (n = 29) that were duplicates of milk samples from which *Staph. aureus* had been isolated previously were thawed at room temperature and included in the sample pool.

Thawed milk samples were vortexed and streaked onto one quarter of blood agar plates using 0.01-mL disposable plastic loops, and plates were incubated at 37°C for 24 h (standard method). A 1-mL aliquot of each sample was plated on Petrifilm Staph Express plates and incubated at 37°C for 24 h following the manufacturer's instructions (Petrifilm method). A 5-mL aliquot of each sample was placed in a separate vial and centrifuged at 2000 × *g* for 15 min. The supernatant was then discarded and a 0.01-mL aliquot of the sediment was spread using a loop on a 5% blood agar plate and incubated at 37°C for 24 h (centrifugation method). The remaining aliquot of the original sample was incubated for 18 h at 37°C in the original plastic vial. When no bacterial growth was observed using the standard method, 0.01 mL of the incubated sample was plated on blood agar and incubated at 37°C for 24 h (incubation method).

All bacterial growth was identified and recorded after 24 and 48 h of incubation. For the standard, centrifugation, and incubation methods, *Staph. aureus* were identified by hemolytic pattern, gram-staining characteristics, positive catalase reaction, positive mannitol agar reaction, and positive tube coagulase test (National Mastitis Council, 1999). Other bacteriological results were recorded as coliforms, *Streptococcus* sp., CNS, *Corynebacterium* spp., *Bacillus* spp., and others as defined by the National Mastitis Council (1999).

Samples were considered contaminated if 3 or more dissimilar colony types were found in the same sample. To meet the objective of this project, cultures were considered negative when <3 colonies were seen on the plate, except for *Staph. aureus*, when the presence of ≥1 colony was considered a positive sample.

For the Petrifilm method, *Staph. aureus* were initially identified following manufacturer's instructions for the Staph Express Count plate and the Staph Express disk. The disk contains a dye and deoxyribonuclease. *Staphylococcus aureus* produces DNA, which reacts with the dye to form a pink zone around colonies. The disk was applied to all Staph Express plates with any evidence of bacterial growth after 24 h of incubation. Positive Petrifilm tests were recorded when a colony was associated with a distinct pink zone after 2 more hours of incubation. When the type of colony growth was diffuse and without apparent individual colonies, a 1:10 dilution was made using sterile distilled water and inoculated on Petrifilm. Results of the diluted sample were used in the analysis. When growth occurred only on Petrifilm, colonies were picked, regrown, and confirmed using standard methods. The Petrifilm results were considered false positives when the standard methods did not result in

confirmation of *Staph. aureus*. Final identification of these colonies was performed using a commercial microbial identification system (BBL Crystal ID; Becton Dickinson, Franklin Lakes, NJ).

Experiment 2

Samples were collected from Farm A. Composite (n = 100) and quarter (n = 386) milk samples were collected by university personnel from cows (n = 100) with high SCC (>200,000 cells/mL) on the current monthly DHI test. Quarter milk samples were collected as described in experiment 1. For composite sampling, a 50-mL graduated vial was used and approximately 5 to 10 mL of milk was collected from each quarter. Samples were cooled immediately and delivered within 24 h to the laboratory. All samples were frozen and analyzed within 15 d of collection. Sample handling and microbiological techniques were the same as experiment 1 except that the centrifugation method was not used.

