Short communication: Diagnostic accuracy of the Petrifilm culture system for identifying colostrum with excessive bacterial contamination in Quebec dairy herds

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ABSTRACT

The objective of this study was to validate the diagnostic accuracy of the Petrifilm culture system (3M, St. Paul, MN) for identifying colostrum with excessive bacterial contamination. An observational cross-sectional study was conducted between October 2015 and February 2016. Two colostrum aliquots were collected during the first meal of 332 calves (33 commercial Holstein dairy farms) in Quebec, Canada. One aliquot per calf was used to quantify the total bacteria count and the total coliform count using standard bacteriological laboratory testing (reference test). These results were dichotomized to identify colostrum with excessive bacterial contamination [aerobic count plate (AC) >100,000 cfu/mL; coliform count plate (CC) >10,000 cfu/mL]. The Petrifilm system was used to quantify both aerobic and coliform contamination of the other colostrum aliquot from each calf. As such, AC and CC were used according to the manufacturer’s recommendations. The area under the curve of the receiver operating characteristic curve of AC and CC compared with the laboratory were 0.83, and 0.95, respectively. Using the optimal threshold of >24,000 cfu/mL for AC results, the Petrifilm system had a sensitivity (Se) of 69%, specificity (Sp) of 86%, and a kappa value of 0.54. Using the optimal threshold of >4,000 cfu/mL for CC results, the Petrifilm system had a Se of 93%, Sp of 90%, and kappa value of 0.64. Overall, these results suggest that the Petrifilm system is an appropriate alternative for identifying colostrum with excessive bacterial contamination.

Key words: bacteria, coliform, colostrum, Petrifilm

Short Communication

An adequate intake of high-quality colostrum is an essential aspect of calf health management. The transfer of colostral immunoglobulins provides temporary protection against infectious diseases, reducing calves’ risks of mortality and morbidity at a young age (Godden et al., 2009). In addition, the benefits of good-quality colostrum are multiple, including improved growth (Robison et al., 1988) and milk production, as well as earlier age at first calving (Faber et al., 2005). To ensure that immunoglobulins are adequately absorbed, avoiding excessive bacterial contamination of the colostrum fed is important (Gelsinger et al., 2015). It is known that bacteria can bind to nonspecific receptors of neonatal enterocytes, thereby decreasing the number of receptors available for the absorption of IgG (Staley and Bush, 1985). It is recommended that colostrum samples contain a total bacterial count (TBC) ≤ 100,000 cfu/mL and a total coliform count (TCC) ≤ 10,000 cfu/mL (McGuirk and Collins, 2004). Colostrum contamination above these thresholds has been associated with increased risk of developing pneumonia (Mellado et al., 2017). The bacterial contamination found in colostrum can originate from different sources, including the mammary gland per se, milking technique, storage and feeding equipment cleanliness, and bacterial overgrowth in stored colostrum (Stewart et al., 2005; Cummins et al., 2017). Currently, the standard test for measuring colostrum contamination is a bacteriological count conducted in the laboratory. This technique requires the sample to be shipped from farm to laboratory and is relatively expensive [~CAN$20 (~US$15)].

If an on-farm culture system such as the Petrifilm system (3M, St. Paul, MN)—which can assess TBC and TCC and is already validated and used for under healthcare management programs (Cameron et al., 2013)—could be used to assess colostrum contamination, it could provide a more timely and affordable [~CAN$8 (US$6)] alternative to the current standard laboratory test. This diagnostic procedure would provide a practical alternative colostrum microbiological
quality monitoring. Therefore, the objective of this study was to validate the performance of an on-farm bacteriological culture system for identifying excessive bacteriological contamination in colostrum fed to newborn calves in Quebec dairy herds.

