Effects of Milk Storage Duration on Various Tests for Abnormal Milk

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Abstract

The influence of age of Schiff’s reagent on the response of the Feulgen-DNA mastitis test was determined in an initial experiment. The average reflectance Feulgen-DNA values were always higher (indicating less DNA) in aged than in fresh Schiff’s reagents, and this difference usually increased with each increase in age of the reagents. The Schiff’s reagent should be discarded after 2 wk, when the color intensity is determined by reflectance spectrophotometry, or after 3-4 wk when the color is scored against a standard color chart.

The pH, California Mastitis Test (CMT), Milk Quality Test (MQT), Michigan Mastitis Test (MMT), catalase test, and reflectance Feulgen-DNA test values were determined on 40 milk samples after zero, one, two, three, and four days of storage. The CMT, MQT, and MMT gelation tests declined 0.5, 0.6, and 0.5 of a score, respectively, between Days zero and four. The pH, catalase, and reflectance Feulgen-DNA values were unaffected by the four-day storage period.

Schalm and Noorlander (9) reported that the California Mastitis Test (CMT) may not be accurate on aged milk if the pH is below 6.3. Similarly, Frank and Pounden (4) observed that milk samples aged a few days produced CMT reactions of lesser intensity than similar tests performed on the day of collection. The catalase test for abnormal milk produced increased quantities of gas as the milk samples aged (4). On the other hand, the Feulgen-DNA test as scored from a color chart was uninfluenced by changes in the pH of milk stored at room temperature for two days (6).

Since the Feulgen-DNA color may be objectively measured by reflectance spectrophotometry (7), it was of interest to determine more precisely the effects of milk storage duration on this and other commonly used tests for abnormal milk. In addition, since the Schiff’s reagent used in the Feulgen-DNA test is not stable for long periods of time, an initial experiment was conducted to determine the shelf life of the reagent prepared with different sources of basic fuchsin.

Methods

Five different sources of basic fuchsin were compared, to determine the shelf life of the Schiff’s reagent. The sources of basic fuchsin were as follows: Fisher Scientific Company, F-98, C.I. no. 42500; Hartman-Leddon Company, 220, C.I. no. 42510; Sigma Chemical Company, Type IV; Allied Chemical Corporation, certified basic fuchsin, 434, C.I. no. 42500; and Allied Chemical Corporation, flagella staining basic fuchsin, 719, C.I. no. 42500. The dye content was 99% in all cases. The Schiff’s reagents prepared from these different sources of basic fuchsin will be referred to as A, B, C, D, and E, respectively.

Five Schiff’s reagents, each utilizing one of the five different sources of basic fuchsin, were prepared as previously described (5). These five original reagents were stored at 5°C, and each compared with a fresh preparation of the respective reagent at weekly intervals for 4 wk. Tests of the original and fresh Schiff’s reagents were performed in duplicate on ten individual quarter milk samples collected fresh each week. This entire experiment was conducted twice.

In the experiment designed to test the effects of aging of the milk on various tests for abnormal milk, a total of 40 individual quarter milk samples was collected. The pH, CMT, Milk Quality Test (MQT), Michigan Mastitis Test (MMT), catalase test, and reflectance Feulgen-DNA test values were determined in duplicate on each milk sample within 30 min after collection (Day zero storage) and after one, two, three, and four days of storage. The assay procedures have been previously de-

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2 Journal Article no. 3731 from the Michigan Agricultural Experiment Station.
scribed (5). The stock samples were stored at 3.3 °C in screw-cap, 0.946-liter Mason jars. Each of the assays was performed at the same time of day throughout the period of the experiment.

Results

The over-all average values for per cent reflectance Feulgen-DNA of fresh Schiff's reagents A, B, C, D, and E on the fresh milk samples for the first experiment were 84.4, 84.0, 83.3, 84.0, and 83.7, respectively (P > 0.05).

Results of the comparison between the fresh Schiff's reagents and the Schiff's reagents after 1, 2, 3, or 4 wk of aging are presented in Table 1. The average reflectance Feulgen-DNA values were always higher (indicating less DNA) in the aged than in the fresh Schiff's reagents. Furthermore, this difference in reflectance units between the fresh and aged Schiff's reagents generally increased with each increase in the age of the Schiff's reagents. Throughout the 4-wk storage period, the reflectance values obtained with the aged Source D reagent more consistently approached perfect agreement (0.0 reflectance unit difference) with the fresh reagent than did the other sources of basic fuchsin. Therefore, Source D was used in subsequent studies.

