THE NEW PLATE COUNT MEDIA IN ROUTINE PLATE COUNTS ON MILK

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A number of experiments were carried out to investigate the differences in bacteria counts on milk samples when the new "milk-free" plating media were compared with the old standard TGEM medium. Variations in type of milk, raw or pasteurized; grade of milk; high or low quality, incubation temperature and laboratory technique were included in these studies. The results obtained were similar to other reported comparative studies. It is likely that over an extended period the average counts with the new media will compare favorably with the old medium.

INTRODUCTION

During the past four or five years there has been much interest in the improvement of media for making standard plate counts on milk and other dairy products. It was desired to have a medium that did not contain or require the addition of skim milk. Preliminary studies and extensive collaborative studies conducted by the Committee on Applied Laboratory Methods of this Association and by the Subcommittee on Methods for the Examination of Dairy Products of the American Public Health Association resulted in the selection of two media which could take the place of the present Standard Tryptone Glucose Extract (Skim Milk) Agar (TGEM). In September 1952 a Committee Report published in the American Journal of Public Health concerning proposed changes for the Tenth Edition of Standard Methods for the Examination of Dairy Products stated that two milk-free plating media will be substituted for the present TGEM medium.

As is the case when changes in any standard procedure are proposed, there are always some workers who desire to investigate in their own laboratories the possible effects of the proposed changes. This desire resulted in a number of preliminary comparisons of the new medium with the standard TGEM medium in routine counts on milk samples by a few control laboratories. The results obtained were somewhat different from what had been expected even though it had been stated that there might be a number of variations on individual samples where the results might be startling. This possibility of unexpected variations and the results of the preliminary routine plate counts prompted further comparisons of the new media and the standard TGEM medium at the National Dairy Research Laboratories. The purpose of this paper is to summarize the more interesting findings resulting from this study with the thought that these comments may be of value in explaining and understanding some of the variations in counts which may occur when the new plating media are used.

EXPERIMENTAL

In these studies it was desired to measure only differences between the various media being studied. Consequently a number of preliminary studies were conducted to investigate the effect of such conditions as type of transfer, number of dilutions, number of plates, etc.

Media

For this study fresh batches of experimental media and standard media were supplied by the Baltimore Biological Laboratories (BBL) and the Difco Laboratories. The experimental BBL medium was their MPH medium which had been used in earlier collaborative studies. The standard medium was the BBL Trypticase Glucose Extract Agar. The experimental Difco medium was designated as Plate Count Agar (PCA). This medium was a modification of an earlier medium in which the yeast extract content was reduced to 0.25 percent. The standard medium was their regular Tryptone Glucose Extract Agar. These media were prepared according to the directions of the manufacturer and were used within 48 hours after preparation.

Milk Samples

The milk samples investigated were taken from the raw milk available at the laboratory which is shipped in from upper New York State and raw milk obtained from a local farm. Some of these raw milks were laboratory pasteurized while other pasteurized

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Plating Procedures

Standard Methods were followed throughout the studies. To avoid any possible psychological bias in the reading of results the plates were extensively randomized in the following manner. When the plates had been prepared from a suitable dilution and poured with the various media, they were recorded by a disinterested operator and distributed in an incubator in such a manner as to minimize temperature differences within the incubator. After 48 hours incubation, the plates were read in random order so that any differences between plates made first and last, or read first and last would not affect the results. In some instances each milk sample was plated by three operators and all plates were read by each operator. Although the actual number of samples examined was small, the experimental design was such that the
results are believed to be statistically valid.

Statistical Analysis
In this study the results were subjected to statistical analysis. Samples were compared by the analysis of variance with suitable checks being made for constancy of error. Logarithms of the counts were used in order to obtain a normally distributed variate. Precision of the results is indicated by the 95 percent confidence interval which must be placed on a datum to give 95 percent confidence of insuring the inclusion of the long range average.

