Improved Methods for Quality Assessment of Raw Milk

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(Received for Publication May 16, 1988)

ABSTRACT

Raw milk samples (203) were obtained from locations throughout Virginia for the purpose of evaluating the presently available techniques, used as is or modified, for enumeration of psychrotrophic bacteria as indicators of the actual quality of milk. Each of the samples was split into two sub-samples, one being tested by various bacterial enumeration methods on the fresh sample, and the other subjected to a preliminary incubation of 13°C or 21°C for 18 h prior to bacterial enumeration. Agar pour plates and Petrifilm dry medium culture plates were both used. Results indicated that the Petrifilm technique was not significantly different from agar pour plate methods as evidenced by correlation values, mean log differences, slopes, and Y-intercepts. The Petrifilm method for the modified (rapid) Psychrotrophic Bacteria Count (mPBC) produced similar overall results to the pour plate method (0.96, -0.283, 1.016, -0.340, respectively) when compared to the standard PBC, and enumeration of colonies on the Petrifilm plates was easier due to the incorporated TTC. A modified Preliminary Incubation Count (mPIC) yielded results not significantly different from those of the PIC (0.94, 0.044, 0.991, 0.169, respectively), and was conducted in less time - 2 d vs. 3 d.

Raw milk quality cannot be defined based upon one or two variables. Quality refers to factors such as flavor, solids level, freezing point, freedom from antibiotics and other inhibitory substances, somatic cell content, and sediment content. Quality also refers to the magnitude of the microbial population and to the types of organisms present (21). Commercial evaluation of the microbial quality of raw milk has long been a problem, while many of the psychrotrophic contaminants grow actively until the point is reached where the udder flora no longer multiples (2,9,15,23). Johns (11) suggested PI at 13°C for 18 h, followed by the Standard Plate Count (SPC), for the purpose of reflecting careless sanitary practices. This method has come to be known as the PI Count or PI Test. Available data indicate that the PI test is a more rigorous test than the SPC when it is used to identify quality or milk handling problems (20). The SPC may continue to be used for regulatory purposes, but it appears use of the PI test has increased dramatically (6).

Bigalke (2) described an ideal test to determine raw milk microbiological quality which would include the following criteria: a test which is rapid and economical; and a test that would reflect the total number of organisms in the milk sample, the number of the psychrotrophic organisms, conditions of production on the farm, and the time and temperature of storage of the raw milk. There is general agreement that the psychrotrophic bacterial count is the most reliable method of indicating conditions of production on the farm. Many argue that refrigeration on the farm makes it difficult to reflect the true conditions of production with the Standard Plate Count (SPC).

Over the years, many methods have been proposed to alleviate some of the problems mentioned herein (3,7,13,14), but new methods are burdened by being compared to imprecise, presently used standard methods. Lack of agreement commonly was viewed as an imperfection in the proposed method without considering the inherent imprecision and inaccuracy of the reference method. The problem is increased when a new method is proposed where there is a fundamental difference in properties being measured even though the goal is the same - to measure the microbial quality of raw milk (15) as indicative of the resultant quality of the finished dairy product.

A modification to standard plating methods which has apparently improved raw milk quality assessment is preliminary incubation (PI). A procedure such as PI, which encourages the growth of bacterial contaminants, offers more hope of detecting milk which is not as good as the initial counts suggest. Preliminary incubation is based upon the theory that, as the holding temperature is lowered, a point is reached where the udder flora no longer multiplies while many of the psychrotrophic contaminants grow actively (12). Standard plating methods have been performed after such preliminary incubations with some success as far as a quality measurement (4,5). Johns (11) suggested PI at 13°C for the 18 h, followed by the Standard Plate Count (SPC), for the purpose of reflecting careless sanitary practices. This method has come to be known as the PI Count or PI Test. Available data indicate that the PI test is a more rigorous test than the SPC when it is used to identify quality or milk handling problems (20). The SPC may continue to be used for regulatory purposes, but it appears use of the PI test has increased dramatically (6).

