APPLICATION OF THE WISCONSIN MASTITIS TEST UNDER FIELD CONDITIONS

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SUMMARY

The Wisconsin Mastitis Test was subjected to a trial in five Wisconsin Department of Agriculture Brucellosis Ring Test Laboratories. The test was conducted by 15 laboratory technicians and was evaluated on two bases, namely (a) reproducibility and (b) correlation of the test results of the technicians with a standard. The reproducibility for the results of each technician and each laboratory was expressed as a coefficient of variability (average pooled C.V. 8.46%) and the correlation was expressed as correlation coefficients (average 0.96). The results of the field trial indicate that the Wisconsin mastitis test can perform as well under field conditions as in a research laboratory.

The principle of estimating products of inflammation in milk from the gel produced by a detergent reagent in the presence of nucleated cells (California Mastitis Test - CMT) (3) has been widely accepted. Jaartsveld (1) presented a method for measuring and recording the degree of gelation present in a CMT-like reaction. The Wisconsin mastitis test (WMT) (5) was introduced as an extension of this principle, to fill a need for an objective screening test with an increased sensitivity for indirectly estimating the cell content of bulk milk samples with a range of cell numbers between 200,000 and 1,500,000/ml.

In the WMT, a 2-ml portion of well mixed milk sample is combined with 2 ml of CMT reagent that has been diluted 1:1 with distilled water. The milk and reagent are combined in a test tube that is fitted with a cap having a center orifice of 1.15 mm diam. Following mixing of reagent and milk, the test tube is held in an inverted position for 15 seconds to permit out-flow through the cap orifice. The test is scored by measuring in millimeters the height of the fluid column remaining in the tube after the tube has been returned to an upright position following out-flow. The agreement between the WMT method and a direct microscope method of estimating cell content of milk samples has been reported (5). Because demands of a large scale screening program would present significantly different laboratory conditions from those found in a research laboratory, it was necessary to know if the WMT would give valuable information under field conditions. It was also necessary to know if the WMT could perform well when conducted by laboratory technicians with limited technical experience or training.

Permission was granted to conduct a trial in the five Brucellosis Ring Test (BRT) laboratories operated by the Division of Animal Health of the Wisconsin Department of Agriculture. These laboratories are located in Mineral Point, Watertown, Green Bay, Black River Falls and Barron, Wisconsin. Three technicians in each laboratory were available for the conduct of the trial.

The trial followed the same pattern in each laboratory and was divided into three sessions: orientation and training, testing for reproducibility and a correlation test. The time spent in each laboratory (parts of 3 days) precluded the use of the same milk samples for all parts of the trial.

MATERIALS AND METHODS

All equipment necessary for the conduct of the WMT, as well as the milk samples necessary for training and reproducibility exercises, was furnished by the authors.

Continuing experience since the introduction of the WMT has led to the conclusion that duplicate WMT examinations of milk samples are desirable. An average of two readings for each milk sample should represent a more accurate result than a single reading. Duplicate tests also permit a check on the technique of the operator (measuring milk and reagent, mixing, timing of outflow and accuracy of reading the scoring gauge). A discrepancy of more than 4 mm between the duplicate readings suggests that the milk sample should be retested. Such a discrepancy can result from obstructions in the cap orifice as well as errors in measuring. All milk samples involved in this trial were submitted to duplicate applications of the WMT and the averaged results were recorded. Those samples with duplicate readings having a discrepancy of more than 4 mm were re-submitted to the testing procedure.

Technicians

The formal education of the 15 BRT laboratory technicians represented a broad range. One received a college undergraduate degree, five completed either a 1st year of college or the University of Wisconsin Farm Short Course, five com-

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Laboratory | Individual | Correlation coefficient | Y intercept on X axis | Slope of regression curve (b) | Standard error of estimate | No. of samples
--- | --- | --- | --- | --- | --- | ---
I | 1 | 0.96 | 0.89 | 0.99 | 2.02 | 100
II | 1 | 0.96 | 1.35** | 0.96 | 1.95 | 100
III | 1 | 0.91 | 5.75** | 0.83** | 2.99 | 100
IV | 2 | 0.91 | 2.28** | 0.83** | 2.40 | 117
V | 3 | 0.94 | 0.83 | 0.88** | 1.97 | 117
VI | 4 | 0.93 | -0.43 | 0.88** | 2.04 | 117
VII | 5 | 0.97 | 0.53 | 0.99 | 1.61 | 118
VIII | 6 | 0.99 | 0.53 | 1.01 | 1.22 | 118

