Comparison of Dry Culture Medium and Conventional Plating Techniques for Enumeration of Bacteria in Pasteurized Fluid Milk

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ABSTRACT

Standard plate counts, psychrotrophic bacterial counts, and coliforms were determined by conventional plating techniques and by Petrifilm™ plates, a dry culture medium, for 48 commercially processed milk samples (24 whole milk and 24 skim milk). The Petrifilm SM plate counts were compared with counts on standard methods agar for the standard plate count, psychrotrophic bacterial count, and rapid psychrotrophic bacterial count. The Petrifilm violet red bile plate counts were compared with counts on violet red bile agar for coliform test with a solid medium and the preliminary incubation method for detection of coliforms. Standard plate counts were determined within 24 h of packaging and after 7, 10, and 14 d of storage at 6.1°C. Psychrotrophic bacterial counts and coliform counts were determined with 24 h of packaging and after 7 d storage. There was a strong linear relationship between Petrifilm SM and standard methods agar plates (excluding counts on samples plated within 24 h of packaging) and for the psychrotrophic bacterial count method. Petrifilm SM had a weak linear relationship with Standard Methods Agar plates for the rapid psychrotrophic bacterial count. Coliform counts determined on Petrifilm violet red bile plates were generally within the same range as counts on violet red bile agar plates. The positive predictive values for the Petrifilm violet red bile plates and violet red bile agar plates were essentially the same for samples plated within 24 h of packaging.

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INTRODUCTION

Agar plating methods for enumeration of bacteria date back to the turn of the century. Recently, several other techniques to enumerate bacteria have been developed. In 1984, Petrifilm SM and violet red bile (VRB) (dry media film) were evaluated for determining total bacterial (3) and coliform (8) counts, respectively, in raw milk. Total bacteria count and coliform count were compared with those obtained by conventional methodology in a collaborative study. These earlier studies indicated that the Petrifilm Plate methods had potential as an alternative to conventional agar plating methods for raw milk analyses.

Petrifilm plates consist of nutrients or selective and differential agents coated on films, along with a cold-water-soluble gelling agent and triphenyl tetrazolium chloride. Colony counts were accomplished by 1.0 ml of appropriate dilutions plated directly onto the Petrifilm Plates, spread of inoculum by pressure to an overlay film with a plastic spreader, and incubation of inoculated plates under the same conditions used for conventional plating techniques.

The primary objective of this research was to evaluate Petrifilm plates as a suitable alternative to conventional plating techniques for enumerating total and psychrotrophic bacteria, and coliforms in commercially pasteurized skim and whole milk. This study incorporated various methods available to the dairy industry today. The standard plate count (SPC) is considered the reference method for estimation of bacterial populations in most dairy products (10). Psychrotrophic bacteria are usually considered responsible for most spoilage problems in fluid milk and therefore are of particular interest to the dairy industry. The SPC and both the psychrotrophic and rapid psychrotrophic bacterial count methods employ standard methods agar (SMA). However, incubation times and temperatures are modified in the latter methods to promote the growth of psychrotrophs (9, 10). Coliform detection is
used in the dairy industry to indicate post-
pasteurization contamination. The coliform test
with a solid medium is a standard method in
the dairy industry today (10). Additionally,
Ledford et al., (5, 6) have shown that the
preliminary incubation method detects coil-
forms that will grow over the shelf-life of fluid
milk and thus provide a good index of storage
quality. Incubation parameters of the latter
method have been modified to enhance coli-
form growth. Both methods employ VRB agar
as the plating medium.

MATERIALS AND METHODS

Experimental Design

The study was designed as a paired com-
parison; all tests were performed in duplicate.
The SMA (BBL Microbiology Systems, Becton
Dickinson and Co., Cockeysville, MD) pour
plate was the reference in evaluation of the
Petrifim SM plate (PSM; Medical-Surgical
Division/3M, St. Paul, MN). The VRB agar
(BBL Microbiology Systems, Becton Dickinson
and Co., Cockeysville, MD) pour plate, with an
overlay, was the reference in evaluation of the
Petrifilm VRBA plate (PVRB; Medical-Surgical
Division/3M, St. Paul, MN).

Sample Selection and Preparation

On the day of processing, whole and skim
milk samples from 24 different commercial
processing plants were collected and trans-
ported to the laboratory on ice. Each sample
was aseptically split and labeled so that aliquots
could be tested. Stored samples were held at
6.1°C representing the mean temperature in
supermarket display cases (7).

Appropriate samples were serially diluted in
phosphate and magnesium chloride dilution
water (10). The primary dilution was prepared
by addition of 11.0 ml of milk to 99.0 ml of
diluent and so forth. Up to three dilutions were
assessed for each sample and test.