Present difficulties of assessing microbiological quality of raw milk in a time period that would allow for effective corrective measures have put an emphasis upon collection of data that is consistent, accurate, and rapid. The objective of this study was to determine which of the presently available enumeration techniques for psychrotrophic bacteria can be used, as is or modified, to provide the most reliable, accurate, and rapid results which will be indicative of the actual quality of raw milk, as related to the standard psychrotrophic bacteria count.
Materials and Methods

Raw milk samples (203) were obtained from locations throughout Virginia. Each of the samples was split into two subsamples, one being tested by various bacterial enumeration methods on the fresh sample and the other being subjected to a preliminary incubation (PI) of 21°C or 13°C for 18 h prior to bacterial enumeration. Bacterial enumerations included: Standard Plate Count - SPC, Coliform Count - CC, Psychrotrophic Bacteria Count - PBC (19), modified (rapid) Psychrotrophic Bacteria Count - mPBC (18), whereby standard methods agar is used and plates are incubated at 21°C for 25 h, Preliminary Incubation Count - PIC (11), and a modified PI Count which used the mPBC vs. the SPC after a PI of 13°C for 18 h - mPIC (Table 1). In all cases, except the PBC and CVT, duplicate samples were enumerated using the Petrifilm dry medium culture plate method (16,17).

Petrifilm plates provide a time-saving method for enumerating bacteria by eliminating the need to prepare media. This sample-ready system consists of nutrients and/or selective and differential agents coated onto films along with a cold-soluble gelling agent and TTC. One milliliter of an appropriate dilution is applied directly to the Petrifilm plate; inoculated plates are incubated under the same conditions used for traditional plating methods. Colonies appear as red dots on a white background on SM plates for SPC; gas bubbles are produced around colonies differentiating coliforms from other gram-negative organisms on VRB plates.

Proper evaluation of each plate count method, as related to the PBC and corresponding agar/Petrifilm methods, was performed by calculation of appropriate correlation, slope, Y-intercept, and mean log difference values.

### TABLE 1. Microbiological methods used in this study.

<table>
<thead>
<tr>
<th>Method</th>
<th>Abbreviation</th>
<th>Preliminary incubation</th>
<th>Plate incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychrotrophic Bacteria Count</td>
<td>PBC</td>
<td>---</td>
<td>7°C/10d</td>
</tr>
<tr>
<td>Modified (rapid) PBC</td>
<td>mPBC</td>
<td>---</td>
<td>21°C/25h*</td>
</tr>
<tr>
<td>Standard Plate Count</td>
<td>SPC</td>
<td>---</td>
<td>32°C/48h</td>
</tr>
<tr>
<td>Coliform Count</td>
<td>CC</td>
<td>---</td>
<td>32°C/24h</td>
</tr>
<tr>
<td>Preliminary Incubation Count</td>
<td>PIC</td>
<td>13°C/18h</td>
<td>32°C/48h</td>
</tr>
<tr>
<td>Modified PIC</td>
<td>mPIC</td>
<td>21°C/18h</td>
<td>21°C/25h*</td>
</tr>
</tbody>
</table>

*Petrifilm method requires an incubation of 48h at 21°C.

### TABLE 2. Relationships of bacterial enumeration methods used for quality assessment of raw milk.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Correlation</th>
<th>Mean Log difference</th>
<th>Slope</th>
<th>Y-intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC*/PSPC*</td>
<td>0.971</td>
<td>-0.110</td>
<td>1.002</td>
<td>-0.117</td>
</tr>
<tr>
<td>CC*/PCC</td>
<td>0.963</td>
<td>0.054</td>
<td>1.017</td>
<td>0.024</td>
</tr>
<tr>
<td>PIC*/PPIC</td>
<td>0.984</td>
<td>-0.362</td>
<td>1.034</td>
<td>-0.556</td>
</tr>
<tr>
<td>mPBC*/mPBC</td>
<td>0.942</td>
<td>-0.553</td>
<td>1.087</td>
<td>-0.871</td>
</tr>
<tr>
<td>PBC*/mPBC</td>
<td>0.944</td>
<td>0.272</td>
<td>0.865</td>
<td>0.684</td>
</tr>
<tr>
<td>PBC*/mPBC</td>
<td>0.961</td>
<td>-0.283</td>
<td>1.016</td>
<td>-0.340</td>
</tr>
<tr>
<td>PIC*/mPIC</td>
<td>0.938</td>
<td>0.044</td>
<td>0.991</td>
<td>0.169</td>
</tr>
<tr>
<td>PIC*/mPIC</td>
<td>0.938</td>
<td>-0.310</td>
<td>0.940</td>
<td>0.093</td>
</tr>
</tbody>
</table>