**. ** Indicate significance at 5% and 1% level respectively, using t tests for Y intercept=0 and b=1.

| Laboratory | Average WMT reading of individuals | Average WMT readings within laboratories | Coefficients of variability
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
| I | 16.83 | 18.49 | 17.73 | 17.68 | 8.3% | 6.6% | 5.9% | 6.9%
| II | 12.84 | 11.71 | 12.56 | 12.37 | 14.2 | 8.6 | 9.7 | 11.3
| III | 15.57 | 15.14 | 16.67 | 15.59 | 5.5 | 5.3 | 4.9 | 5.2
| IV | 9.16 | 9.70 | 10.54 | 9.80 | 13.7 | 8.2 | 7.3 | 9.8
| V | 12.36 | 12.81 | 11.41 | 12.19 | 10.3 | 8.0 | 8.8 | 9.1

The orientation and training of the three technicians in each laboratory was completed in approximately 1 hour. An explanation of the purpose and theory of the WMT was offered, followed by a demonstration of calibration and testing procedures. Each technician was then invited to conduct as many calibration and testing procedures as he felt necessary to become familiar with the appropriate technique.

Reproducibility test

Reproducibility tests permit an expression of the ability to repeat the same results when the procedure is repeatedly applied to the same samples. The reproducibility is expressed as coefficients of variability (4) and the lower the coefficient of variability, the more reproducible the test. A low coefficient of variability engenders confidence in the mechanism of the test. To compare the reproducibility of the WMT when conducted under field conditions with the reproducibility reported from studies in a research laboratory (2) ten composite milk samples with California mastitis test (CMT) reactions varying from trace (T) through 2 plus (+ +) were chosen. From these 10 composite samples, three racks of 20 samples each were prepared. Each rack contained duplicate aliquots of each of the 10 composite milk samples. In each rack, samples numbered 11 through 20 were duplicates of samples numbered 1 through 10. The order of the 20 samples in each rack was randomized following a random-digits table. Each technician was assigned one rack of samples and was requested to apply the WMT to each sample. After this assignment was completed, the order of the samples was arranged in a different random order and the same technician was requested to reapply the WMT to his 20 samples. This entire procedure was repeated three more times permitting a total of 100 WMT observations for each technician with a grand total of 1500 observations for the entire trial.

Correlations

The major purpose of the WMT is to estimate the cell content of milk. To determine if this function could be performed as well under field laboratory conditions as in a research laboratory, 547 bulk milk samples were examined. Technicians from each BRT laboratory were requested to...
collect bulk milk samples representing a minimum of 100 farms in their respective areas. The number of samples examined in the five BRT laboratories were: 100, 117, 118, 100 and 112 respectively, making a total of 547. These milk samples were subjected to the WMT simultaneously by the three BRT laboratory technicians and the senior author.

For the correlation phase of the trial, the WMT results of the senior author were used as a standard with which to compare the WMT results of the 15 technicians. Comparisons were expressed as correlation coefficients (4).

**RESULTS**

**Reproducibility**

The average WMT results from 100 milk samples recorded by each technician, along with the average WMT readings within each laboratory, the coefficients of variability of each technician and the pooled coefficients of variability (4) for each laboratory are presented in Table 2. The coefficients of variability were computed for each individual in each laboratory using the variation within the 10 replicate tests within samples for that individual.

Any significant difference among the mean squares of individual technicians in each laboratory; among mean squares of “individuals x samples”; and among the variances of the three individuals in each laboratory (Table 3) was determined from an analysis of variance (4) of the data collected from the reproducibility portion of the trial. No comparison of the WMT results between laboratories was possible since different milk samples were supplied to each laboratory.

Although the differences among the average WMT readings of the three individuals in each laboratory were small, there was a significant difference within each laboratory as determined by the F value for the mean squares of individuals. Because large numbers of observations were collected, these small differences could be demonstrated.

Differences among samples for these several individuals tended to remain quite constant throughout the trial. This was especially evident in laboratories II through V as shown by the mean squares of “individuals x samples” (Table 3).