Tests Performed

The SMA pour plates and (PSM) plates were
employed for the following tests: 1) SPC (10)
on the samples within 24 h of and 7, 10, and 14
d after packaging; 2) psychrotrophic bacterial
count (PBC) (10) and 3) rapid psychrotrophic
bacterial count (RPBC) (9) on samples within
24 h of and 7 d after packaging. The VRBA
pour plates with an overlay and PVRB plates
were employed for coliform enumeration
according to the following tests: 1) Coliform
test with a solid medium (COLI) (10) on
samples within 24 h of and 7 d after packaging
and the preliminary incubation method for
detection of coliforms (PI) (5) on samples
within 24 h of packaging. For each coliform
enumeration test, five colonies (when available)
were selected randomly from each of the
plating media and confirmed in 2% brilliant
green lactose bile broth (BGLB; BBL Micro-
biology Systems, Becton Dickinson and Co.,
Cockeysville, MD).

Figure 1 gives a complete schematic de-
scription of the testing program.

Statistical Analyses

Bacterial counts were first converted to
log_{10} to match more nearly the underlying
statistical assumptions. Standard regression
methods (11) were used to construct least
squares regression lines and 95% confidence
limits. Repeatability was calculated as coef-
icient of variation between replicates (standard
deivation expressed as percentage of mean).
For analysis of coliform data, a positive predic-
tive value was calculated as the proportion of
the number of colonies identified as coliforms
by a confirmatory test compared with the
number of colonies considered to be coliforms
by the presumptive test.

RESULTS AND DISCUSSION

Statistical analysis indicated that the type of
milk (whole or skim) had no effect on the
relationship between dry culture medium and
conventional plating techniques. Therefore,
combined data for whole and skim milk are
provided.

The SMA mean log counts for the SPC were
significantly higher than PSM tested on d 0, 7,
and 14. Differences between SMA and PSM
were less on d 7 and 14 (significantly different
at P<.05 but not P<.01). The slope and inter-
cept of the regression line of the PSM and SMA
for d 0 were significantly different from 1 and
0, respectively, indicating a lack of equivalence
of the two methods when samples were plated
within 24 h of packaging. No differences
were significant for slopes or intercepts for d 7
Each Milk Sample
Three Dilutions (when appropriate) Plated in Duplicate
(Whole and Skim from Each of 24 Plants)

Petrifilm SM and Standard Methods Agar

SPC (d 0, 7, 10, 14)  
PBC (d 0, 7)  
RPBC (d 0, 7)

Plate and Incubate at 32 ± 1°C for 48 h
Plate and Incubate at 7 ± 1°C for 10 d
Plate and Incubate at 21°C for 25 h

Record All Counts

Petrifilm VRB and Violet Red Bile Agar

Coli (d 0, 7)  
PI (d 0)

Incubate Sample at 37°C for 6 h

Plate and Incubate at 32 ± 1°C for 24 ± 2 h

Confirm 5 Colonies in BGLB

Incubate at 32 ± 1°C for 48 ± 3 h

Record Results

Figure 1. Schematic of Testing Program. Abbreviations: SPC, standard plate count; PBC, psychrotrophic bacterial count; RPBC, rapid psychrotrophic bacterial count; VRB, violet red bile; COLI, coliform count; PI, preliminary incubation coliform count, and BGLB, brilliant green lactose broth.

to 14 (Table 1). These data are presented in Figure 2, 3, 4, and 5 with regression lines and 95% confidence limits.

The difference between PSM and SMA counts on d 0 may be related to the construction of the PSM plate as well as the flora of the samples. Lower counts on PSM are likely related to the comparative repair and growth rate of injured organisms on SMA and PSM. Small changes in medium have had significant effects on counts (1, 4, 12). The changes in growth medium from an agar plate to a thin gel contained between two films may be sufficient alteration in the microenvironment to change the repair rate of some microorganisms. To use PSM plates to test pasteurized milk additional time for the repair and growth of injured organisms may be necessary. In a trial with 10 fresh milk samples, increase of incubation time from 48 to 72 h resulted in no significant increase in SMA, but PSM count increased so as not to be significantly different from SMA incubated for either 48 or 72 h (P<.01). Incubation time and temperature are also critical factors, as indicated by the similarity of the PBC results for PSM and SMA on samples within 24 h of packaging. Although PBC counts on PSM tended to be slightly lower than SMA, there was a strong linear relationship.

Although the results for SPC in this study differ from those in the collaborative study (2), the samples in the collaborative study were entirely different in their preparation and make-up than the commercially pasteurized samples used in this study. Milks in the collaborative study were sterilized and then spiked...
TABLE 1. Comparison of standard plate counts for whole and skim milks by standard methods agar (SMA) and Petrifilm SM (PSM) plates.

