A SIMPLE QUANTITATIVE CATALASE PROCEDURE FOR ABNORMAL MILK

J. J. JANZEN AND W. C. COOK

Department of Dairy Science
Clemson University, Clemson, S. Carolina 29631

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SUMMARY

A simple, quantitative catalase test for detecting abnormal milk has been described. This procedure utilizes a 10 ml. B-D Yale glass syringe with a 23 ga 1/2 inch Luer-Lok needle. The needle tip is plugged by inserting into a No. 00 rubber stopper. Five ml. of milk and 0.5 ml. of 3% H2O2 solution are measured into the syringe barrel. The syringe is then inverted, and stopper loosened to allow the discharge of all entrapped air from the syringe. The syringe is then reclosed, set upright, and incubated at 73°F for 3 hours. The volume of O2 released is measured as ml. O2 (directly on the scale) and converted to % O2 by multiplying the reading by 20.

This test provides a simple, efficient, and reproducible procedure for reporting catalase activity on the basis of percent oxygen evolved during a standardized test period. Comparison of the Clemson and Wisconsin Catalase test procedures indicated consistently lower values for the latter. Possible reasons for this variation are mentioned.

The catalase test has been used for many years as a measure of the leucocyte content of milk. This test is based on the decomposition of hydrogen peroxide by the enzyme catalase which is present in milk.

Milk normally contains some catalase, however, udder infections increase the catalase activity of milk. This increase is due largely to leucocytes, body cells, blood and bacteria, especially staphylococci and aerobic spore formers.

Fermentation tubes of various types have been used to measure the amount of oxygen liberated by the catalase present in the milk (5). Inverted tubes with standardized orifices in the cap have also been used (6). Similar techniques using centrifuge tubes fitted with stoppers and glass tubes also have been used (4). All of these procedures, while varying in simplicity, have certain drawbacks. Many do not measure all the oxygen liberated.

Garrison and Patrick (3) described a quantitative technique which permitted the measurement of total volume of liberated oxygen. This procedure required the use of a glass test-tube and a special agar plug. Willits and Babel (7) have described a disc flotation technique which is simple, rapid, accurate and requires very little equipment. Nageswararao, et al. (4) report a close relationship between the inverted tube method and the Warburg procedure, on samples ranging from 18 to 50% oxygen.

Most catalase tests that have been proposed are relatively simple. They all propose to measure the amount of oxygen released by the action of the enzyme catalase on a standard hydrogen peroxide solution. The temperature and time of incubation also must be specified. All proposed tests require rigid standardization, but the results are not always reported in units that can be readily converted to a standard base, such as percent oxygen. This paper will describe a simple test for measuring the total oxygen liberated from the hydrogen peroxide by the enzyme catalase in the sample of milk being tested.

EXPERIMENTAL PROCEDURE

Materials.

This procedure utilized 10-cc B-D Yale Locking syringes with 23-gauge 1/2-inch Luer-Lok needles. The needle tips were plugged by insertion into rubber stoppers No. 0 or No. 00. A test-tube rack was used to store the syringes during the performance of the test. Mohr pipettes, graduated to 0.1 ml, were used for measuring the 3% H2O2 solution and the milk samples. A 3% H2O2 solution was prepared from a 30% stock supply. One incubator set at 72°F, and a time clock were used. Figure 1 displays the component parts of this catalase test. Figure 2 presents the completed test following incubation.

Figure 1. The component parts of the Clemson catalase test; flask with 3% H2O2; (1) plunger, (2) syringe barrel, (3) Luer-Lok needle plus stopper, (4) Jeb tube milk sample, (5) assembled syringe, (6) mohr pipettes graduated in 0.1-ml increments.

2Technical Contribution No. 651, South Carolina Agricultural Experiment Station. Published by permission of the director.
Clemson catalase test showing syringes, in rack, following incubation at 72 F for 3 hours and ready for reading.

