Validation of commercial luminometry swabs for total bacteria and coliform counts in colostrum-feeding equipment

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ABSTRACT

A sufficient quantity and quality of colostrum must be fed quickly to the newborn calf while minimizing bacterial contamination. Adenosine triphosphate bioluminescence swabs offer a potential rapid on-farm alternative to assess bacterial contamination of colostrum. The objective of this study was to validate the Hygiena (Camarillo, CA) AquaSnap Total (AS), SuperSnap (SS), PRO-Clean (PC), and MicroSnap Coliform (MS) swabs as well as visual hygiene assessment for detection of elevated bacterial counts in or on colostrum-feeding equipment. From April to October 2016, 18 esophageal tube feeders, 49 nipple bottles, and 6 pails from 52 dairy farms in Ontario were evaluated for cleanliness. Following visual hygiene assessment, sterile physiological saline (15 mL) was poured into each piece of equipment, mixed for 2 min to ensure total surface coverage, and poured into a sterile collection container through the feeding end. The fluid was split into equal aliquots, with one being evaluated by conventional culture and the other evaluated using the luminometry swabs. Nonparametric receiver operator curves were used to compare the test performance of the luminescence reading (relative light units; RLU) from each type of swab to conventional bacterial culture. The area under the curve comparing the AS swab to total bacterial count (cut point >100,000 cfu/mL) was 0.89, and using a cut point of 631 RLU correctly classified 84% of samples with a sensitivity of 88% and a specificity of 77%. The area under the curve comparing the MS swab to total coliform count (cut point >10,000 cfu/mL) was 0.85, and using a cut point of 44 RLU correctly classified 89% of samples with a sensitivity of 83% and a specificity of 90%. Visual hygiene assessment, PC and SS swabs were not reliable indicators for feeding equipment cleanliness. The results suggest that the AS and MS swabs can be used as an alternative to traditional

laboratory bacterial counts to evaluate cleanliness of colostrum-feeding equipment.

Key words: calf, colostrum, contamination

INTRODUCTION

Colostrum is an integral component of calf health management. Reduced mortality and morbidity (Wells et al., 1996; Windeyer et al., 2014; Raboisson et al., 2016), improved growth (Faber et al., 2005; Furman-Fratczak et al., 2011), earlier age at first calving (Faber et al., 2005) and increased milk production (DeNise et al., 1989; Faber et al., 2005) have all been shown to be benefits of successful passive transfer of colostrum antibodies. Despite the known importance of colostrum, failure of passive transfer remains an issue in both female (Trotz-Williams et al., 2008; Beam et al., 2009) and male dairy calves (Pardon et al., 2015). A contributing factor is the cleanliness of colostrum being fed to calves. Bacterial contamination of colostrum [i.e., total bacterial count (TBC) of >100,000 cfu/mL or total coliform count (TCC) of >10,000 cfu/mL (McGuirk and Collins, 2004; Morrill et al., 2012)] is common. Contaminated colostrum introduces bacteria that can bind or block IgG absorption (Staley and Bush, 1985; Baintner, 2007; Cummins et al., 2017) and can produce disease (Godden, 2008; Houser et al., 2008), so it is fundamental to feed colostrum with low bacterial counts.

Several critical control points can prevent contamination of colostrum (Stewart et al., 2005). The feeding process and equipment, whether nipple bottle or esophageal tube feeder, can introduce bacteria to colostrum. Although Stewart et al. (2005) failed to demonstrate a significant difference in bacterial contamination after passing colostrum through a tube feeder, the study was conducted on a dairy farm with excellent sanitation of equipment and concluded that if the feeding equipment is not appropriately cleaned between uses it could represent a source of bacterial contamination for colostrum.