*Standard plate count.
*Petrifilm plate method.
*Coliform count.
*Preliminary incubation count.
*Modified psychrotrophic bacteria count.
*Psychrotrophic bacteria count.
*Modified preliminary incubation count.
at least >0.900 (0.865). This was also the case with the Y-intercept (0.684). The Petrifilm mPBC method appeared to give similar, though somewhat superior, results vs the pour plate mPBC when compared to the standard PBC (0.961, 0.283, 1.016, -0.340, respectively).

A modified Preliminary Incubation count (mPIC), pour plate and Petrifilm methods, yielded results not significantly different from those of the standard PIC by using the mPBC procedure after a preliminary incubation of 13°C for 18 h. The pour plate method produced very acceptable data (0.938, 0.044, 0.991, 0.169, respectively), and in less time than the PIC - 2 d vs. 3 d. The Petrifilm method also generated similar data (0.938, -0.310, 0.940, 0.093, respectively), but offered no incubation time savings. A method including a 21°C PI for 18 h followed by mPBC was found to be valuable in assessing raw milk quality as indicative of production conditions, but the correlation to PBC was low (0.63), and numbers were an average of 10 times higher than the PIC.

**DISCUSSION**

Proper comparison of microbial enumeration methods requires not only correlation value evaluation, but it is imperative that one consider the validity of the "line of best fit" which better represents the true value of the obtained data. This is accomplished by evaluating the slope and Y-intercept of such a line, and by reviewing the magnitude of the mean log difference. If the mean log difference is unacceptably large, and if the slope and Y-intercept are much removed from 1.000 and 0.000, respectively, (as determined by the individual user), the correlation coefficient is of little value.

The Petrifilm dry medium culture method would appear to be a very viable alternative to the conventional agar pour plate method, and would also be much less labor intensive. It was not only acceptable method for the Standard Plate Count and the violet red bile coliform count (CC), but was also found useful for the modified (rapid) psychrotrophic bacteria count and the Preliminary Incubation count. This is important in today's dairy industry which is more interested in ultimate milk and dairy product quality as determined by the presence or absence of psychrotrophic bacteria in raw milk (10), and less enamored with information provided by SPC or CC.

This keen interest in psychrotrophic contaminants also points out the need for an estimation procedure which provides data in less than 10 d, namely the mPBC. This very under-utilized method apparently has not received attention worthy of a procedure with such a high correlation coefficient, along with acceptable previously discussed parameters, and results available in 25 h. The Petrifilm mPBC, even though requiring 48 h, is a tremendous improvement over the standard PBC of 7°C for 10 d. It becomes evident that the optimum growth temperature of psychrophils (20-25°C) (18) is also the preferred enumeration temperature.

The purpose of the Preliminary Incubation count is to estimate the number of psychrotrophic bacteria in raw milk which is indicative of the resultant quality of the finished dairy product. This information is not provided by the SPC or CC. Again, by using an incubation temperature preferred by psychrophils - 21°C vs 32°C - comparable data were obtained in less time when using identical preliminary incubations of 13°C.

Is the PI count providing all the information expected of such widely used technique? There is overwhelming evidence that the PIC is clearly superior to the SPC in evaluating the microbiological quality of raw milk (1,12,21), but FDA study (22) does not support this conclusion stating, "Evaluation of the data does not favor either procedure - PIC or SPC - as being a better indicator of sanitary and production conditions on dairy farms." Therefore, work remains to provide an acceptable test method by which the number of psychrotrophic bacteria and, hence, the quality of raw milk are determined. One alternative we have investigated in our laboratory uses a 21°C/18 h preliminary incubation followed by the mPBC. This method proved successful in monitoring raw milk quality, but resultant data were approximately 10 times higher than corresponding PIC data, therefore a need would exist to re-evaluate "acceptable" bacterial levels.

**ACKNOWLEDGMENT**

This research was funded by the Virginia State Dairymen’s Association and the Virginia Agricultural Council. The authors appreciate the support of Ms. Sue McAllister and 3M Corporation for making available Petrifilm supplies and technical advice.

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