THE RESAZURIN TEST—ITS USE AND PRACTICABILITY AS APPLIED TO THE QUALITY CONTROL OF RAW MILK

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An earlier report by Ramsdell, Johnson, and Evans discussed the use of Resazurin as a chemical indicator for determining the sanitary quality of milk. On the basis of the above investigation, it was concluded that the use of Resazurin gave more information as to the quality of milk than any other chemical indicator now in use. Further work on this dye was conducted by C. K. Johns. Coincident with this latter research, investigations on Resazurin were initiated in these laboratories. It is the purpose of this paper to present data which indicate, first, that the Resazurin test is of added value as an indicator of quality over the Methylene Blue test as well as having the advantage of consuming much less time than the latter test, and second, that the Resazurin test is a valuable adjunct to microscopic diagnosis in routine quality control of milk.

EXPERIMENTAL

Of primary interest was a comparison of the sensitivity of Resazurin to that of Methylene Blue, the standard plate method, and the microscopic Breed smear. Accordingly, the producer samples were obtained at the weigh can and all of the above-mentioned tests were run on each milk. The Resazurin test was run according to the following procedure:

1. One tenth of one cc. of 0.05 per cent Resazurin dye solution (Eastman) was measured into a sterile test tube.
2. Milk samples were obtained directly from the weigh can, using a 10 cc. narrow bore-type dipper.
3. Samples were incubated for one hour at 98° F., in a covered water bath.
4. The samples were read and recorded as quickly as possible after the incubation period.

A total of 305 samples were collected for this test over a period of five weeks. Although the determinations were made in unison, they are presented separately for purposes of clarity.

Of the 220 samples remaining blue at the end of one hour of incubation with Resazurin, 201, or 91 per cent, were incapable of reducing the Methylene Blue.
TABLE 1
Comparison of color of Resazurin dye at the end of one hour with reduction time of Methylene Blue

<table>
<thead>
<tr>
<th>NO. OF SAMPLES</th>
<th>COLOR OF RESAZURIN AFTER 1 HR.</th>
<th>REDUCTION TIME OF METHYLENE BLUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7 hrs. or longer</td>
</tr>
<tr>
<td>220</td>
<td>Blue</td>
<td>201</td>
</tr>
<tr>
<td>30</td>
<td>Purple Pink</td>
<td>21</td>
</tr>
<tr>
<td>32</td>
<td>Sl. Pink</td>
<td>26</td>
</tr>
<tr>
<td>14</td>
<td>Pink</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>Vivid Pink</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>White</td>
<td></td>
</tr>
</tbody>
</table>

Methylene Blue dye in seven hours or more. Purple pink and slight pink color shades, denoting some reduction with the Resazurin dye, did not, in many cases, affect the reduction time of Methylene Blue. This may be accounted for from the fact that staphylococci have a particular aptitude for reducing the Resazurin as compared to other types of bacteria commonly found in milk. Leucocytes encountered in colostral and mastitis-infected milk also cause rapid decolorization. The latter statement was particularly emphasized in the seven samples which were vivid pink on the Resazurin test at the end of one hour. Four of these samples which were unable to decolorize Methylene Blue in seven or more hours had a high leucocyte content. This bespeaks the sensitivity of Resazurin to physiologically abnormal and pathological milks as previously demonstrated.

TABLE 2
Comparison of color of Resazurin dye at the end of one hour with standard plate count

<table>
<thead>
<tr>
<th>NO. OF SAMPLES</th>
<th>COLOR OF RESAZURIN AFTER 1 HR.</th>
<th>STANDARD PLATE COUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Less than 25,000</td>
</tr>
<tr>
<td>220</td>
<td>Blue</td>
<td>160</td>
</tr>
<tr>
<td>30</td>
<td>Purple Pink</td>
<td>7</td>
</tr>
<tr>
<td>32</td>
<td>Sl. Pink</td>
<td>17</td>
</tr>
<tr>
<td>14</td>
<td>Pink</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>Vivid Pink</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>White</td>
<td>1</td>
</tr>
</tbody>
</table>

The standard plate counts on these milks were less than 25,000 bacteria per cubic centimeter (Table 2). With five exceptions, milks incubated with Resazurin which remained unchanged in color at the end of the hour had a standard plate count of less than 200,000 per cc. The similarity in correlation of the purple pink and slight pink shades indicates the desirability of classifying these two colors together. One of the two samples completely reduced on Resazurin to white had a standard plate count of less than 25,000; however, the microscopic count was over 200,000, actually uncountable, as may be seen from Table 4. Again, the effect of staphylococci and high leucocyte milk on the reducing time of the dye was encountered, making
RESAZURIN TEST

Further correlations of this new test with previously known standards rather difficult. As may be seen from Tables 2 and 3 the Resazurin test is comparable to the Methylene Blue test in regard to the standard plate count.

| TABLE 3 |

Comparison of reduction time of Methylene Blue with bacterial plate count

<table>
<thead>
<tr>
<th>NO. OF SAMPLES</th>
<th>REDUCTION TIME OF METH. BLUE</th>
<th>STANDARD PLATE COUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Under 25,000 to 50,000</td>
</tr>
<tr>
<td>253</td>
<td>7 hrs.</td>
<td>174</td>
</tr>
<tr>
<td>19</td>
<td>6 hrs.</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>5 hrs.</td>
<td>2</td>
</tr>
<tr>
<td>23</td>
<td>Under 5 hrs.</td>
<td>4</td>
</tr>
</tbody>
</table>

