Investigations on Possible Use of Mastitis-Screening Tests in Dairy Herd Improvement Association Central Laboratories

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Abstract

A trial designed to answer questions relative to the use of mastitis-screening test in the DHIA program was conducted in two phases. In the preliminary phase, weekly bucket-milk samples were collected for 11 wk from 4 to 17 cows, to test the effects of preservatives, age of sample, and temperature of storing sample on the Wisconsin Mastitis Test (WMT) and the catalase test. Of the preservatives tested, boric acid showed the least detrimental effect on the screening tests. There was a consistent decrease in WMT reading with increasing age of the sample. Extreme temperatures, such as freezing and incubating at 28 C overnight, had an adverse effect on both screening tests.

The second phase was designed to test further the effect of boric acid and age of sample on the screening tests, and to compare the WMT and catalase test readings with leucocyte counts. Bucket samples from 47 cows were collected three times at 3-wk intervals. Each sample was tested three consecutive days following sampling. Unpreserved, refrigerated milk showed a gradual decrease in WMT reading with increased age. Boric acid addition caused an initial depression in WMT readings of fresh milk, followed by a daily decrease similar to that found in unpreserved milk. The effects of boric acid and age of sample were more predictable for the WMT than for the catalase test, due to the high variability of the latter.

The correlation between the leucocyte count and WMT readings on fresh and preserved milk for each of three days following sampling ranged from .81 to .90. The leucocyte-catalase test correlation ranged from .81 to .67.

Mastitis-screening tests have been developed as indirect methods of estimating the number of leucocytes in milk. The principal use of the tests have been at cow-side or on bucket milk, to detect cows producing abnormal milk, and in quality-control work on bulk herd milk. Interest is developing in use of a screening test in the DHIA program, to provide a monthly mastitis test on individual cows. A monthly test may offer little of value in terms of immediate diagnosis for treatment of clinical cases of mastitis. However, it may offer some long-range managerial benefits. A monthly test would indentify consistently abnormal cows that perhaps seldom reach the clinical stage of mastitis, but may be responsible for high readings on quality-control tests on bulk herd milk. A significant change in the monthly test or a gradual increase in screening test readings may indicate improper operation of the milking machine or faulty milking procedures or other management problems.

If a screening test is to be incorporated into the DHIA program, it would be desirable to use one milk sample for all tests in the DHIA laboratory. At this point, several questions arise: Is there a milk preservative compatible with the milk fat, protein, and solids-not-fat tests as well as the screening test to be used? Since DHIA samples are not tested immediately in all cases, does age of the sample have any effect on the screening test? DHIA samples are subjected occasionally to extreme temperatures. Do these extreme temperatures affect the screening tests? The work reported here is an attempt to answer these questions. It was conducted in two phases, a general preliminary phase and a more extensive, application phase.

The preliminary study was designed to examine the effects of various milk preservatives, age of samples, freezing the samples, and holding the samples unpreserved and unrefrigerated, on two mastitis-screening tests. The Wisconsin Mastitis Test and the catalase test were selected because of their objective measurements, simplicity, and relatively inexpensive equipment requirements.

Boric acid evolved from the preliminary study as the preservative showing the greatest possibility of being used in a joint DHIA-mastitis-screening test program. A more extensive study was conducted to test further the effects of aging and the use of boric acid as a pre-
serving on the Wisconsin Mastitis Test and the catalase test. Results of these two tests were also compared with the microscopic leucocyte count on refrigerated control samples.

Experimental Procedure

Preliminary study. Bucket milk samples of a PM milking were collected weekly for 11 wk. Initially, four cows were selected on the basis of monthly cow-side CMT records on the University of Wisconsin experimental herd. The cows selected represented the four CMT categories of 0, T-1, 2, and 3, as designated by Schalm and Noorlander (10). In succeeding weeks, additional cows were added at random until a total of 17 cows was tested the last 7 wk. No consideration was given the monthly CMT reading in selecting the additional cows.