A cross-sectional observational study was conducted using colostrum samples collected from commercial Holstein dairy herds located near the bovine ambulatory clinic of the Faculté de médecine vétérinaire of the Université de Montréal (St-Hyacinthe, QC, Canada). Herd owners were recruited by convenience based on their interest in participating in a research study on colostrum contamination. Data collection was performed between October 2015 and February 2016. The Animal Care Committee of the Université de Montréal approved this study (protocol number 16-Rech-1854). To participate, each herd owner was asked to collect two 10-mL samples ( aliquots) of the first colostrum meal fed to each calf; samples were collected directly from the feeding bottle, bucket, or esophageal feeding tube immediately before the first feeding. The colostrum samples were frozen by the farmers (−18°C) and kept frozen until the bacteriological analysis. For each calf enrolled, one colostrum aliquot was sent to the diagnostic laboratory service for standard bacteriological culture (Université de Montréal), whereas the other was cultured in the in-house laboratory of the bovine ambulatory clinic (Université de Montréal) using the 3M Petrifilm system. A sample size of 300 calves was estimated based on fitting most scenarios to find a kappa value that was at least moderate (>0.4, using a true kappa value from 0.5 to 1.0 with a 0.1 increment and various scenarios of contamination prevalence varying between 10 and 50% (10% increment steps)] with a statistical power of 80% and a type 1 error of 5% (IRR package, N.cohen, kappa argument, R software (R 4.0.3), R Foundation for Statistical Computing, Vienna, Austria).

At the diagnostic laboratory service (Université de Montréal), the colostrum samples were thawed and mixed. The samples were transferred, using sterile pipette tips, into sterile plastic tubes to perform 1:10 and 1:1,000 dilutions with sterile water. Using a calibrated 10-μL loop, 2 Columbia agar plates (one for 1:10 dilution and the other for 1:1,000 dilution) and 1 MacConkey agar plate (1:10 dilution) were inoculated. The Columbia agar plates were incubated for 48 h at 35 ± 2°C in an atmosphere enriched with 5% CO2, and the MacConkey agar plates were incubated for 24 h at 35 ± 2°C under aerobic conditions. After incubation, colony counting of the agar plates was performed. The resulting count was then multiplied by 1,000 for the 1:10 dilution and by 100,000 for the 1:1,000 dilution, to obtain the number of cfu/mL of the colostrum samples.

At the in-house laboratory, 2 culture media from the Petrifilm system were used. The first was the aerobic bacteria count plate (AC, 3M Petrifilm Aerobic Count Plate), composed of standard methods nutrients, a cold water–soluble gelling agent, and an indicator that simplifies colony counting (3M Food Safety, 2017a). The second was the coliform count plate (CC, 3M Petrifilm Coliform Count Plate), containing violet red bile nutrients, a cold water–soluble gelling agent, and an indicator that facilitates colony counting (3M Food Safety, 2017b). Once the samples were thawed at room temperature, they were thoroughly mixed and transferred to sterile plastic tubes to perform a 1:1,000 dilution with sterile water. One milliliter of the diluted solution was placed on the AC and the CC. The plates were incubated at 38°C for 48 h in the case of the AC and 24 h in the case of the CC. The colony-forming units were quantified; red-colored colonies were counted to enumerate the TBC, and red-colored colonies with gas trapped around were counted to enumerate TCC (3M Food Safety, 2017a,b).

Statistical analyses were performed using SAS (version 9.4, SAS Institute Inc., Cary, NC) and MedCalc Statistical Software (version 19.0.3, MedCalc Software Bvba, Ostend, Belgium). Descriptive statistics were computed for TBC and TCC. The test characteristics of the 3M Petrifilm system were calculated using 2 × 2 frequency tables (Proc FREQ in SAS). Sensitivity (Se) was defined as the proportion of colostrum with excessive contamination, as determined by the standard bacteriological culture (TBC > 100,000 cfu/mL and TCC >10,000 cfu/mL), which was classified as excessively contaminated by the Petrifilm system (i.e., Petrifilm value > Petrifilm threshold). Conversely, specificity (Sp) was defined as the proportion of colostrum with non-excessive contamination (TBC ≤ 100,000 cfu/mL and TCC ≤10,000 cfu/mL), as determined by the reference test that was classified as not excessively contaminated by the Petrifilm system (i.e., Petrifilm value < Petrifilm threshold). Using these frequency tables, Se, Sp, positive predictive value, and negative predictive value were calculated.