From these results it may be said that data as to the bacterial quality of milk obtained from one hour of incubation with Resazurin are fully as reliable as seven hours' incubation with Methylene Blue. Moreover, Resazurin is more sensitive to physiologically abnormal and pathological milks than is Methylene Blue. The correlation between Resazurin sensitivity and microscopic Breed count is better than that obtained through comparison with the standard plate method, as might be expected, due to the ability of the microscope to diagnose leucocytes, staphylococci, etc.

| TABLE 4 |

Correlation between colors developed after one hour of incubation with Resazurin and microscopic Breed count

<table>
<thead>
<tr>
<th>NO. OF SAMPLES</th>
<th>COLOR OF RESAZURIN AFTER 1 HR.</th>
<th>MICROSCOPIC BREED COUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Under 25,000 to 50,000</td>
</tr>
<tr>
<td>166</td>
<td>Blue</td>
<td>148</td>
</tr>
<tr>
<td>25</td>
<td>Purple Pink</td>
<td>20</td>
</tr>
<tr>
<td>32</td>
<td>Sl. Pink</td>
<td>22</td>
</tr>
<tr>
<td>11</td>
<td>Pink</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>Vivid Pink</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>White*</td>
<td>2</td>
</tr>
</tbody>
</table>

* High leucocytes.

It, therefore, cannot be stated that an accurate or semi-accurate bacteria count can be obtained from Resazurin because it is very sensitive to milk that is abnormal from causes other than bacteria count. Yet, the very sensitivity of Resazurin to these other quality defects in milk should be characterized as an asset.

If reliance were placed on the classification of milk according to the various color shades of Resazurin formed at the end of the hour of incubation considerable discrepancy between laboratories could be expected unless color standards were used. Microscopic diagnosis of all samples showing partial or complete reduction therefore seemed desirable. Moreover, in many plants
microscopic examination of individual producers’ milk constitutes a great portion of the time spent in laboratory control. A preliminary test which would be capable of segregating those milks which are satisfactory from those which are not, prior to microscopic diagnosis, would place emphasis on poor milks and also save a considerable amount of time.

THE RESAZURIN TEST AS A PRELIMINARY TEST FOR MICROSCOPIC DIAGNOSIS

All producers’ samples were incubated with Resazurin for one hour. At the termination of this period the samples were examined and Breed smears were made directly from those tubes in which the dye was partially or completely reduced. This method was adopted inasmuch as it obviates the necessity of double samples and since we were more interested in determining the types of bacteria rather than the total number of bacteria. The slight growth obtained during the incubation period was of value particularly in those cases where *Streptococcus agalactiae* was the cause of reduction. The practical application of this procedure is demonstrated in Table 5, a duplication of a test sheet from one of our country stations. This table represents the number of samples examined out of 100 producer samples taken.

![Image of Table 5](image)

In most cases where there was a color change, as in the above table, poor quality was verified by the microscope. Occasionally samples will change
to a purplish pink and may even turn pink, yet under the microscope no abnormality is observed. The cause of this has not as yet been entirely determined. However, previous investigations\(^1\) state that the Resazurin dye is reduced more rapidly by milk with a high catalase and chloride content and these factors may be responsible.

As may be observed from Table 5, the time saved through the use of the Resazurin-microscope combined procedure was equivalent to the time necessary to smear, diagnose and wash up the slides for 86 microscopic samples.

An approximation of the amount of time saved in our other country laboratories is given in Table 6.

<table>
<thead>
<tr>
<th>STATION</th>
<th>NO. OF SAMPLES TAKEN IN 1 WK.</th>
<th>MICROSCOPIC ANALYSES MADE 1 WEEK</th>
<th>ESTIMATED TIME SAVED 1 WEEK</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>475</td>
<td>75</td>
<td>13 hrs.</td>
</tr>
<tr>
<td>B</td>
<td>600</td>
<td>100</td>
<td>16 hrs.</td>
</tr>
<tr>
<td>C</td>
<td>260</td>
<td>40</td>
<td>6 hrs.</td>
</tr>
<tr>
<td>D</td>
<td>400</td>
<td>60</td>
<td>11 hrs.</td>
</tr>
<tr>
<td>E</td>
<td>365</td>
<td>55</td>
<td>10½ hrs.</td>
</tr>
<tr>
<td>F</td>
<td>260</td>
<td>40</td>
<td>6 hrs.</td>
</tr>
<tr>
<td>G</td>
<td>240</td>
<td>35</td>
<td>5½ hrs.</td>
</tr>
<tr>
<td>H</td>
<td>120</td>
<td>20</td>
<td>2 hrs.</td>
</tr>
</tbody>
</table>

Thus, the introduction and use of the procedure in our country laboratories has given us quicker segregation of poor quality milk, placed more emphasis on poor quality milk, allowed more time for follow-up and, because of this, increased the frequency with which the tests can be run. For these reasons the combined Resazurin-microscopic diagnosis seems to be of singular aid in a general quality improvement program.

These preliminary thoughts on the use and practicability of Resazurin are presented that they might stimulate further discussions and research on a test which warrants further investigation.

**CONCLUSIONS**

1. Information as to the sanitary quality of milk can be obtained in one hour through the use of the Resazurin test which is comparable to that obtained in seven hours using the Methylene Blue test.

2. The Resazurin test is superior to the Methylene Blue test in that it is extremely sensitive to physiologically abnormal and pathological milks.

3. The Resazurin test is a valuable adjunct to microscopic diagnosis in eliminating the time normally spent in diagnosis of good milks, thereby allowing more time for detection of the source of the trouble with poor milks.

**ACKNOWLEDGMENTS**

We wish to express our appreciation to Mr. H. L. White and to Mr. L. A. Cooley for their suggestions and cooperation during this work.