Several methods are used to evaluate the cleanliness of feeding equipment. Visual assessment is a frequently

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Table 1. Visual hygiene assessment scoring chart used to assess contamination of colostrum feeding equipment

Score	Overall hygiene score	Milk/colostrum	Fecal material
1	Feeding equipment is visibly clean (i.e., no fecal material or milk/colostrum debris residue).	No milk/colostrum debris residue is visible.	No fecal material is visible.
2	Trace amounts of manure, milk/colostrum residue, or both, are visible.	Trace amounts of milk/colostrum residue are visible.	Trace amounts of manure residue are visible.
3	Manure, milk/colostrum residue, or both, is clearly visible.	Milk/colostrum residue is clearly visible.	Manure is clearly visible.
4	Manure contamination, milk/colostrum residue, or both, is extensive.	Milk/colostrum residue is extensive.	Manure contamination is extensive.

used method, but it has questionable efficacy to identify bacterial contamination (Malik, 2003; Willis et al., 2007). The most efficacious method to monitor bacterial contamination is through laboratory culture (McGuirk and Collins, 2004; Godden, 2008). The challenge with this method is that the results may not be available for several days, making timely demonstration of a cleanliness problem difficult.

Adenosine triphosphate bioluminescence offers a potential rapid on-farm alternative to traditional bacterial culture counts. This technology is well established in the food service industry and its use is rising in the health care industry (Shama and Malik, 2013). As ATP serves as an energy source for all plant, animal, and microbial cells, its presence indicates the presence of OM including bacterial contamination (Finger and Sischo, 2001; Amodio and Dino, 2014). Commercially available ATP swabs contain luciferase, an enzyme that catalyzes a chemical reaction which produces light proportional to the amount of ATP present (Finger and Sischo, 2001). The light intensity is captured by a luminometer that quantifies the intensity in relative light units (RLU; Shama and Malik, 2013).

The objective of this study was to validate the Hygiena (Camarillo, CA) AquaSnap Total (AS), Super-Snap (SS), PRO-Clean (PC), and MicroSnap Coliform (MS) swabs, as well as visual hygiene assessment, to detect elevated bacterial counts in colostrum feeding equipment.

MATERIALS AND METHODS

Experimental Design

This cross-sectional diagnostic accuracy study was conducted on dairy farms in Ontario supplying male calves to 2 veal operations. Each dairy farm was visited once, and during the farm visit, feeding equipment that was used to administer colostrum was randomly selected for further evaluation. The equipment was chosen by flipping a coin for each piece of available equipment until only a single piece of equipment had a result of

heads. Random selection was done for nipple bottles, pails, and esophageal tube feeders if available to a maximum of one per farm of each type of equipment. The surfaces of the equipment that would normally come into contact with the colostrum were scored visually (Table 1), receiving a hygiene score of 1 to 4 on the basis of gross contamination of the equipment surface(s) with milk residue or fecal material. Following visual assessment, 15 mL of sterile physiological saline (Veto-quinol Lavaltrie, Quebec, Canada) was dispensed into the equipment using a sterile 20-mL syringe. The fluid was gently mixed ensuring coverage of all the surface areas for 2 min and then poured into a sterile container (Fisher Scientific, Waltham, MA). The container was put immediately into a cooler with ice and transported to the University of Guelph where it was split into equal aliquots, with one submitted to the bacteriology laboratory at the Animal Health Laboratory for culture and the other evaluated using the luminometry swabs.

Bacterial Analysis

Total bacterial counts and TCC were performed with the 3M aerobic colony count (ACC) Petrifilm (3M, St. Paul, MN) and 3M coliform count (CC) Petrifilm (3M). The wash fluid was diluted serially in 9-mL tubes of PBS to create the following dilutions for ACC: 1:100, 1:1,000, 1:10,000, and 1:100,000; and the following dilutions for CC: neat (undiluted wash fluid), 1:10, 1:100, and 1:1,000. One-milliliter aliquots of each dilution were pipetted onto the respective ACC Petrifilm and CC Petrifilm. The ACC Petrifilm was incubated at 35°C for 48 h and following incubation, all red-colored colonies were counted to enumerate the TBC. The CC Petrifilm was incubated at 35°C for 24 h, and following incubation, all red-colored colonies with associated gas bubbles were counted to enumerate the TCC.