Experiment 3

Quarter milk samples (n = 332) were obtained from all lactating cows (n = 88) located on a commercial farm (Farm B) that used a robotic milking system. The herd was selected because of a suspected problem with subclinical mastitis caused by *Staph. aureus*. Cows were restrained in headlocks and milk samples were collected by university personnel using aseptic procedures as recommended by the National Mastitis Council (1999). Immediately after collection, a 1-mL aliquot of each milk sample was spread on a Petrifilm Staph Express Count plate following manufacturer's directions. Samples were incubated at 37°C for 24 h. *Staphylococcus aureus* were identified following manufacturer's instructions for the 3M Staph Express Count plate and the 3M Staph Express disk. The disk was applied to all Staph Express plates with evidence of bacterial growth after 24 h of incubation (n = 320). Petrifilm plates with evidence of zones associated with colonies (n = 127) were recorded (weak or distinct) after 2 more hours of incubation. All colonies that formed weak or distinct pink zones on Petrifilm were picked and regrown on blood agar plate. *Staphylococcus aureus* were identified by hemolytic pattern, gram-staining characteristics, positive catalase reaction, growth on mannitol, and positive tube coagulase test. Final identification of CNS was performed using a commercial microbial identification system (BBL Crystal ID).

Experiment 4

Composite milk samples (n = 240) were collected from all lactating cows on a commercial farm (Farm C) in Wisconsin. Milk samples were collected in the milking parlor by university personnel after premilking cow preparation and before attachment of the milking unit using aseptic procedures as recommended by the National Mastitis Council (1999). Samples were cooled immediately and delivered within 8 h to the laboratory. A 1-mL aliquot of each milk sample was spread on each of a Petrifilm Staph Express Count plate and a Petrifilm Aerobic count plate following manufacturer's directions (3M). Petrifilm Staph Express plates were initially read after incubation at 37°C for 24 h by 3 readers with varying levels of experience. Readers A and B had some previous experience with interpretation of Petrifilm, whereas reader C had no experience with the product. Plates were read independently without consultation between readers. Readers recorded the number of colonies (0, 1 to 50, 51 to 150, >150), colony color (red-violet, black, blue-green, other), and colony size (tiny, normal, large, irregular). After the initial reading of the plates, the disk was applied to all Petrifilm Staph Express plates that contained evidence of bacterial growth (≥1 cfu/mL) and plates were incubated at 37°C for a further 2 h. Plates with disks were independently read by the same 3 readers. Readers recorded the intensity of pink zones surrounding colonies (none, weak, distinct). When 2 of 3 readers agreed that colonies formed a weak or distinct pink zone on Petrifilm, the suspect colonies were picked and regrown on blood agar plate. *Staphylococcus aureus* were identified by hemolytic pattern, gram-staining characteristics, positive catalase reaction, growth on mannitol, and positive tube coagulase test. Final identification of CNS was performed using a commercial microbial identification system (BBL Crystal ID).

Colonies from Petrifilm Aerobic Count plates that contained >25 cfu/mL were picked and regrown on blood agar and MacConkey agar. Identification of bacteria was performed using microbiological procedures as outlined by the National Mastitis Council, (1999). The morphology and hemolytic patterns of bacterial colonies were determined and organisms were differentiated using standard microbiologic methods. *Staphylococcus aureus* were identified using mannitol and coagulase reactions, *Streptococci* were differentiated using the CAMP test, esculin reactions and agglutination, and gram-negative bacteria were identified using MacConkey agar, motility, indole and ornithine reactions, and triple sugar iron slants.

Statistical Analyses

Statistical analyses for experiments 1 and 2 were performed using the McNemar test for paired data

using SAS statistical package (SAS Institute, 1999). Statistical significance was defined at $P \leq 0.05$. In experiment 1, the relative sensitivity was defined as the probability of isolating *Staph. aureus* using a single technique compared with the probability of isolation using the 4 microbiological techniques (gold standard). Two samples with false-positive Petrifilm results (based on standard microbiological methods) were excluded from the data to compare prevalence of *Staph. aureus*.

In experiment 2, the prevalence of *Staph. aureus* results was compared on a cow basis and quarter basis. When quarter samples were used, cows were considered positive if *Staph. aureus* was isolated from any quarter. The cow status for composite sampling was determined using results of the composite sampling. The gold standard was defined based on the parallel *Staph. aureus* isolation of the 3 methods together. Four cows with false-positive results on the Petrifilm method were excluded from the calculations of prevalence.