Results
Preliminary studies were conducted to determine the most suitable experimental procedures to follow which would result in the most accurate measurement of differences between the standard media and the experimental media. The type of transfer from the dilution bottle to the plate was found to give the same precision whether one ml or one-tenth ml was used. The method of making the dilutions was found to be a source of variation in the results in that one or two dilutions produced the same precision but as the number of dilutions was increased to three and beyond, the precision decreased. This lack of precision was also evident in duplicate plates poured from the same dilution bottle when the number of dilutions was increased to three or beyond. For these studies all plates for the different media were made from the same dilution bottle when the number of dilutions was increased to three or beyond. For these studies then all plates for the different media were made from the same dilution bottle to increase the precision of the comparisons between the media. Although the operators did tend to show differences in counting levels, these differences did not affect the media comparisons.

Laboratory Differences
At the start of these investigations data obtained by a routine testing laboratory in which the experimental media were compared with the standard plating media were submitted for analysis. Later samples of raw and pasteurized milk were plated in our laboratory using the experimental media and the standard media. These results, shown in table 1, indicate that the routine testing laboratory obtained lower counts with the experimental media than with the standard media. Analysis of the standard media counts showed that the experimental media counts were slightly higher than the standard media counts. These results were all obtained on raw milk samples.

Temperature of Incubation
Inasmuch as laboratories in some areas were using 37°C as the incubation temperature for their plate counts, a comparison was made in which duplicate plates of each medium were incubated at 37°C, 35°C and 32°C. The results summarized in table 2 show that the same media differences were obtained at each temperature. It was thought that perhaps lower temperatures might result in relatively higher media differences but this difference was not observed.

Media Differences
Following these preliminary experiments a limited number of media comparisons were made using the two experimental media (MPH and PCA), standard TGEM agar from BBL, and Difco raw and pasteurized milk samples.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Routine lab</th>
<th>Research lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGEM</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PCA</td>
<td>81</td>
<td>114</td>
</tr>
<tr>
<td>MPH</td>
<td>89</td>
<td>123</td>
</tr>
</tbody>
</table>

Table 2—Comparison of Plating Media Productivity at Several Incubation Temperatures

<table>
<thead>
<tr>
<th>Medium</th>
<th>37°C</th>
<th>35°C</th>
<th>32°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGEM</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PCA</td>
<td>109</td>
<td>104</td>
<td>117</td>
</tr>
<tr>
<td>MPH</td>
<td>117</td>
<td>122</td>
<td>111</td>
</tr>
</tbody>
</table>

Table 3—Comparison of Plating Media Productivity on Different Milk Samples at 35°C

<table>
<thead>
<tr>
<th>Medium</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>Log avg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCA</td>
<td>83</td>
<td>83</td>
<td>95</td>
<td>117</td>
<td>107</td>
<td>78</td>
<td>90</td>
<td>.....</td>
<td>.....</td>
<td>92</td>
</tr>
<tr>
<td>P</td>
<td>105</td>
<td>141</td>
<td>119</td>
<td>100</td>
<td>.....</td>
<td>.....</td>
<td>.....</td>
<td>101</td>
<td>143</td>
<td>118</td>
</tr>
<tr>
<td>MPH</td>
<td>89</td>
<td>106</td>
<td>100</td>
<td>158</td>
<td>77</td>
<td>94</td>
<td>81</td>
<td>.....</td>
<td>.....</td>
<td>93</td>
</tr>
<tr>
<td>P</td>
<td>115</td>
<td>148</td>
<td>128</td>
<td>147</td>
<td>.....</td>
<td>.....</td>
<td>.....</td>
<td>112</td>
<td>116</td>
<td>114</td>
</tr>
</tbody>
</table>

R = Raw milk
P = Pasteurized milk

In all cases TGEM count = 100%

Our laboratory results showed that the experimental media counts were slightly higher than the standard media counts. These results were all obtained on raw milk samples.

The results obtained with the experimental media summarized in table 3 show that with both experimental media the raw milk counts were slightly lower than counts obtained with the standard media but the differences were not significant. However, the pasteurized milk counts were significantly higher with the experimental media than with the standard media. Stated in another way, the differences between the new media and the standard media are greater for pasteurized milk samples than for raw milk samples. It is interesting to note that these results compare favorably with those reported by Pessin and Robertson and Buchbinder et al.