The chi-square test for homogeneity of variances was used to compare the variances of individuals within a laboratory (Table 3). The residuals for all technicians were examined and were found to have essentially normal distributions. Only two technicians (Individuals I in laboratories II and IV) were significantly more variable than the other technicians in their respective laboratory (Table 2).

**Correlation**

Correlation coefficients provide a standardized statistical tool for measuring how two variables vary together, or expressed in another way, a measure of the intensity of association of two variables. A perfect functional linear relationship between two variables may be expressed as a correlation coefficient of 1. The agreement between the WMT results of the various BRT laboratory technicians and the standard used, expressed as correlation coefficients, ranged from 0.91 to 0.98 (Table 1) with an average of 0.96. To further illustrate the agreement, the Y axis intercept point, the slope of the regression curve and the standard error of estimate were determined from the results of each technician. Significance at the 5% and 1% levels of any differences between the Y intercept points was zero and the slope of the various regression curves (b) = 1 was determined by application of t tests. These data are summarized in Table 1.

As an illustration of the value of these descriptive quantities, a scatter diagram is presented (Figure 1) showing: The correlation between the WMT results of technician nine (X axis) and the standard (Y axis); the regression equation, the standard error of estimate, the regression curve, and the point of intercept of the regression curve on the Y axis. If the results represented on the X axis (technician’s WMT results) were identical with those represented on the Y axis (standard), the slope of the regression curve would be 1, the Y intercept would be zero and all points would fall on the regression line.

**Table 3. Statistics for Determining Significance of Deviations from the Mean and Homogeneity of Variances**

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Mean squares of:</th>
<th>Error</th>
<th>Chi square</th>
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<tbody>
<tr>
<td>I</td>
<td>69.05**</td>
<td>1.52</td>
<td>5.36</td>
</tr>
<tr>
<td>II</td>
<td>34.63**</td>
<td>1.95</td>
<td>33.98**</td>
</tr>
<tr>
<td>III</td>
<td>21.67**</td>
<td>0.67</td>
<td>0.03</td>
</tr>
<tr>
<td>IV</td>
<td>48.11**</td>
<td>0.93</td>
<td>13.06**</td>
</tr>
<tr>
<td>V</td>
<td>51.00**</td>
<td>1.22</td>
<td>5.67</td>
</tr>
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* and ** Indicates significance at 5% and 1% respectively. Significance of mean squares of individuals determined from F value = Individuals / Samples.

Similarly, the significance of individuals x samples is determined from F value = Individuals / Samples. Test of the homogeneity of variances among the three individuals in each laboratory is expressed by Chi square values. The degrees of freedom for individuals, individuals x samples, and error were 2, 18 and 270 respectively for data from laboratories II through V. For data from laboratory I, the degrees of freedom were 2, 38 and 240.

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*Average r was computed from z transformation (4).*

*Random selection.*
The average correlation coefficient of 0.96 indicates a very high agreement between the WMT score of the technicians and the standard used. Twelve technicians produced results in which there was no significant difference between the respective Y intercepts and zero. As an example of a significant difference, individual number 3 in laboratory I (Table 1) had a Y intercept of approximately 6 mm. Similarly, the slope of the regression curve for the same individual was significantly different from 1 (b=0.83), indicating that he read one unit for each 0.83 units of the standard. This large Y intercept indicates that this individual was reporting results consistently lower than the standard. The slope of the regression line being less than 1 indicates that the difference was more pronounced at the lower end of the scale than at the upper end.

The two greatest standard error of estimates were found for the same individuals whose Y intercepts were farthest from zero. Ten technicians produced results in which there was no significant difference between the slope of the respective regression curves (b) and 1.

CONCLUSIONS

These data permit some general statements. Individuals produced WMT results with a high degree of consistency. Similarly the variation between individuals was greater than the variation within individuals. The technicians conducting the WMT produced results showing excellent agreement with the standard used. Further, the WMT in the hands of these technicians proved to be highly reproducible. The design of the trial permitted a highly critical examination of the WMT under field conditions. Consequently, even minor differences between individuals conducting the test were demonstrated. When translated into cell numbers, however, the importances of these differences is questionable. As an illustration, the greatest difference between individual average WMT readings within any laboratory (Table 2) was 1.6 mm, which represents approximately 70,000 cells per milliliter of milk when this measurement is made in the central range of the WMT scale.

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REFERENCES