**Procedure.**

Be sure the glassware is clean, and the plunger and barrel of each syringe properly matched (both barrel and plunger have matching numbers). Attach the needle to the syringe tip and insert the needle into the rubber stopper. Introduce 5.0 ml of well-mixed milk into the syringe barrel. Add 0.5 ml of 3% H.O. solution with a mohr pipette. Insert syringe plunger into the barrel, invert the syringe assembly, loosen the needle seal with a gentle half-twist, and slowly push up the plunger to evacuate all the air within the syringe. Reclose the needle seal. Return the syringe to an upright position, and set it in the test-tube rack and incubate it at 72 F for 3 hours. The released O₂ will collect at the top of the milk, displacing the plunger. Measure the volume of O₂ released by subtracting the milk level reading from the base-of-plunger reading. Calculate the ml O₂ by multiplying the ml of O₂ by 20. (Convert the ml O₂ reading to a basis of 10 ml of milk and multiply by 10 to obtain percent).

**RESULTS AND DISCUSSION**

This procedure has been used over a period of one year and has been found to be practical, easy to perform and replicable. Replicability results are presented in Table 1.

The results reported in Table 1 were obtained using 10-ml syringes. These syringes were calibrated in 0.2-ml increments. The use of 30-ml syringes also has been investigated and is presently being used on another research project. This larger syringe permits the use of a 10-ml sample of milk plus 1 ml hydrogen peroxide. The 30-ml syringe has the advantage of allowing the measurement of volumes of O₂ well over 100%. This may occur in some samples high in leucocytes and/or bacteria. The major disadvantage of using a 30-ml syringe is the lack of accuracy in reading the scale, since the smallest graduations are in 1-ml increments.

Disposable 12-cc Roehr* monoject plastic syringes have been tried in lieu of glass syringes. The replicability of this test was poor when the plastic syringes were used. The rubber-tipped plungers offered considerable and varying resistance to movement within the syringe barrel. The resistance appeared to be the major cause for the variable results.

Comparisons were made between the Wisconsin Catalase Test (1) and the Clemson Catalase test described in this paper. The Wisconsin procedure used the 12-cc Roehr plastic syringe, 9-ml milk, and 1 ml 3% H.O. The 12-cc. Roehr plastic syringe represents an improvement over the earlier screw-top tube method as used by Corbett (2). The incubation temperature and time was the same for both procedures (72 F for 3 hrs). It should be noted that the Wisconsin procedure allows for the milk-peroxide mixture to drip from the tip as O₂ is generated within the syringe. This allows for varying volumes of milk to be expelled, depending on the catalase activity within the sample. In the case of the Clemson procedure,

![Figure 2](http://meridian.allenpress.com/journals/app pair=411701994584093420157_1,19935350_29965269/411701994584093420157_1.png)

*Roehr Products Co. Inc., Deland, Florida.

**Table 1. Replicability of the Clemson Catalase Test**

<table>
<thead>
<tr>
<th>Raw milk samples tested</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>%O₂</td>
<td>44</td>
<td>32</td>
<td>72</td>
<td>44</td>
<td>36</td>
<td>44</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>Total replications</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>No. agreeing</td>
<td>9</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>No. disagreeing</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Maximum variation (% O₂)*</td>
<td>+4</td>
<td>0</td>
<td>+4</td>
<td>0</td>
<td>0</td>
<td>+4</td>
<td>+4</td>
<td>0</td>
</tr>
</tbody>
</table>

*Plus (+) indicates the varying test was higher than the accepted % O₂ level for the respective sample.

**Table 2. Comparison of Clemson and Wisconsin Catalase Tests**

<table>
<thead>
<tr>
<th>Range of variations‡ between the 2 procedures</th>
<th>Milk samples tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>(% O₂)</td>
<td>(No.)</td>
</tr>
<tr>
<td>0.0</td>
<td>24</td>
</tr>
<tr>
<td>2.0 - 4.0</td>
<td>85</td>
</tr>
<tr>
<td>6.0 - 10.0</td>
<td>80</td>
</tr>
<tr>
<td>12.0 - 20.0</td>
<td>51</td>
</tr>
<tr>
<td>22.0 - 30.0</td>
<td>18</td>
</tr>
<tr>
<td>32.0 and over</td>
<td>11</td>
</tr>
<tr>
<td>Total milk samples tested</td>
<td>267</td>
</tr>
</tbody>
</table>

*The Wisconsin test reading, where different, were always lower than the Clemson test readings.
none of the milk is lost and all the \( O_2 \) is retained within the syringe.