In experiment 3, specificity and predictive values were estimated using epidemiological software (Win Episcope 2.0; available at http://www.clive.ed.ac.uk) based on formulas derived from standard epidemiological methods (Thrushfield, 1995; Martin et al., 1987). Three records with incomplete laboratory results were excluded from statistical analysis. False positives were defined as colonies that exhibited a weak or distinct pink zone on Petrifilm (after application of the disk) but were identified as other pathogens using standard microbiological methods. The association between zone intensity and accuracy of identification of *Staph. aureus* was determined using χ^2 analysis (WinEpiscope 2.0).

In experiment 4, specificity and the association between zone intensity and accuracy of identification of *Staph. aureus* was estimated as in experiment 3. The association among readers and results of Petrifilm was evaluated using χ^2 analysis (WinEpiscope 2.0).

RESULTS

Experiment 1

The estimated prevalence of *Staph. aureus* in the milk samples ranged from 5.4 to 8.2% and the sensitivities of the various methods ranged from 65.6 to 87.5%. *Staphylococcus aureus* was isolated from a maximum of 18 (62.1%) of the frozen duplicate samples that had been added to the sample pool. The use of composite milk samples and the extended duration of freezing (>1 yr) probably contributed to the low rate of recovery. The estimated prevalence of *Staph. aureus* was highest for samples processed using the centrifuged, incubated and Petrifilm methods ($P < 0.05$) (Table 1). The sensitivity of the standard method was significantly lower than that of the incubation and Petrifilm methods ($P < 0.05$).

Table 1. Prevalence and relative sensitivity of *Staphylococcus aureus* according to different microbiological methods.

	Staphylococcus aureus results (n = 389)		
Microbiological method	Number isolated	Prevalence, %	Sensitivity, %
Standard[1]	21	5.4^{d}	65.6^{b}
Centrifugation[2]	24	6.1^{cd}	75.0^{ab}
Incubation[3]	27	6.9^{bc}	84.4^{a}
Petrifilm[4]	28	7.2^{bc}	87.5^{a}
Gold standard[5]	32	8.2^{a}	

[a,b,c]Values with different letters in the same column differ significantly ($P \leq 0.05$).

[1]Milk (0.01 mL) streaked on blood agar plate (NMC, 1999).

[2]Centrifugation of 5 mL of milk followed by incubation of 0.01 mL of the sediment on blood agar.

[3]Preincubation of whole milk sample for 18 h at 37°C followed by culture as standard.

[4]Petrifilm Staph Express Count Plate (3M, Minneapolis, MN). Two false-positive results were excluded.

[5]All methods combined.

The use of preincubation increased the recovery of most pathogens from quarter samples obtained from Farm A ($P < 0.05$) (Table 2). There was no significant difference in the rate of contamination among methods, and the prevalence of isolation of pathogens was highest for incubated samples. Centrifugation resulted in increased recovery of *Bacillus* compared with the standard method, whereas incubation increased the recovery of all microorganisms except for *Staph. aureus* and *Corynebacterium* sp.

The use of Petrifilm Staph Express increased recovery of *Staph. aureus* compared with other microbiological methods ($P < 0.05$) (Table 2). The use of Petrifilm resulted in a 62.5% increase in positive *Staph. aureus* samples compared with the standard method ($P = 0.03$) (Table 2).

Experiment 2

The estimated prevalence of *Staph. aureus* was 86% higher for quarter samples processed using Petrifilm compared with composite samples processed using the standard method (Table 3). Although the prevalence of *Staph. aureus* was numerically higher for quarter samples, there was no significant difference in estimated prevalence of *Staph. aureus* between quarter and composite samples for samples processed using any method (Table 3). There was a tendency for both quarter samples ($P = 0.06$) and composite samples ($P =$

Table 2. Prevalence of bacterial isolation from quarter samples of one commercial dairy herd (Farm A) according to different methods (n = 360).