Immediately after the samples were collected in the milking parlor, they were taken to the laboratory and divided into subsamples for the different treatments. Each experimental subsample had a control counterpart that was refrigerated until tested. Not all treatments continued throughout the test period. As soon as evidence of a deleterious effect of a treatment was obtained, the treatment was discontinued.

The following preservatives were tested at the rate specified per 150 ml of milk: formaldehyde (six drops 37%); mercuric chloride (one 2-gr tablet); sodium dichromate (one ½-gr tablet); hydrogen peroxide (1.5 ml 10% H₂O₂); boric acid (1.5 g). Samples containing preservatives were stored at room temperature (approximately 25 C) until tested.

The effects of extreme temperatures on samples were tested by freezing one set of subsamples, and incubating another set in a water bath at 28 C for 17-20 hr, or until tested. This was an attempt to simulate field conditions where DHIA samples are subjected to extreme temperatures. To eliminate the possible effects of preservatives, none was used in the temperature trial samples.

To test the effects of age of sample on screening tests, three sets of subsamples were refrigerated. Another set was preserved with boric acid and stored at room temperature. One set of the refrigerated samples was tested each day for three successive days following sampling. A different set was used each day, to eliminate any possible effects from warming the sample for testing, then refrigerating it again. In other words, once the refrigerated subsample had been warmed to room temperature and tested, it was discarded. The subsample preserved with boric acid was likewise tested for three consecutive days and results compared with the refrigerated control samples.

The Wisconsin Mastitis Test and the catalase test were applied to all subsamples. The WMT procedure described by Thompson and Postle (12) was followed. This test has a range in readings from 3 to 36 mm. The minimum reading of 3 mm, obtained from extremely high-quality milk, is the result of a natural film that adheres to the wall of the test tube during the testing procedure. The procedure for the catalase test as described by Burch (3) was followed with two modifications: a) 15-ml graduated centrifuge tubes with rubber stoppers containing 4 mm-diameter soft glass tubing were used rather than the 16- by 125-mm screw-type test tubes; b) 3 cc of 3% H₂O₂ were used rather than 1 cc. A fresh supply of 3% H₂O₂ was prepared for each test period from a 30% stock solution.

Duplicates were run on all samples the first 4 wk, to check the procedure for repeatability. Tests were begun approximately 17 hr after the samples were collected, except for the tests on the effect of aging. They were begun 17, 41, and 65 hr after sampling. All samples were standardized to a temperature of 24 ± 2 C before tests were applied.

The Orange G dye method (14), the Golding method (6), and the Babcock test were used to test the effects of boric acid as a preservative on the protein, solids-not-fat, and milk fat tests, respectively.

Application to herd conditions. Bucket-milk samples of a PM milking were collected from 47 cows in the experimental herd at the University of Wisconsin. This included all of the cows in lactation for the entire test period, from June 14 to August 2, 1965. To facilitate sampling and running the tests, the herd was divided. One-half of the herd was tested 1 wk, and the second half the following week, followed by 1 wk of no testing. This scheme was repeated three times, so that each cow was tested three times at 3-wk intervals. The 3-wk intervals were used in an attempt to measure the individual cow differences in screening tests from test period to test period, as would be available in a DHIA program. The interval was reduced to 3 wk rather than a full month, to obtain three test periods during the time allotted to the experiment.

Immediately after the samples were collected in the milking parlor, they were taken to the laboratory and divided into subsamples. Three sets of subsamples were refrigerated, one of which was tested each day for three consecutive days. Another set was preserved with boric acid (1 g/100 ml of milk), stored at room temperature, and divided into subsamples.
temperature, and tested each day for three consecutive days. Tests were begun approximately 17, 41, and 65 hr after samples were collected. The WMT and catalase tests, as explained in the preliminary study, were applied to all samples.

In addition, a slide was prepared, using the Levowitz and Weber stain, of the one-day-old refrigerated control sample. A microscopic leucocyte count was made and compared with the screening tests. The mean of 25 fields per slide, counted at random, was used to compute leucocyte numbers.