<table>
<thead>
<tr>
<th>Days of storage at 6.1°C</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>No. of samples</td>
<td>47</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>Mean log SMA count/ml</td>
<td>2.82</td>
<td>5.34</td>
<td>6.29</td>
</tr>
<tr>
<td></td>
<td>(0.57)</td>
<td>(2.22)</td>
<td>(2.51)</td>
</tr>
<tr>
<td>Mean log PSM count/ml</td>
<td>2.38</td>
<td>5.04</td>
<td>6.19</td>
</tr>
<tr>
<td></td>
<td>(0.41)</td>
<td>(2.24)</td>
<td>(2.47)</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>.78</td>
<td>.98</td>
<td>.99</td>
</tr>
<tr>
<td>Slope</td>
<td>.56</td>
<td>.99</td>
<td>.98</td>
</tr>
<tr>
<td>Intercept</td>
<td>.79</td>
<td>-.23</td>
<td>.05</td>
</tr>
</tbody>
</table>

1 Standard deviations are in parentheses.

with viable mixed cultures of *Escherichia coli* and *Staphylococcus aureus* or *E. coli* and *Streptococcus lactis*. These organisms are somewhat different from the psychrotrophic organisms (notably *Pseudomonas* and *Enterobacter species*) commonly found in commercially processed fluid milk supplies. Samples for the collaborative study would not likely have contained injured organisms. If the commercially processed samples in the latest study were sterilized and spiked with similar organisms, the results seemingly would be comparable.

Duplicate SMA and PSM plates with actual numerical counts within the 30 to 300 count range were examined for repeatability. No significant difference was found between the overall repeatability of PSM (CV = 3.4%) and SMA plate (CV = 2.6%).

Comparisons of PBC and RPBC are provided in Table 2. For the PBC, SMA mean log counts were significantly higher than PSM at d 0, but counts at this time were very low. At d 7, no significant difference was found. Slopes and intercepts from the regressions for both days were not significantly different from 1 and 0, respectively. A strong linear relationship between SMA and PSM was evident for the PBC method. The SMA counts tended to be slightly higher, but slopes close to 1 indicated that the
difference between PSM and SMA was constant over the entire range of counts.

The regression and correlations for the RPBC method indicated a poor linear relationship between SMA and PSM. Slopes were significantly different from 1 at .51 (d 0) and .64 (d 7). The SMA counts were significantly higher than PSM at each day. The RPBC method gives small pinpoint colonies on SMA, and such colonies may be too small to be detected on PSM. Additional research on a limited number of determinations (n = 26) employing an incubation time of 48 h at 21°C gave counts not statistically different from the SMA RPBC method. There is a need for further research to establish the exact incubation time required for a rapid psychrotrophic count using PSM; PSM cannot be considered a suitable alternative to SMA for the RPBC method employing 25 h of incubation.

Duplicate SMA and PSM plates for PBC and RPBC with actual numerical counts within the

### Table 2. Comparison of psychrotrophic bacterial counts for whole and skim milks by standard methods agar (SMA) and Petrifilm SM (PSM) plates.

<table>
<thead>
<tr>
<th>Days of storage at 6.1°C</th>
<th>Psychrotrophic bacterial count</th>
<th>Rapid psychrotrophic bacterial count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>No. of samples</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Mean log SMA count/ml</td>
<td>1.02</td>
<td>5.17</td>
</tr>
<tr>
<td>(1.13)</td>
<td>(2.64)</td>
<td></td>
</tr>
<tr>
<td>Mean log PSM count/ml</td>
<td>.90</td>
<td>5.03</td>
</tr>
<tr>
<td>(1.09)</td>
<td>(2.71)</td>
<td></td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>.97</td>
<td>.98</td>
</tr>
<tr>
<td>Slope</td>
<td>.93</td>
<td>1.01</td>
</tr>
<tr>
<td>Intercept</td>
<td>-.05</td>
<td>-.18</td>
</tr>
</tbody>
</table>

1 Standard deviations are in parentheses.
TABLE 3. Levels of presumptive and confirmed coliform counts for whole and skim milks by violet red bile agar (VRBA) and Petrifilm VRB (PVRB) plates.