	Method			
	Standard,[1] %	Centrifugation,[2] %	Incubation,[3] %	Petrifilm,[4] %
Staphylococcus aureus	2.2[b]	2.5[b]	2.5[b]	3.6[a]
Coliforms	9.4[b]	7.7[b]	16.3[a]	—
Streptococcus spp.	3.3[b]	3.6[ab]	5.2[a]	—
CNS	6.3[b]	8.0[b]	24.6[a]	—
Corynebacterium spp.	0.5	1.4	1.4	—
Bacillus	1.1[c]	3.6[b]	12.1[a]	—
Others[5]	0.5[b]	1.9[ab]	2.5[a]	—
Contaminated[6]	1.1	1.9	1.4	—
No growth	77.9[c]	73.5[b]	42.8[a]	—

[a,b,c]Values in the same row with different superscripts differ significantly ($P \leq 0.05$).

[1]Milk (0.01 mL) streaked on blood agar plate (NMC, 1999).

[2]Centrifugation of 5 mL of milk followed by streak of 0.01 mL of the sediment on blood agar.

[3]Preincubation of whole milk sample for 18 h at 37°C followed by culture as standard.

[4]Petrifilm Staph Express Count Plate (3M, Minneapolis, MN). Two false-positive results were excluded.

[5]Others = *Pseudomonas* spp. and yeasts.

[6]Contaminated = 3 or more different colony types.

0.07) processed using the standard method to have lower prevalence compared with the gold standard.

Experiment 3

Staphylococcus aureus was isolated (and confirmed using standard methodologies) in 19.6% of quarter samples and from at least one quarter of 45.5% of the cows. No evidence of weak or distinct pink zones was seen in 193 (60.3%) Petrifilm plates that received disks; these samples received no further processing.

Table 3. *Staphylococcus aureus* prevalence as determined by various microbiological methods and utilizing either composite or quarter milk samples.

	Staphylococcus aureus prevalence by cow status		
Microbiological method	Composite samples, % (n = 96)	Quarter samples,[1] % (n = 370)	P-value[2]
Standard[3]	7.3[b]	9.4[ab]	0.500
Incubation[4]	8.3[ab]	10.4[ab]	0.625
Petrifilm[5]	9.4[ab]	13.5[a]	0.125
Gold standard[6]	12.5[ab]	14.6[a]	0.687

[a,b,c]Values with different superscripts differ significantly ($P \leq 0.05$).

[1]For quarter sampling, a cow was considered positive if any quarter showed a positive result.

[2]Contrast between composite and quarter samples.

[3]Milk (0.01 mL) streaked on blood agar plate (NMC, 1999).

[4]Preincubation of whole milk sample for 18 h at 37°C followed by culture as standard.

[5]Petrifilm Staph Express Count plate (3M, Minneapolis, MN). Four false positive results were excluded.

[6]All methods combined.

Of all colonies that exhibited distinct pink zones, 93.5% had microbiological characteristics diagnostic of *Staph. aureus* (Table 4). In this high prevalence population, the occurrence of a distinct pink zone surrounding a colony was highly specific for *Staph. aureus* and had a specificity of 98.5% (Table 4). The use of a weak pink zone to diagnose *Staph. aureus* resulted in a high rate of false-positive results (22.4%). The intensity of the pink zone was strongly associated with the odds of confirming *Staph. aureus* ($P < 0.001$). Colonies exhibiting distinct pink zones were 120 times more likely to be confirmed as *Staph. aureus* compared with colonies that exhibited weak pink zones. A variety of staphylococci was associated with the development of weak pink zones. *Staphylococcus haemolyticus* (n = 16) and *Staph. simulans* (n = 7) were the most commonly isolated staphylococci.