Luminometry Swabs

The PC, SS, AS, and MS swabs were used according to the manufacturer's directions (Hygiena) on each

Table 2. Descriptive summary of total bacterial count and total coliform count in colostrum feeding equipment measured using Petrifilm (3M, St. Paul, MN) in a diagnostic laboratory

Equipment type		Total bacterial count			Total coliform count		
		≤100,000	>100,000	>1,000,000	≤10,000	>10,000	>50,000
Nipple bottle	No.	19	30	20	39	10	4
	%	39	61	41	80	20	8
Esophageal feeder	No.	5	13	8	15	3	3
	%	28	72	44	83	17	17
Pail	No.	6	0	0	4	2	2
	%	100	0	0	67	33	33
Overall	No.	30	43	28	58	15	9
	%	41	59	38	79	21	12

sample of wash fluid. The product instructions can be found at <https://www.hygiene.com/food-and-beverage-atp-tests/>.

The Ensure luminometer (Hygiene) was used for all swabs except the PC that was assessed based on a color scale (green = clean; gray = caution; light pink = harmful; and dark purple = dangerous) provided with the swab.

Sample Size Calculation

The prevalence of bacterial contamination of colostrum feeding equipment was expected to be 50%. The sensitivity and specificity of the luminometry swabs were estimated to be 71 and 83%, respectively (Finger and Sischo, 2001). Using the method described by Buderer (1996) and assuming the clinically acceptable width of the 95% confidence intervals for sensitivity and specificity was to be no larger than 15%, the sample size required was determined to be 70 samples.

Statistical Analysis

Total bacterial count was used as the reference method when evaluating the diagnostic performance of visual hygiene score, SS swab, AS swab, and PC swab, and TCC was used as the reference method when evaluating the MS swab and the visual hygiene score. As the $\log_{10}$ bacteria counts were not normally distributed as identified by a significant Shapiro-Wilk test, a Spearman rank correlation coefficient (r) was calculated and a correlation plot was created to describe the relationship between the swabs and the $\log_{10}$ bacteria counts based on the respective reference method. Nonparametric receiver operator characteristic (ROC) curves were generated to assess the diagnostic performance when using a cut point of 100,000 and 10,000 cfu/mL for TBC and TCC, respectively. Diagnostic test characteristics [sensitivity (Se), specificity (Sp), and predictive values] were calculated at the cut point, which correctly classified the most tests. All statistical

analyses were performed using STATA 14 (StataCorp LP, College Station, TX).

RESULTS

Descriptive Statistics

A total of 52 dairy farms were visited from April to October of 2016. The participating farms had an average of 135 lactating cows (range: 35 to 520 lactating cows) and 73% housed their lactating cows in free-stall barns. During the visits, 18 esophageal tube feeders, 49 nipple bottles, and 6 pails were evaluated. Of the colostrum feeding equipment tested, 41% had a TBC of ≤100,000 cfu/mL and 79% had a TCC of ≤10,000 cfu/mL. A descriptive summary of the TBC and TCC is in Table 2.

AquaSnap

A strong positive correlation was observed between the AS swab RLU and $\log_{10}$ TBC ($r = 0.81$; $P < 0.01$; $n = 73$; Figure 1). The area under the ROC curve (AUC; Figure 2) was 0.89 (95% CI: 0.82–0.96), correctly classifying 84% of samples at a cut point of 631 RLU. Using this cut point, Se, Sp, and predictive values were calculated (Table 3).

SuperSnap

No significant correlation was observed between the SS swab RLU and $\log_{10}$ TBC ($r = -0.04$; $P = 0.71$; $n = 73$). The AUC was 0.49 (95% CI: 0.35–0.64), and using a cut point of 64 RLU, 63% of samples were correctly classified. The Se, Sp, and predictive values are presented in Table 3.

MicroSnap

The MS swab RLU had a moderate positive correlation with the $\log_{10}$ TCC ($r = 0.42$; $P < 0.01$; $n =$

Figure 1. Relationship between log₁₀ total bacterial count (TBC) determined by Petrifilm (3M, St. Paul, MN) and relative light units (RLU) determined by a commercial luminometry assay (AquaSnap, Hygiena, Camarillo, CA).

Figure 2. Receiver operator characteristic (ROC) curve comparing relative light units determined by a commercial luminometry assay (AquaSnap, Hygiena, Camarillo, CA) to total bacterial count determined by Petrifilm (3M, St. Paul, MN).

73; Figure 3). The AUC (Figure 4) was 0.85 (95% CI: 0.69–1.00), correctly classifying 89% of samples at a cut point of 44 RLU. The Se, Sp, and predictive values using this cut point are in Table 3.