A receiver operating characteristic curve (Mensik et al., 1978) was built to identify the Petrifilm system thresholds (one for AC and one for CC) with the optimal combination of Se and Sp (i.e., maximum sum of Se + Sp) and to determine the discrimination ability of the test by calculating the area under the curve (Meale et al., 2017). A frequency table was used to compare the optimal Petrifilm system threshold with the TBC and TCC results (Proc FREQ in SAS).

To visualize the agreement between the Petrifilm system and the standard laboratory results, a correlation...
The firstcolostrum meal of 332 calves was collected from 33 Holstein commercial farms (1 to 18 samples per farm, median = 12 samples per farm, mean = 10 samples per farm). Colostrum samples were frozen in the hour following colostrum meal 94% of the time. Table 1 presents the Se, Sp, positive predictive value, negative predictive value, and kappa values of the Petrifilm system optimal threshold to identify colostrum with excessive bacterial contamination in colostrum based on the standard bacteriology test results of TBC > 100,000 cfu/mL and TCC > 10,000 cfu/mL. The area under the curve of the receiver operating characteristic curve for AC compared with the standard bacteriology test (>100,000 cfu/mL) was 0.83 (Figure 1A). The area under the curve for CC compared with the standard bacteriology test (>10,000 cfu/mL) was 0.95 (Figure 1B).

According to the laboratory results (reference standard test), TBC and TCC for the 332 colostrum samples ranged from 0 to 5,000,000 cfu/mL (median: 200,000 cfu/mL) and 0 to 5,000,000 cfu/mL (median: 0 cfu/mL), respectively. The proportions of excessively contaminated colostrum with aerobic bacteria (TBC > 100,000 cfu/mL) and coliforms (TCC > 10,000 cfu/mL) were 50% and 13% in this study, respectively. When using the Petrifilm system, TBC and TCC ranged from 0 to 1,740,000 cfu/mL (median: 15,000 cfu/mL) and 0 to 1,000,000 cfu/mL (median: 0 cfu/mL), respectively. The proportions of excessively contaminated colostrum with aerobic bacteria (TBC > 24,000 cfu/mL) and coliforms (TCC > 4,000 cfu/mL) were 42% and 20%, respectively. The agreement between the Petrifilm system counts and the laboratory counts were moderate for TBC and strong for TCC using optimal thresholds (Table 1). The intraclass correlation values were 0.57 (0.47–0.66) for TBC and 0.64 (0.55–0.71) for TCC, indicative of moderate reliability. The agreement and disagreement between the laboratory results (reference standard test) and TBC and TCC are presented in Figure 2.

The present study is one of the first to describe the diagnostic accuracy of an on-farm bacteriology culture system to identify excessive aerobic and coliform bacterial contamination in colostrum. Our results showed that the Petrifilm system offers a good alternative to the traditional bacteriology laboratory test. The intraclass correlation values indicated a moderate reliability of AC and CC when evaluated on a continuous scale. Our results showed that the Petrifilm system tended to underestimate the bacterial concentration of colostrum when compared with standard laboratory results (Figure 2A). This observation was supported by the fact that there was a negative bias at high TBC values. This implies that, to maximize the accuracy of the diagnostic test, a lower threshold than that used by the laboratory test (reference test) should be used. The outcome for underestimating bacterial concentration of colostrum with the Petrifilm system is that producers may unknowingly feed some contaminated colostrum.

When used to properly identify colostrum samples with excessive bacterial contamination, the Petrifilm system using an AC threshold of >24,000 cfu/mL and a CC threshold of >4,000 cfu/mL had high Sp (86% and 90%, respectively). A test with a high Sp decreases the risk of having colostrum falsely classified as excessively contaminated (false positive). The Se was moderate for AC (69%) and good for CC (93%). A test with good Se decreases the chances of having colostrum falsely classified as non-excessively contaminated (false negative). As a result, dairy producers using the Petrifilm system may estimate the proportion of excessively contaminated colostrum samples with the idea that few contaminated colostrum samples