Experiment 4

Staphylococcus aureus was confirmed in 5.4% of composite milk samples. Complete agreement among readers was seen for 136 of 225 plates that had bacterial growth (60.7%). Two of 3 readers agreed that weak (n = 21) or distinct (n = 21) pink zones were seen on 42 (17.5%) of 240 plates (Table 5). The relative specificities were 87.2 and 96.0% for weak and distinct pink zones, respectively. The positive predictive value was less than experiment 3 because of the lower prevalence of infection with *Staph. aureus*. Interpretation of Petrifilm Staph Express count plates was influenced by reader (Table 6). Readers varied in their assessment of number of colonies, colony size and presence or in-

Table 4. Microbiological results of milk samples showing evidence of weak or distinct pink zones on Petrifilm.[1]

		Positive for microbiological method			Diagnosis			Test characteristics[3]		
Zone intensity	n	Hemolysis	Mannitol	Coagulase	*Staph. aureus*	CNS	Other[2]	Specificity	PV+	PV–
Experiment 3[4]										
Weak	62	38	34	8	7	43	12	77.6[6]	52.4[6]	
Distinct	62	57	60	54	58	3	1	98.5	93.5	97.3
Experiment 4[5]										
Weak	21	5	5	1	1	17	3	87.2[6]	31.0[6]	
Distinct	21	11	12	12	12	9	0	96.0	57.1	99.5

[1]Petrifilm Staph Express Count Plate (3M, Minneapolis, MN).

[2]No growth on blood agar, and a variety of environmental contaminants.

[3]Specificity is defined as the probability that colonies that are not *Staph. aureus* do not have the specified zone (experiment 3); predictive value of a positive test (PV+) is defined as the probability that colonies with the specified zone are confirmed as *Staph. aureus*; predictive value of a negative test (PV–) is defined as the probability that colonies without the specified zone are definitely not *Staph. aureus*.

[4]Quarter milk samples; total n = 317; negative samples for *Staph. aureus*: n = 252; 65 samples positive for *Staph. aureus*; 3 samples not used because of missing data.

[5]Composite milk samples; total n = 240; negative samples for *Staph. aureus*: n = 227; 13 samples positive for *Staph. aureus*.

[6]Values calculated taking into account weak + distinct results.

tensity of pink zones. There was no significant association among readers and identification of colony color.

Of the remaining samples (n = 196), 111 samples had no growth or <25 cfu/mL growth on Petrifilm Aerobic Count plates. A variety of major and minor mastitis pathogens were identified: CNS (n = 43), *Streptococcus uberis* (n = 5), other environmental Streptococci (n = 8), coliforms (n = 4), mixed minor pathogens (n = 4), *Listeria monocytogenes* (n = 1), and some probable contaminants (bacillus, yeasts, fungi, etc.; n = 20). Confirmation of IMI would require further diagnostic efforts that were not part of the objective of this project.

DISCUSSION

Rapid and accurate diagnosis of mastitis pathogens is essential for mastitis control. In mild or moderate cases of clinical mastitis, identification of pathogens can be used to guide treatment decisions (Siamak et al., 2001; Ruegg, 2004). Sensitive and rapid diagnostic methods are also useful to assist in identification of cows when segregation programs are used to control contagious mastitis (Hogan et al., 1989). In this study, Petrifilm Staph Express Count plates were more sensitive than standard microbiological methods, and re-

Table 5. Interpretation and microbiological outcomes of zone intensity of Staph Express Petrifilm by 3 independent readers.