PRO-Clean

The PC swab color scale had a negligible negative correlation with the log₁₀ TBC ($r = -0.19$; $P = 0.12$; $n = 69$). The ROC curve generated an AUC of 0.49, and

using a cut point of a gray color or greater, only 44% of samples were correctly classified. The Se, Sp, and predictive values using this cut point are in Table 3.

Visual Assessment

Visual inspections on all farms were completed by a single observer. A weak positive relationship was observed between overall hygiene score and log₁₀ TBC (r

Table 3. Diagnostic test characteristics of luminometry swabs and visual hygiene assessment, relative to bacterial culture using Petrifilm (3M, St. Paul, MN) in a diagnostic laboratory

Item	Reference method ¹	TP ² (%)	Cutpoint ³	Sensitivity ⁴ (%)	Specificity ⁵ (%)	NPV ⁶ (%)	PPV ⁷ (%)
AquaSnap ⁸	TBC	58.9	631 RLU	88.4	76.7	84.5	82.1
SuperSnap ⁸	TBC	58.9	64 RLU	83.7	30.0	56.3	63.2
MicroSnap ⁸	TCC	16.4	44 RLU	83.3	90.2	96.5	62.4
PRO-Clean ⁸	TBC	57.1	≥Grey	15.0	83.3	40.5	56.5
Overall visual ⁹	TBC	58.9	≥3	30.2	80.0	44.3	68.5
Fecal visual ¹⁰	TCC	16.4	≥3	0.0	93.4	39.4	0.0

¹TBC = total bacterial count determined using Petrifilm (3M) in a diagnostic laboratory; TCC = total coliform count determined using Petrifilm (3M) in a diagnostic laboratory.

²True prevalence (TP) = percentage of samples tested with a TBC >100,000 cfu/mL or TCC >10,000 cfu/mL.

³RLU = relative light units.

⁴The probability of testing positive for bacterial contamination using luminometry swabs or visual hygiene assessment, given the feeding equipment has a level of TBC >100,000 cfu/mL or TCC >10,000 cfu/mL.

⁵The probability of testing negative for bacterial contamination using luminometry swabs or visual hygiene assessment, given the feeding equipment has a level of TBC ≤100,000 cfu/mL or TCC ≤10,000 cfu/mL.

⁶Negative predictive value = probability the feeding equipment has a level of TBC ≤100,000 cfu/mL or TCC ≤10,000 cfu/mL, given the feeding equipment tested negative for bacterial contamination according to luminometry swabs or visual hygiene assessment.

⁷Positive predictive value = probability the feeding equipment has a level of TBC >100,000 cfu/mL or TCC >10,000 cfu/mL, given the feeding equipment tested positive for bacterial contamination according to luminometry swabs or visual hygiene assessment.

⁸Hygiena, Camarillo, CA.

⁹Overall hygiene score created through visual assessment of feeding equipment (Table 1).

¹⁰Fecal material score created through visual assessment of feeding equipment (Table 1).

Figure 3. Relationship between $\log_{10}$ total coliform count (TCC) determined by Petrifilm (3M, St. Paul, MN) and relative light units (RLU) determined by a commercial luminometry assay (MicroSnap, Hygiena, Camarillo, CA).

= 0.28; $P = 0.02$; $n = 73$). The AUC was 0.61 (95% CI: 0.44–0.79), correctly classifying 71% of samples at a cut point of 3 or greater. Using this cut point, Se, Sp, and predictive values were generated (Table 3). The correlation between fecal hygiene score and $\log_{10}$ TCC was negligible ($r = -0.05$; $P = 0.69$; $n = 73$), and the AUC was 0.45 (95% CI: 0.33–0.57), correctly classifying 78% of samples at a cut point of 3 or greater. The Se, Sp, and predictive values were generated using this cut point (Table 3).

Figure 4. Receiver operator characteristic (ROC) curve comparing relative light units determined by a commercial luminometry assay (MicroSnap, Hygiena, Camarillo, CA) to total coliform count determined by Petrifilm (3M, St. Paul, MN).

