MEMBRANE FILTER METHOD FOR DETERMINATION OF
COLIFORMS IN PASTEURIZED AND CERTIFIED MILK

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The use of the membrane-filter method for determination of coliform organisms in pasteurized and certified milk permits a more rapid counting of coliform colonies with the elimination of much of the plating procedure used with the conventional determination methods. Results of analysis for coliform content can be preserved on the membranes for future reference. Large quantities of milk can be examined rapidly through the use of the centrifuge in the method outlined.

Endo-agar was used in this work as the differential agar medium.

INTRODUCTION

The coliform organisms, principally the species belonging to the E. coli and A. aerogenes groups, include the aerobic and facultative anaerobic, non-spore forming and Gram negative bacteria, which ferment lactose with formation of gas and acid. These organisms are found almost any place in nature and they grow in the soil, on feed, on floors, intestinal tract of animals and man, etc. As lactose fermenting, they will grow rapidly in milk or cream and also in residual amounts of milk products left in the production or manufacture utensils. Being so widely present in the environment of the dairy farm and plant, it is not surprising that they are commonly present in raw dairy products.

The non-spore forming coliform organisms are mostly destroyed during pasteurization. However, some strains may survive these temperatures. The organisms may also gain the entrance to the finished product directly from various sources during processing such as cracks and crevices in equipment, ineffective sterilization of equipment, or indirectly by hand or air contamination. When once in milk, they may grow and multiply under optimal conditions. The presence of coliform bacteria does not necessarily indicate a health hazard as they are not pathogenic, nor does it necessarily indicate contamination with fecal material. The relative numbers in a product may, however, indicate the care and sanitation exercised in handling and processing.

In recent years, various states have developed standards on the number of coliforms permitted in pasteurized milk. The City Health Departments of Chicago, Columbus, and Buffalo have established and are now enforcing standards of a maximum of 10 coliforms in 1 ml milk. This standard is recommended by the U.S. Public Health Service in their forthcoming ordinance and code. The City of New York has special standards for summer — 2 coliforms in 1 ml — and for winter milk 0.3 coliform in 1 ml. Albany, N. Y. allows a maximum of 0.36 coliform in 1 ml pasteurized milk or 36 in 100 ml. Many other cities which until this time have had no standards are working to establish some. Various rigid coliform standards have already been established in Europe.

Milk containing more than 30 coliforms in 1 ml cannot be sold in Germany as certified; in England, 100 ml of certified milk must not contain more than 10 coliforms, and grade A pasteurized milk cannot contain any coliforms.

HISTORICAL

The identification and isolation of coliform organisms is usually made with two bacteriological tests; the presumptive and the completed. These procedures require considerable time and a large amount of bacteriological equipment, especially to properly evaluate quantitatively the number of coliforms present in pasteurized milk. The membrane-filter method to be described, has the advantage of showing on one petri dish the number of coliforms present in a large amount of milk.

The membrane-filters were first used to determine the number of coliforms in water. The purpose of these experiments was to obtain on one membrane disc the

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bacteria present in a large amount of water, and simultaneously to avoid using of a large amount of bacteriological equipment. The results of these water tests were satisfactory; the E. coli, A. aerogenes, Typhus and Paratyphus group bacteria, genus Eberthella and Salmonella were easily cultivated and showed good growth on the membrane filters, using standard differentiation agar as medium, while at the same time other bacteria were hindered in their development.

The membrane-filter exists in a very thin and uniform colloidal form, which is made from cellulose or from cellulose esters with pores 1.5 to 0.5 microns wide. The filters are white and the surface is smooth and shining. They are resistant to 20 percent H₂SO₄ solution, 20-25 percent HCl solution, 25 percent Nitric acid solution and 1 – 3 per cent NaOH solution. The membrane-filters can be used only for filtration of water and water solutions, and are not resistant to organic solvents or solutions, such as ether, chloroform, acetone, etc. However, they can be washed for a short time with diluted alcohol. The filter is best preserved in distilled water, containing a few drops of disinfectant.

**EXPERIMENTAL**

After experiments for determination of coliforms in water, the following method was developed for testing pasteurized milk to determine coliform organism content using the membrane filters.