Reader			Consensus zone intensity[2]	No.	Number confirmed[1]		
A	B	C			*Staph. aureus*	CNS	Other
Distinct	Distinct	Distinct	Distinct	6	3	3	0
Distinct	Distinct	Weak	Distinct	1	1	1	0
Distinct	Weak	Distinct	Distinct	1	1	1	0
No zone	Distinct	Distinct	Distinct	1	1	1	0
Weak	Distinct	Distinct	Distinct	12	6	3	0
Weak	Weak	Weak	Weak	10	1	8	1
Weak	Distinct	Weak	Weak	1	0	1	0
Weak	Weak	Distinct	Weak	1	0	1	0
Weak	Weak	No zone	Weak	4	0	3	1
No zone	Weak	Weak	Weak	5	0	4	1
No zone	No zone	No zone	None	120	NA[3]	NA	NA
No zone	No zone	Weak	None	4	NA	NA	NA
No zone	No zone	Distinct	None	2	NA	NA	NA
No zone	Weak	No zone	None	56	NA	NA	NA
Weak	No zone	No zone	None	1	NA	NA	NA
No disk applied			None	15	NA	NA	NA
			Total	240	13	26	3

[1]Confirmation using traditional microbiological techniques.

[2]Zone intensity assigned for analysis (2 of 3 readers agreed).

[3]NA = Not applicable.

Table 6. Association between reader and interpretation of Petrifilm.[1]

Outcome	Reader A n	Reader A %	Reader B n	Reader B %	Reader C n	Reader C %	χ^2	*P*-value
No. of colonies								
0	22	9.2	16	6.7	10	4.2	15.9	0.01
1 to 50	132	55.0	154	64.2	136	56.7		
51 to 150	25	10.4	31	12.9	25	10.4		
>150	61	25.4	39	16.3	69	28.8		
Color of colonies								
Red-violet	8	3.3	17	7.1	10	4.2	4.02	0.13
Other	232	96.7	223	92.9	230	95.8		
Colony size								
Normal	156	65.0	145	60.4	180	75.0	12.0	0.002
Other	84	35.0	95	39.6	60	25.0		
Zone								
None	188	83.6	125	55.6	181	80.4	68.1	<0.001
Weak	29	12.9	79	35.1	21	9.3		
Distinct	8	3.6	21	9.3	23	10.2		

[1]Staph Express Count Plate (3M, Minneapolis, MN).

sults were obtained in less time and with less labor and expertise. When the Petrifilm method was used, *Staph. aureus* were confirmed in 24 to 26 h, whereas confirmation using standard methods took at least 48 h.

The increased sensitivity of Petrifilm for isolation of *Staph. aureus* may be a result of the larger inoculum volume used with that method. In this study, the detection limit was decreased from 100 to 1 cfu/mL. The low numbers of microorganisms shed by infected mammary glands and the intermittent shedding pattern of *Staph. aureus* in milk supports the use of lower detection limits for *Staph. aureus* (Sears et al., 1990). Increased sensitivity for *Staph. aureus* can be useful in segregation programs because of more efficient detection of infected cows. Conversely, the risk of false-positive results is increased when lower detection limits are used. The use of aseptic sampling technique and proper handling and storage of samples are essential. Diagnostic results of multiple samples obtained from the same quarter are more reliable than diagnosis based on a single sample and could be used in situations where larger economic values are involved (such as in culling of high genetic merit animals). The significance of isolation of a single colony of *Staph. aureus* from a 1-mL inoculation is unknown and in some cases will not reflect infection status of the gland. It is important to have additional information (such as monthly SCC values and the individual cow history of clinical mastitis) to make informed decisions regarding the significance of isolation of such few colonies of a suspected pathogen.

No significant difference was observed between results of samples processed using centrifugation or standard methodology. This finding is in agreement with those of Zecconi et al. (1997) when comparing *Staph. aureus* results in 3 of the 6 herds studied. Zecconi et al. (1997) inoculated the whole sediment from a 10-mL milk sample centrifugation and in this study, only 0.01 mL from a 5-mL sample sediment was plated; therefore, detection limits were different. Although centrifugation does enhance recovery of *Staph. aureus*, the use of this technique is extremely labor intensive and may not be practical for many laboratories.