Results and Discussion

Results of the preliminary study are summarized in Table 1. Significance was determined by the paired “t”-test. Of the preservatives used, boric acid was the only one that showed possibility for further testing with regard to the WMT and catalase screening tests. Although boric acid had a depressing effect on the WMT, this effect was consistent. The initial depressing effect was further demonstrated in the trials on effects due to aging. On Day 1 there was a significant difference in WMT reaction between the fresh and preserved milk. However, on Days 2 and 3 there was no significant difference between fresh and preserved milk. This indicates that after the initial effect, milk preserved with boric acid has a normal deterioration rate similar to unpreserved milk, as far as the WMT is concerned.

The nonsignificant effect of boric acid on the catalase test in 53 samples was due to both positive and negative variation of the experimental samples from the control. There was a highly significant negative effect of boric acid on the catalase test in the trials on the effect of aging. However, the effects of aging were not consistent. The cause of the decrease in catalase reading on Day 2 is unknown, but may be attributed to the variability of the test.

The study of the effect of temperature on the screening tests was prompted by reports from the field that the catalase test on frozen samples was not consistent, and from the fact that DHIA samples are occasionally subjected to extreme temperatures.

Freezing had a highly significant effect on the WMT. The statistically nonsignificant effect on the catalase test was due both to positive and to negative variability and agrees with the unofficial field reports. Of the 30 samples tested, the catalase reading of the frozen samples was higher than the control on 12 samples, and lower than the control on 15 samples.

Incubation at 28°C overnight resulted in a
highly significant decrease in the WMT reading. The highly significant increase in the catalase test was probably due to bacterial growth. It is apparent that extreme temperatures imposed on milk samples will affect the reliability of the WMT and catalase tests.

Ashworth, Seals, and Erb (1) and Natzke (9) have reported the effects of various preservatives on the protein, SNF, and milk fat tests. Natzke found boric acid to be ineffective as a long-term preservative. Our studies suggested that as a short-term preservative, up to three days, it had possibilities. A small trial of eight samples, shown in Table 2, revealed a nonsignificant effect of boric acid on the Orange G dye and Babcock tests. However, it caused a significant increase in SNF and could not be used on milk employed for SNF testing.

Table 2 contains the analysis of variance for the WMT and catalase tests, using data from 47 cows sampled three times at 3-wk intervals. Each sample was tested three consecutive days following sampling. All first-order interactions were computed. For brevity in Table 3, the nonsignificant interactions were combined with the error term.

The highly significant breed effect may be explained by the small and unequal number of cows per breed, along with an abnormal distribution of ages and stages of lactation. All lactating cows in the herd were included in the trial without regard for breed, age, or stage of lactation. Consequently, there were 8 Ayrshires, 7 Jerseys, 13 Guernseys, and 19 Holsteins involved.

| Table 3
| Analysis of variance for Wisconsin Mastitis Test and catalase test |
| Source | df | WMT | Catalase |
| Breed | 3 | 451.18** | 1,409.12** |
| Lactation number | 2 | 1,179.61** | 4,688.64** |
| Testing period | 2 | 127.44* | 1,207.11** |
| Fresh or preserved milk | 1 | 673.79** | .12 |
| Days following sampling | 2 | 452.61** | 8.76 |
| Breed x lactation number | 6 | 163.51** | 3,830.92** |
| Residual | 329 | 33.76 | 297.47 |
| Coefficient of variability | 62.70% | 93.40% |

* Significant at the .05 level of probability.
** Significant at the .01 level of probability.

Lactation numbers were categorized into first, second, and third or more lactations. Samples were collected three times at 3-wk intervals. Samples were tested each day for three consecutive days following sampling.

The effect due to lactation number is consistent with previous reports. Marshall and Edmondson (8) and Braund and Schultz (2) report an increase in the California Mastitis Test reading with increasing age and lactations of the cow. There were 10 cows in their first lactation, 14 in their second, and 23 in their third or more lactation in the study.

The significant breed x lactation number interaction appears to be abnormal. An examination of the raw data shows the young cows of one breed, with small numbers of cows in the sample, having above-normal WMT and catalase readings. This appears to be a sampling error and would not necessarily be characteristic of the breed.