Buchbinder et al. noted the fact that the new experimental medium appeared to be more productive (gave higher counts) for pasteuriz-
ed milk than for raw milk. These authors suggest that this difference may be due to the fact that pasteurized milk, unlike raw milk, contains bacteria which are injured but not destroyed during pasteurization and which grow in the presence of accessory food substances supplied by the yeast. This also may be a possible explanation of the results noted in our studies on pasteurized milk samples.

**Milk Differences**

In analyzing the results of this comparative study, it was observed that there also was considerable variation in the counts obtained with the various media with different milks on different days regardless of whether the milk was raw or pasteurized. It can be seen in Table 3 that with some samples the differences between the experimental media and the standard media are greater in some instances than in others, for example, samples 8 and 9. In this instance sample 8 was a laboratory pasteurized sample checked immediately after pasteurization while sample 9 was a commercially pasteurized milk sample which had been refrigerated for several days after pasteurization and undoubtedly had an entirely different flora (probably psychrophiles) than the first sample. The same observation can be made on pasteurized milk samples 1 and 2 where the first was a laboratory pasteurized high quality milk from a local farm tested within 5 hours after milking and the second was a laboratory pasteurized poor quality milk sample from upper New York State which was anywhere from 24- to 48-hours old at the time of testing.

Note also milk sample 4, a fresh local raw milk in which the raw counts on the new media are significantly higher than those on TEGEM. This difference is greater than any observed on all other raw milk samples and would indicate an entirely different flora in this milk. It should be remarked that this variation from the over-all trend significantly exceeds sampling error.

It would appear that the new experimental media contain better nutrients than the standard media for the growth of various milk bacteria. The increased colony size obtained with the new media also indicates that there is this nutrient difference. These observations would indicate that there is need for further investigation concerning the ability of the new media to support growth of different types of bacteria which may be present in both raw and pasteurized milk.

**Summary**

In summary, these brief preliminary studies in general substantiate the published results of the earlier collaborative studies on the comparison of new experimental media and the standard TEGEM medium. It was found that for statistical analysis of results great care must be taken in the planning, operation, and interpretation of comparative studies so that only media differences are measured. The greatest variations encountered might be explained by differences in the milks investigated—whether the milk was raw or pasteurized, a good milk or poor milk—differences in the bacterial flora and their response to the nutrients available in the new media. It is likely that over an extended period the average of counts obtained with the new media will compare favorably with counts obtained with the present standard TEGEM medium. In any case, the new media are an improvement over the present medium, especially from the point of view of ease in counting of plates.

**BIBLIOGRAPHY**


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residues when they are suspected in damaging or questionable amounts at harvest. Residue removal is to be avoided except as a means of last resort. Some studies in residue removal are in progress. There is some reason to believe, for example, that the toxic properties of certain insecticidal chemicals may be eliminated by the heat encountered during the canning process.

Important progress has been made in developing satisfactory analytical methods for determining residues. Chemical analyses, infrared spectrophotometry, and bio-assays are available for most insecticidal chemicals. Each method has its strong points. Most satisfactory results are probably obtained where bio-assays are run in conjunction with other methods. In many cases methods now in use are sensitive to 1 part or less in 10,000,000.

The many problems associated with residues resulting from the use of insecticides for the control of insects are by no means completely solved but tremendous progress has been made in the last few years. Education of farmers in the proper use of insecticides will greatly reduce these hazards. With more efficient insecticidal chemicals now coming into use, applications for insect control can generally be made at a time and at a rate of application coupled with the use of proper formulations to preclude largely the possibility of trace residues at harvest.

**BILL BRYANT HOSPITALIZED**

Mr. C. B. A. (Bill) Bryant is hospitalized at the Community Health Center, Hillsdale, Michigan, suffering with a case of pneumonia. This situation necessitates the cancellation of his many speaking engagements for the next two months. Now is the chance for his many friends to drop him a line of greeting specifying "no answer expected."