The results of both tests performed on identical milk samples are summarized in Table 2. The data in Table 2 indicate considerable variation between these two tests. It must be recognized, however, that the test data are not directly comparable because of the inherent error in expressing the Wisconsin test results as percent.

**CONCLUSION**

The catalase test described appears to provide a simple, efficient and reproducible procedure that allows for reporting of results on a percentage basis. Reporting results on the basis of "% \( O_2 \) evolved" under a standard set of conditions provides for easy comparison of results between laboratories.

**ACKNOWLEDGMENTS**

Appreciation is expressed to Mrs. Florence Crawford for her untiring efforts in the performance of these tests and collection of the data.

**REFERENCES**


**CERTIFIED RAW MILK, WHAT IS IT?**

L. D. Searing

Seattle-King County Health Department
Seattle, Washington

Certified Raw Milk was sold in the Seattle and King County area many years ago. It disappeared over twenty-five years ago, at the beginning of World War II because of difficulty in obtaining adequate qualified help.

Now, with the adoption of the new King County Milk Sanitation Resolution, Certified Raw Milk is again permitted. Some of the dairies have shown interest in qualifying for the sale of this product.

The definition of "Certified Raw Milk" in the King County Resolution is as follows:—"Certified Raw Milk which conforms with requirements of the American Association of Medical Milk Commissions, Inc., in force at the time of production and is produced under the supervision of a Medical Milk Commission, recognized and approved by the American Association of Medical Milk Commissions, Inc., reporting monthly to the Director." (of Public Health).

Standards for Certified Raw Milk are higher than for Grade A Raw Milk. Weekly samples of all Certified Milk and milk products are required for butterfat and total solids tests and bacteria and coliform analysis. Certified Milk shall have a bacterial count of not more than 10,000 colonies per ml and a coliform count of not more than 10 per ml. Monthly veterinary inspection of the milking herd must be made. All milking animals must be tested for tuberculosis annually and for brucellosis semi-annually. Monthly medical examination of dairy workers is required and sanitary inspections are required at least once per month.

A certified milk program requires the organization of a Medical Milk Commission. A Medical Milk Commission may be appointed by the Medical Society, by public health officials, or by the Council of the American Association of Medical Milk Commissions. The Medical Milk Commission elects its own officers and appoints a Physician, a Veterinarian, a Laboratory Director, and a Sanitarian to enforce the Methods and Standards.

In contrast to the above requirements for Certified Milk, Grade A raw milk standards require only four samples of milk and milk products each six months for butterfat, total solids, and bacterial analysis. No coliform analysis is required. The bacterial standard is 20,000 per ml or twice as lenient as for Certified Milk. No Veterinary inspection of the herd is required. No medical examination of the dairy worker is required, merely a chest X-ray every two years. Sanitary inspection is required only once in six months. Annual tuberculosis and brucellosis tests are required.

The question might be asked—"after twenty-five years without Certified Milk in this area, with the development of a fine Grade A pasteurized milk supply, with the development of excellent pasteurization equipment and controls, with ninety-eight per cent of the milk sold being pasteurized, why is Certified Raw Milk necessary?" Certified Raw Milk represents a compromise in the Health Department's efforts to do away with Raw Milk entirely. A small segment of our population sincerely believes that raw milk is more nutritious and believes it is their constitutional right to buy it. Certified Raw Milk preserves this right but assures a safer product.

With the necessary higher costs of producing Certified Raw Milk it is anticipated that the sale will drop appreciably and that eventually only pasteurized milk will be available.