<table>
<thead>
<tr>
<th>Method</th>
<th>Day</th>
<th>Medium</th>
<th>Ranges of coliform count per milliliter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>Presumptive data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COLI¹</td>
<td>0</td>
<td>VRBA</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>PVRB</td>
<td>38</td>
</tr>
<tr>
<td>COLI</td>
<td>7</td>
<td>VRBA</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>PVRB</td>
<td>27</td>
</tr>
<tr>
<td>PI²</td>
<td>0</td>
<td>VRBA</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>PVRB</td>
<td>22</td>
</tr>
<tr>
<td>Confirmed data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COLI</td>
<td>0</td>
<td>VRBA</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>PVRB</td>
<td>39</td>
</tr>
<tr>
<td>COLI</td>
<td>7</td>
<td>BRBA</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>PVRB</td>
<td>29</td>
</tr>
<tr>
<td>PI</td>
<td>0</td>
<td>VRBA</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>PVRB</td>
<td>23</td>
</tr>
</tbody>
</table>

¹ Coliform test with a solid medium.
² Preliminary incubation method for detection of coliforms in commercially processed milk samples.

30 to 300 count range were examined for repeatability. No significant difference was found between the overall PSM (CV = 3.8% and 2.8%) and SMA repeatability (CV = 3.2% and 2.2%) for the PBC and RPBC.

Coliform counts were very low for the samples tested; therefore, each sample was cross-classified according to presumptive and confirmed counts per milliliter by method, day, and medium (Table 3). No trends were evident in the COLI d 0 and 7 data. The PI data indicates a slightly higher trend to classify samples as having >150 coliforms/ml counts on VRBA.

The positive predictive value (representing the proportion of confirmed and presumptive colonies) was also calculated for each method, day, and medium (Table 4). For COLI, d 0, values were not significantly different at .86 (PVRB) and .81 (VRBA). The positive predic-

TABLE 4. Positive predictive values for coliforms in whole and skim milks confirmed from Petrifilm VRB (PVRB) and violet red bile agar (VRBA) plates.

<table>
<thead>
<tr>
<th>Method</th>
<th>Day</th>
<th>Medium</th>
<th>No. of colonies selected for confirmation (A)</th>
<th>No. of colonies selected and confirmed (B)</th>
<th>Positive predictive value (B/A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COLI¹</td>
<td>0</td>
<td>PVRB</td>
<td>29</td>
<td>25</td>
<td>.86</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>VRBA</td>
<td>36</td>
<td>29</td>
<td>.81</td>
</tr>
<tr>
<td>COLI</td>
<td>7</td>
<td>PVRB</td>
<td>88</td>
<td>78</td>
<td>.89</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>VRBA</td>
<td>98</td>
<td>51</td>
<td>.52</td>
</tr>
<tr>
<td>PI²</td>
<td>0</td>
<td>PVRB</td>
<td>135</td>
<td>113</td>
<td>.84</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>VRBA</td>
<td>137</td>
<td>111</td>
<td>.81</td>
</tr>
</tbody>
</table>

¹ Coliform test with a solid medium.
² Preliminary incubation method for detection of coliforms in commercially processed milk samples.
live value for PVRB (.89) was significantly higher than VRBA (.52) for COLI d 7. Two factors possibly attribute for the significant difference in positive predictive values. Although coliforms and noncoliforms grow on PVRB, the only colonies counted and subject to confirmatory tests for coliforms are those producing gas. Thus, confirmation rates from PVRB should be high. No such selection of colonies can be made for confirmation from VRBA plates. Furthermore, studies in our laboratory showed that 30% of aged milk samples tested for coliforms gave presumptive colonies growing on VRBA plates, which isolation and identification showed were *Pseudomonas* species (5). Additionally, in unpublished data in connection with studies of the preliminary incubation method for coliforms, 51 of 292 isolates from VRBA plates of aged milk samples were either of the genera *Serratia* or *Hafnia*, which would grow on VRBA but not produce gas in BGLB. In view of these findings, the lower positive predictive value for d 7 VRBA plates is not surprising. The positive predictive value for PVRB and VRBA plates by the PI method were not significantly different (PVRB = .84 and VRBA = .81). Thus, differences in positive predictive values were only observed in aged milk samples. No coliform analyses were performed after d 7.

**CONCLUSIONS**

The following conclusions can be drawn from this research: 1) a strong linear relationship exists between SMA and PSM plates employed for the SPC (d 7 to 14) and PBC methods; 2) PSM performance is comparable on whole and skim milk, indicating no fat content bias for normal fluid milk; 3) PSM plates, particularly for fresh (d 0) samples, gave lower counts than agar pour plates; 4) PSM plates are not a suitable alternative to SMA for the RPBC method employing the suggested incubation time; 5) PVRB and VRBA counts are generally within the same range; 6) PVRB and VRBA positive predictive values are essentially the same for samples plated within 24 h of packaging.

The PSM could be considered an alternative to conventional plating techniques employed for SPC (d 7 to 14) and probably d 0 by using a 72 h incubation, psychrotrophic bacterial counts, coliform tests with solid medium, and preliminary incubation detection of coliforms for evaluation of fluid milks.

**REFERENCES**