Results of the experiment designed to determine the effects of aging of the milk sample on the pH and on the response of several tests for abnormal milk are given in Table 2. The average pH ranged between 6.79 and 6.95, but the linear regression of pH on age of milk was not significantly different from zero (P > 0.05).

The CMT, MQT, and MMT assays declined 0.5, 0.6, and 0.5 of a score between Days zero and four. The linear regressions of the scores on age were significant at the 10, 5, and 15% levels of probability, respectively. The catalase and Feulgen-DNA tests were not significantly affected (P > 0.90 and P > 0.40, respectively) by the four-day storage period.

Discussion

The five different sources of basic fuchsin used in fresh Schiff's reagent did not produce different staining intensities in milk. De la Torre and Salisbury (2) observed that different aged batches of Schiff's reagent, prepared from the same dye, resulted in different color intensities of Feulgen-stained bovine spermatozoa. However, unlike the results of de la Torre and Salisbury (2) and those of Salisbury et al. (8), which showed an increased Feulgen-DNA content in bovine spermatozoa with aged Schiff's reagent, we observed a decrease in DNA values in milk somatic cells with advancing age of the stain. This discrepancy may have been caused by differences in the types of cells studied or

### Table 1

<table>
<thead>
<tr>
<th>Age of Schiff's reagent (wk)</th>
<th>Basic fuchsin source</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td></td>
<td>0.5</td>
<td>0.4</td>
<td>1.0</td>
<td>0.6</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.2</td>
<td>0.7</td>
<td>1.8</td>
<td>0.3</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>2.3</td>
<td>2.0</td>
<td>2.0</td>
<td>1.0</td>
<td>1.8</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>3.8</td>
<td>2.4</td>
<td>2.3</td>
<td>0.8</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*The reflectance unit obtained with aged Schiff's reagent minus the reflectance unit obtained with fresh Schiff's reagent.


### Table 2

<table>
<thead>
<tr>
<th>Mastitis test</th>
<th>0</th>
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<th>2</th>
<th>3</th>
<th>4</th>
</tr>
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<tr>
<td>pH</td>
<td>6.80</td>
<td>6.79</td>
<td>6.96</td>
<td>6.87</td>
<td>6.86</td>
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<tr>
<td>CMT</td>
<td>1.9</td>
<td>1.8</td>
<td>1.8</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td>MQT</td>
<td>2.3</td>
<td>2.2</td>
<td>2.1</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>MMT</td>
<td>3.6</td>
<td>2.3</td>
<td>2.3</td>
<td>2.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Catalase (% O₂)</td>
<td>49.6</td>
<td>45.8</td>
<td>48.0</td>
<td>48.9</td>
<td>50.2</td>
</tr>
<tr>
<td>Feulgen-DNA (% reflectance)</td>
<td>82.6</td>
<td>81.5</td>
<td>81.4</td>
<td>80.6</td>
<td>80.5</td>
</tr>
</tbody>
</table>
by differences in preparing the stain. The data suggested that the Schiff’s reagents of the present experiments should be discarded after 2 wk, when the color intensity is determined by reflectance spectrophotometry. However, depending upon the source of basic fuchsin, the Schiff’s reagent may be used for 3-4 wk when the color is scored from a color chart (5), because a reflectance value difference of 5.0 between two samples is the minimum detected by the color chart method.

The progressive decrease in gel formation in the CMT, MQT, and MMT assays with advancing age of the milk sample agreed with results of Frank and Pounden (4). That no significant increase occurred in the catalase score with age of milk is contrary to their results on aged samples collected from a bulk tank. However, it is suggested that perhaps bacterial growth was more restricted in the Mason jar storage conditions of the present experiment than in the bulk tank system (4). Since pH did not change appreciably with age, the hypothesis that bacterial growth was not excessive in the present study is supported.

No changes in response were observed in the reflectance Feulgen-DNA assay after aging a given milk sample for four days. This suggests that Feulgen-DNA assay values probably would not be significantly affected in mixed bulk tank samples aged as long as four days. Both the gelation (1, 6) and the Feulgen assays (3) are based on measurement of DNA. The reason for the discrepancy in the results of these two types of tests with advancing age may be that the gel tests require a relatively intact DNA molecule to form the gel (1); whereas, the Feulgen-DNA test requires hydrolyzation of the DNA molecule (3). Thus, breakdown in the DNA molecule of milk somatic cells, with time, should not affect the Feulgen-DNA assay.

References