In this study, using our definition of contamination, preincubation increased the recovery of major mastitis pathogens without increasing the number of contaminated samples. Sol et al. (2002) also reported improvement in isolation of major mastitis pathogens when using a similar method. The relative sensitivity of Petrifilm plates was similar to the incubation method. The use of either method would result in increased isolation of *Staph. aureus* but the incubation method requires a longer period until diagnosis. The use of incubation did result in isolation of more minor pathogens.

The use of composite milk samples is often preferred by farmers to reduce the cost of diagnosis (Ruegg and Reinemann, 2002). The relative sensitivity of a single composite milk sample used to detect *Staph. aureus* has been estimated to be 63% (Lam et al., 1996). In this experiment, analyzing composite milk samples with the standard method resulted in the lowest sensitivity, and this method should be avoided in situations when false-negative results are undesirable (such as when introducing new animals to a herd or in segregation programs). Lam et al. (1996) improved the relative sensitivity of testing composite milk samples by using multiple samples and by utilizing a greater inoculum volume.

In this study, the sensitivity of Petrifilm Staph Express was equivalent to traditional techniques. Petrifilm may be preferred in situations where rapid decisions are required or when a sample-ready system is preferable (such as on-farm culture programs). In herds that have segregation programs to control *Staph. aureus*, evaluation of milk samples obtained from postpartum cows is often recommended. The use of Petrifilm in this situation may result in quicker diagnosis and allow better implementation of segregation plans.

The manufacturer's interpretive criteria for use of Petrifilm Staph Express suggest that the appearance of red-violet colonies on the initial incubation is presumptive evidence for diagnosis of *Staph. aureus*. Results of experiment 4 do not support this recommendation for milk samples and demonstrate the necessity of using the Staph Express disk for confirmation even when red-violet colonies are the only colonies present. Of plates classified as containing red-violet colonies (n = 35), only 8 were confirmed as *Staph. aureus*. Therefore, assessment of results of Petrifilm Staph Express should include the use of the confirmatory disk.

Diagnosis of *Staph. aureus* using Petrifilm Staph Express is dependent upon the ability of individuals to observe variations in colony color after the secondary incubation. Different intensities of pink zones appear around colonies after incubation with the Staph Express disk. In this study, no attempt was made to train individuals or standardize interpretive criteria of the zones. Results from this study demonstrate that the ability to accurately and carefully read zone intensity highly influences the specificity of this test. Thus, it is important that people reading the plates have excellent visual abilities and the ability to discern colors. In cases where different individuals will be reading plates, it is important that adequate training be performed to standardize the interpretation of color associated with the confirmatory test. Training should include observation of Petrifilm Staph Express plates (with and without the confirmatory disks) that have been inoculated with milk known to contain both *Staph. aureus* and CNS. In circumstances where false-positive results are highly undesirable, results of positive Petrifilm tests should be confirmed using traditional laboratory methods.

The use of Petrifilm products for on-farm culture programs is appealing because of the simplicity of use compared with standard microbiological methods. Variability observed in reading the Petrifilm plates demonstrates that standardization and training of personnel reading the plates is fundamental for achieving reliable results. Periodic assessment of accuracy of on-farm methods by submission of duplicate samples to specialized microbiology laboratories is recommended to minimize errors. Under these circumstances, the use of the Petrifilm method on farms would reduce the time between obtaining the milk sampling and obtaining results, and would allow farmers to use the results in control and treatment programs in herds with mastitis suspected to be caused by *Staph. aureus*.

CONCLUSIONS

Results from all 4 experiments show potential for the use of Petrifilm Staph Express Count plate as a diagnostic tool in a herd evaluation program when *Staph. aureus* is the pathogen of interest. Petrifilm was highly sensitive but varied in specificity according to interpretive criteria and level of training of people that read the plates. Results of these experiments highlight the importance of confirming some positive results of Petrifilm (weak pink zones) using standard laboratory methods in situations when high sensitivity and specificity are required. Results also suggest standardization of interpretive criteria is fundamental to achieve consistent results.

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