The significant effect due to testing period may be attributed to the progressively advanced stage of lactation. Even though there was only a 6-wk period between the first and third tests, the data tend to agree with earlier work. Braund and Schultz (2) showed an increase in positive reactions to the CMT as cows passed mid-lactation. Garrison et al. (5) report an increase in cell count with advancing lactation. Under normal herd conditions and over a period of time, where fresh cows are being added to the herd and dry cows are being removed, one might expect a nonsignificant effect due to testing period.

The effects of boric acid and aging on the
Mastitis Tests

WMT and catalase tests are the main items of importance in this study. The data in Table 3 showed a significant effect of boric acid on the WMT. Boric acid caused a consistent decrease in WMT reading. Of the 423 samples tested, 61.5% of the boric-acid-preserved samples had a lower WMT reading, and 11.8% had a higher WMT reading than their control counterparts. The 26.7% having the same WMT reading are, in general, the higher-quality samples, with low WMT readings that boric acid cannot further reduce.

Boric acid showed no statistically significant effect on the catalase test. This is due to lack of consistency in effect. Of the 423 samples, 51.8% of the preserved samples had a lower catalase reading, and 38.5% had a higher catalase reading than their control samples. This high degree of variability is emphasized further by the computed coefficient of variability of 93.4% for catalase, compared with 62.7% for WMT.

The significant effect due to days following sampling or the age factor is in agreement with work reported by Frank and Pounden (4) and Tucker and Paape (13). They report a progressive decrease in gel formation with advancing age of the sample, in tests based on DNA.

The specific decreases expressed as least-squares means are shown in Table 4. After the initial significant decrease in WMT due to boric acid, there is a consistent decrease with aging not significantly different from the normal decrease in fresh milk. This suggests the possibility of developing a correction factor that could be applied to the WMT, depending upon age of the sample and whether it is preserved with boric acid.

The same consistent decrease was not obtained from the catalase test, due to its high variability. Table 4 must not be construed to mean that the catalase test is unaffected by boric acid or by age. The stability in the mean catalase reading with age of the sample is also in agreement with Tucker and Paape (13).

Luedeeke (7) and Spence and Simon (11) have studied the relationship between CMT, catalase, and cell counts. Thompson and Postle (12) compared the WMT with leucocyte counts. Table 5 shows the correlation between cell counts and the WMT and catalase tests. The leucocyte counts were correlated with the WMT and catalase readings on fresh milk preserved by refrigeration and milk preserved with boric acid and tested for three consecutive days. The results are in close agreement with Thompson and Postle (12).

This work suggests that a monthly mastitis test on individual cows applied to the DHIA Central Laboratory has potential value as a management guide. If the same milk samples were to be used for the milk fat or protein test and the mastitis test, it would necessitate: a) selection of an appropriate mastitis test, b) use of a preservative compatible to all tests used, and c) consideration of the age of the sample. With respect to these three items, our data a) favored the WMT over the catalase test, b) showed boric acid to be a satisfactory short-term preservative compatible to the WMT, milk fat, and protein tests, and c) showed that samples should be tested within three days of collection, due to gradual reduction with age in WMT reading. The rather consistent daily decrease in WMT reading with increasing age of sample suggests the possibility of using a correction factor for age of sample. There is a need for additional experience with this procedure under actual field conditions.

References

TABLE 5
Correlation of microscopic leucocyte counts with Wisconsin Mastitis Test and catalase test on fresh and preserved milk one, two, and three days after sampling (N=141)

<table>
<thead>
<tr>
<th></th>
<th>Fresh milk* (days)</th>
<th>Preserved milkb (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>WMT and leucocytes</td>
<td>.84</td>
<td>.86</td>
</tr>
<tr>
<td>Catalase and leucocytes</td>
<td>.65</td>
<td>.67</td>
</tr>
</tbody>
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* Samples preserved by refrigeration.

b Samples preserved with 1 g boric acid/100 ml milk.