DISCUSSION

This study demonstrates that bacterial contamination of colostrum feeding equipment is common and that luminometry swabs provide a reasonable alternative to traditional laboratory culture to assess contamination. The study also demonstrates that visual hygiene assessment of feeding equipment is a poor indicator of bacterial contamination. The level of bacterial contamination could be overestimated because the majority of sampling occurred in the summer and season of sampling has been demonstrated to be a risk factor for elevated bacterial contamination of colostrum (Fecteau et al., 2002). Pouring liquid through the colostrum feeding equipment was used to mimic the passage of colostrum and may provide more reliable results when compared with dry swabbing as this method ensures total coverage of the surface area of the feeding equipment. However, no literature is available comparing the 2 methods to evaluate colostrum feeding equipment and more research is necessary to identify the best method for this purpose.

With 59% of feeding equipment wash fluid having >100,000 cfu/mL TBC and 21% having >10,000 cfu/mL, cleanliness of the feeding equipment should be improved. As Stewart et al. (2005) noted, if feeding equipment is not cleaned appropriately it could lead to contaminated colostrum. The proper method to clean feeding equipment is to disassemble equipment, rinse in lukewarm water, place in hot water detergent, scrub all surfaces with a brush, rinse in hot water with acid sanitizer, and let dry. If this procedure is followed, the amount of contamination should be limited when colostrum is fed through the equipment (Stewart et al., 2005). It is interesting to note that the nipple bottles and tube feeders had a higher amount of TBC contamination, whereas the pails had higher TCC contamination. This could reflect the difficulty in disassembly and cleaning of the nipple bottles and tube feeders and the inability to reach the difficult-to-clean areas. The pails are likely more prone to fecal contamination as the open tops lead to greater chance of manure splashing into the pails.

Visual assessment was an unreliable indicator of feeding equipment cleanliness. When using visual assessment, many false negatives occurred, demonstrating that many pieces of equipment that appeared clean were in fact contaminated. These findings are similar to what has been described in hygiene assessment of surfaces in hospitals, where visual assessment was a poor indicator of standards of cleanliness (Griffith et al., 2000; Cooper et al., 2007). As many producers and industry advisors use visual assessment as a method to evaluate hygiene, it is important to dispel this method.

Adenosine triphosphate bioluminescence provides a viable alternative to both visual assessment and bacterial culture. The ability to perform the entire process in minutes and immediately demonstrate the level of contamination to producers makes these swabs a practical mechanism to evaluate the cleanliness of feeding equipment. This technology, despite the initial cost of the luminometer, could be used as part of a calf health monitoring program provided by herd advisors.

The AS and MS performed well when compared with TBC and TCC, respectively. Both swabs had positive moderate to strong correlation to their specific bacteria counts and showed a reasonable performance based on Se, Sp, and predictive values. The SS swab did not perform well. The SS had a poor Sp with many of the negative samples having positive test results. To increase the Sp, the RLU cut point could be increased; however, this would be at the expense of sensitivity. The main purpose of the PC swab was to detect protein residue as a surrogate measure of surface contamination, and cross-reaction with colostrum and milk residues could explain the swab's overall poor performance in identifying bacterial contamination of colostrum feeding equipment.

The performance of the AS and MS swabs was similar to other studies evaluating ATP bioluminescence as a tool to identify microbial contamination in food stuff such as liquid milk and bottled water (Deininger and Lee, 2001; Luo et al., 2009; Meighan, 2014) and hospital equipment (Sciortino and Giles, 2012; Alfa et al., 2013). The reduced level of Sp that was seen with the AS, SS, and PC could be due to somatic cells or other nonmicrobial sources of ATP creating a bioluminescence background (Bottari et al., 2015). However, as it is a greater risk not to identify a truly contaminated sample than to have a false positive, the higher levels of sensitivity may overcome the limitation of low Sp.

CONCLUSIONS

Most the colostrum feeding equipment evaluated had bacterial contamination above the suggested target of >100,000 cfu/mL of TBC and >20% exceeded the target of >10,000 cfu/mL of TCC. The AS and MS performed well when evaluating wash fluid from the equipment and provide a rapid on-farm alternative to traditional laboratory methods to quantify the degree of bacterial contamination of colostrum feeding equipment.

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