**A. Equipment.**

1. **Vacuum filter.** The filter apparatus consist of a 1000 ml filtering flask with a rubber stopper and a Buchner type funnel with or without fritted disc. Also a Morton filter apparatus or any suitable bacteria filter apparatus can be used.

2. **Membrane filter.** Membrane filter by R. Zsigmondy obtained from Membranefiltergesellschaft, Sartorius-Werke A. G., Goettingen, Germany, were used for the experimental work.

3. **Petri dishes, differential agar for coliforms, cotton, and alcohol.**

**B. Sterilization of the bacteria filter apparatus.**

A swab of cotton is dipped in alcohol, ignited, and all parts of the apparatus sterilized with the burning cotton. It is best to work the vacuum pump simultaneously in order to sterilize the inside of the apparatus. The apparatus can be also sterilized in an autoclave.

**C. Preparation of the membrane filters.**

The filter must be boiled in distilled water for a period of 20 minutes. This operation will remove the disinfectant and the air contained in the pores and at the same time will adequately sterilize the filter. The pores will then be free for the capillary action and give free entry to the agar, so that the agar can cover the surface of the filters in a thin layer.

**D. Preparation of milk samples.**

Warm the milk sample to 40°C and centrifuge in sterile centrifuge bottle for 10 minutes at 2000-2500 rpm.

Draw off the milk and wash the remaining sediment with approximately 50 ml of distilled sterile water.

Shake vigorously and centrifuge as before.

Again draw off the water layer; dissolve the remaining sediment with about 100 ml of sterile distilled water and shake vigorously to break up the sediment particles.

**F. Filtration.**

The membrane filter is handled with sterile tweezers and placed on the filter apparatus plate. The entire surface of the membrane should rest on the filter plate, with the printed number up. Using vacuum, filter the dissolved sediment through the membrane. After the filtration is finished remove the membrane, dry the bottom care-fully over a hot plate, and place on an agar plate, previously prepared by pouring approximately 10 ml of agar into sterile petri dish. The agar should be hard before use. In placing the membrane on the agar, avoid building up of air bubbles under the filter. To discourage the development of surface colonies, pour an additional 4 ml of agar over the surface.

**RESULTS**

After 24 hours incubation at 37°C the cultures on the filter are counted and reported as the number of coliforms in the amount of the initial material. The differentiation of the coliform colonies from other bacteria, which are mostly hindered in their growth, occurs in the same way as in regular standard plating. Any kind of differential agar may be used as the medium; however, during these experiments the best results were obtained with the use of Endo-agar. The coliform cultures appear deep red, while the other colonies appear white. After the readings are made the membrane-filters may be dried in an oven at 60°C and preserved; no further growth will occur but the initial colonies remain intact.

The data in table 1 shows some of the results obtained by this method during the preliminary experiments. The milk samples were not commercially pasteurized, but pasteurized in the laboratory.

The gas tube test results are reported as a percentage of positive tubes of five tubes used for each dilution. Membrane filter results are reported as the average colony count of five membrane filters for each size sample of milk reported.

Sterile water blanks in place of milk samples were run under controlled laboratory conditions to determine the extent of possible contamination of a selected sample that could occur with the membrane method employed for coliform detection. Since all the sterile water blank examinations made showed
zero coliform, it was established to the satisfaction of the author that coliform content secured on milk samples tested was from the milk source and not a result of technical contamination.

**Summary**

Preliminary work on the use of a membrane-filter method for determination of coliforms organisms is presented. This method permits more rapid counting of coliform colonies present and eliminates much of the plating heretofore necessary. The results of the counting can be preserved directly on the membranes for future reference. The use of the centrifuge in the method outlined permitted actual examination of large quantities of milk.

**Acknowledgment**

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Part of the experimental work was done at the Bacteriological Institute of the South Germany Dairy Experimental Station, Weilenstephan, Germany.

**References**

9. Membranefilter Ges. Bakterienfiltrations-Apparat "COLI 5".

**Table 1 — Preliminary Comparative Data**

<table>
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<th>Sample</th>
<th>Percent of tubes showing gas develop.</th>
<th>Number of colonies on membrane filters</th>
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**Influence of DDT Wettable Powder**

(continued from page 5)

**References**