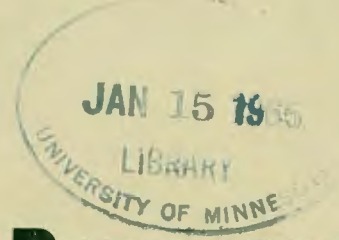


Minnesota

Dairy Products Processor



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MINNESOTA ABNORMAL MILK CONTROL PROGRAM

Minnesota is embarking on an abnormal milk control program developed out of the deliberations of the Minnesota Mastitis Council. The state organization is an affiliate of the National Mastitis Council, and represents all groups interested in mastitis control: producers, processors, veterinarians, milking machine manufacturers, regulatory agencies, and educational institutions.

The council's responsibility is to use the best information available in making recommendations for the control of mastitis. A procedure for control of all abnormal milk has been recommended; state and local regulatory agencies may adopt this procedure as a part of their control effort. If the program is to succeed, uniformity of action and testing is essential, as is the cooperation of all groups represented by the council.

RECOMMENDED PROCEDURE FOR ABNORMAL MILK CONTROL

The Minnesota Mastitis Council recommends the following procedure for control of abnormal milk:

- I. Once each month milk from producer herds will be checked for abnormality with the catalase test.
- II. For herds in which the catalase test shows 30 percent or more of oxygen the following program will be in effect:
 - A. Each quarter of all milking cows shall be tested, using the California Mastitis Test or other approved test. Each cow producing abnormal milk will be identified. The producer will be requested to withhold milk from such cows until further tests indicate that it is normal.
 - B. A fieldman or other qualified person shall visit the herd at milking time.
 - C. The producer will be encouraged to consult qualified people to advise him on improved milking practices, milking machine operation and inspection, and herd health.
- III. Regulatory Action
 - A. If a patron has three consecutive monthly catalase tests of 30 percent or more oxygen, the regulatory agency responsible for inspection of the supply of milk shall request from the producer a letter from the producer's veterinarian certifying that the herd is under professional supervision for correction of abnormal milk.

- B. The producers will be requested to forward the letter from the veterinarian to the regulatory agency within 10 days after receiving written request. To affirm that the milk is satisfactory for human consumption, only milk from cows approved by the attending veterinarian may be marketed.

THE CATALASE TEST

Catalase is a compound found in all milk usually at low concentrations. It is present also in blood cells, both red (erythrocytes) and white (leucocytes). Mastitis infection is followed by an increase in leucocytes in milk and, therefore, catalase content.

Positive reactions by the catalase test occur in milk from:

1. Cows with mastitis
2. Stripper cows (milked less than twice daily)
3. Fresh cows (less than 5 days after calving)
4. Cows suffering from any systemic illness
5. Cows with injuries causing blood to be present in the milk

The above factors must be considered in interpreting results of the catalase test and, to some extent, other routine mastitis tests. All are abnormal milk problems, but corrective measures will vary accordingly.

DIRECTIONS FOR MAKING CATALASE TEST

I. Equipment and Reagents:

- A. Hydrogen Peroxide--Stock solution (30 percent) from which a 3-percent solution is made up fresh prior to testing. Because hydrogen peroxide loses strength during storage, obtain stock solution from chemical supply houses that can assure fresh stock. Purchase only small amounts, use within a short period of time, and always keep refrigerated.
- a. Three-percent hydrogen peroxide is prepared by diluting one part of 30-percent solution to nine parts of distilled water. Make up only that amount which will be used in 2-3 hours.
- B. Test tubes: 15 ml., screw cap--outside diameter 16 mm., length 125 mm. Plastic caps can be purchased with 1/8 inch diameter hole in the cap. All caps must have this hole. Regular caps can be bored from inside out with 1/8 inch diameter drill.

C. Sterile distilled water.

II. Procedure

1. Add 10 ml. of milk to test tube.
2. Add 1 ml. of 3-percent hydrogen peroxide.
3. Fill tube to overflowing with distilled water.
4. Place cap on to finger tightness. (If automatic syringes are used for measuring or filling, cap may be placed on tube after milk addition and steps completed through hole in the top.)

5. Invert tube(s), being careful not to permit air bubble to enter.
6. Incubate sample(s) at room temperature (not less than 70° F.) for 3 hours.
7. Measure volume of gas in tube and record.

Catalase has the ability to break down hydrogen peroxide to water and oxygen. Amount of oxygen liberated as noted by length of air space in the tube is proportionate to amount of catalase present.

III. Construction of Measuring Device

1. Cut an L-shaped ruler from a piece of rigid cardboard.
2. Add 1 ml. of water to test tube and mark this level on the ruler just above the base of the "L."
3. Add 9 ml. of water and mark this level on ruler.
4. Divide area between these two marks into 9 equal parts. The measuring device then consists of 10 major divisions equivalent to 10- through 100-percent oxygen levels. Read results directly.

IV. Determining Concentration of 3-percent Hydrogen Peroxide

If the stock solution is fresh no question should exist concerning concentration of 3 percent hydrogen peroxide made from it. Where some doubt of freshness exists, the following procedures may be used:

To an Erlenmeyer Flask:

1. Add 5 ml. 10-percent potassium iodide solution
2. Add 2 ml. glacial acetic acid
3. Add 1 ml. of hydrogen peroxide solution (approximately 3 percent)
4. After partial titration, add a few drops of starch indicator
5. Titrate to starch end point (blue-black to colorless) with 0.1 normal sodium thiosulfate.

Then: Percent hydrogen peroxide = ml. $\frac{0.1 \text{ normal sodium thiosulfate}}{5.9}$

Final concentration should be between 2.8 and 3.2 percent hydrogen peroxide.

Preparation of starch indicator

1. Make a starch suspension by stirring 1 gram of soluble starch into 10 ml. of distilled water.

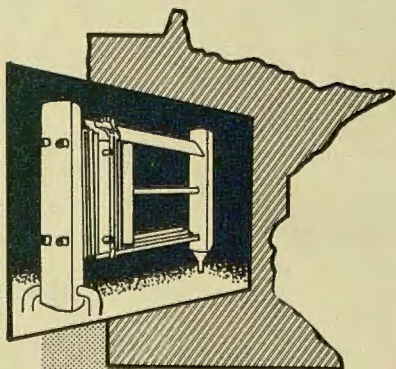
2. Pour this well-mixed suspension into 100 ml. of boiling distilled water, stir thoroughly, and continue heating for 2 minutes.
3. Cool. If a precipitate settles out, pour off solution above it and use this as the indicator.
4. Always prepare fresh indicator.

PRECAUTIONS IN MAKING A CATALASE DETERMINATION

Milk on which a catalase test is made must be fresh. Some bacteria contain catalase and excessive growth will contribute to the catalase content.

Preservatives should not be added to milk prior to making a catalase test; they will retard catalase activity.

I would be interested in learning about any problems that develop in making catalase determinations or in the abnormal milk program as such. Several organizations, agencies, producers, and processor groups are vitally interested in the outcome of this program and all are prepared to assist in resolving problems.



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