<table>
<thead>
<tr>
<th>Petrifilm test</th>
<th>Threshold (cfu/mL)</th>
<th>Se (%)</th>
<th>Sp (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&gt;24,000</td>
<td>69 (62–76)</td>
<td>86 (80–91)</td>
<td>83 (77–89)</td>
<td>73 (67–79)</td>
<td>0.54 (0.45–0.63)</td>
</tr>
<tr>
<td>CC&lt;sup&gt;2&lt;/sup&gt;</td>
<td>&gt;4,000</td>
<td>93 (84–100)</td>
<td>90 (86–93)</td>
<td>55 (43–67)</td>
<td>99 (98–100)</td>
<td>0.64 (0.53–0.75)</td>
</tr>
</tbody>
</table>

<sup>1</sup>AC = 3M Petrifilm aerobic count plate; 3M, St. Paul, MN.

<sup>2</sup>CC = 3M Petrifilm coliform count plate; 3M, St. Paul, MN.
would be missed. However, the false positive fraction may be a problem in herds with a very low prevalence of excessive contamination. Given that neither test is 100% accurate, their use at the individual sample level may lack accuracy. However, obtaining the apparent prevalence of contaminated colostrum from multiple samples would be useful to assess the true prevalence of contamination (Dohoo et al., 2007). Therefore, the use of the Petrifilm system would be more relevant at the herd level, similar to monitoring the transfer of passive immunity (Lombard et al., 2020). Monitoring the bacterial contamination of colostrum administered to calves is an important step in colostrum management protocols (Godden et al., 2019). If excessive bacterial contamination is a problem, the next step would be to review the colostrum harvesting, storage, handling, and feeding processes.

From a practical point of view concerning the use of the Petrifilm system, counting the number of aerobic bacteria with AC is facilitated by the presence of an indicator dye that colors the colonies in bright pink (3M Food Safety, 2017a). Furthermore, counting coliforms with CC is facilitated by the presence of gas trapped around the red colonies, which makes it easier to identify coliforms (3M Food Safety, 2017b). Furthermore, identification of samples with >24 colonies for AC and >4 colonies for CC makes it easy to identify colostrum with excessive contamination. As a result, producers and farm employees would need only minimal training to use this system. Cameron et al. (2013) reported a high level of agreement between the producer-derived Petrifilm system results and the automated Petrifilm reader results when used in milk for the identification of intramammary infection. One may speculate that the same would be true of colostrum contamination, but this remains to be confirmed.

In this study, excessive aerobic bacterial contamination of the first colostrum meal fed to calves occurred frequently, as 50% of the colostrum samples had TBC > 100,000 cfu/mL, as measured by the reference method. These results were higher than previously reported in Quebec, where 36% of the colostrum samples analyzed with tryptocase soy agar were contaminated (Fecteau et al., 2002), as well as slightly higher (43%) than those reported in a United States study (Morrill et al., 2012). With regard to methods, as the colostrum samples are collected by the farmer, the method used by each person may vary between farms (not standard), and further bacterial contamination during the collection process could have occurred. The higher prevalence observed in this study may be partly due to the fact that the colostrum samples are collected by the farmer, so the method used by each person may vary from farm to farm (nonstandard). Additional bacterial contami-

![Figure 1](image-url)
Figure 2. (A) Agreement (purple squares) and disagreement (red squares) between total bacteria count and standard bacteriology test (reference test). (B) Agreement (purple squares) and disagreement (red squares) between coliform count and the standard bacteriology test (reference standard test).
nation during the collection process might also have occurred.

Season has been shown to be a risk factor for identifying excessive colostrum contamination on farms, contamination levels being lower during the winter than the summer (Fecteau et al., 2002). The current study was not designed to compare the prevalence of colostrum contamination between seasons, but it should be noted that most of our samples were collected during the cold season.

In conclusion, we found that, despite the Petrifilm system and standard bacteriology test methods failing to give identical results, the Petrifilm system was an appropriate alternative method for identifying colostrum with excessive bacterial contamination on dairy farms. This information is especially useful for dairy producers and veterinary clinics that are already using the Petrifilm system for other purposes.

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