Methods for the Bacteriological Examination of Milk Bottle Caps, Hoods, and Closures *

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A considerable amount of study has been made of the sanitary and bacteriological condition of glass and paper containers for milk, cream, and other dairy products. Conditions with respect to caps, hoods, and closures used in the packaging of these foods have, by comparison, received little attention. There is sometimes a tendency to ignore the fact that the cap or closure is really a part of the milk container and, as such, often comes in direct contact with food products. Some laboratories prefer, therefore, to test the complete package. When studied in this way, milk bottle rinses give the combined counts from containers and closures. With this procedure it is possible also to determine contamination from the capper.

Certain investigations of this subject are concerned chiefly with the protection which various types of closures afford bottled milk or cream. (1, 2, 3, 4). Similar problems in the case of other dairy products such as cheese and ice cream are also receiving consideration.

Quite apart from matters of sanitary protection, questions are frequently asked concerning the sanitary and bacteriological condition of the cap or closure itself. It is sometimes necessary, therefore, to test these products independently of the containers. Certain studies of this problem have already been carried out (5, 6), particularly by J. W. Rice who pioneered in the field. Feeling that more detailed investigations of suitable bacteriological methods for testing these products are needed, especially contact methods for the determination of surface contamination, further studies of the subject have been undertaken at this laboratory.

There are many types of caps and covers for milk bottles (7) including the common disc or plug caps; caps which cover to some extent the bottle lip or rim; hoods and closures made of paper, paperboard, metal, transparent sheeting, synthetic plastics, or films having cellulose, rubber, wax, or resinous bases, the majority of which not only cover the pouring lip but are sealed around the neck of the bottle.

Various methods have been developed for determining the bacteriological content of disc caps, hoods, and closures. These include the following procedures:

1. Disintegration tests for paper and paperboard products.
2. Rinse methods.
3. Contact culture methods for hoods and metal foil used as receptacles for nutrient media.
4. Milk bottle blank method.

Disintegration Tests. This method is useful for estimating bacterial counts in paper and paperboard used in the manufacture of these products. The test is also applicable to disc caps where it is desirable to know the bacteriological condition of the interior of the paperboard cap as well as the amount of surface contamination.

Determination of Surface Contamination.

a) Milk Bottle Blank Method. It is sometimes desirable to determine the bacteriological counts of cap and closure surfaces, particularly the surfaces which come in direct contact with perishable

* Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 424, October 9, 1940.

foods such as milk and milk products. Various rinse and contact culture methods have been employed but one of the most satisfactory procedures involves the use of an unblown milk bottle blank** (Figure 1) having a normal cap seat and a concavity of about 25 ml. capacity.

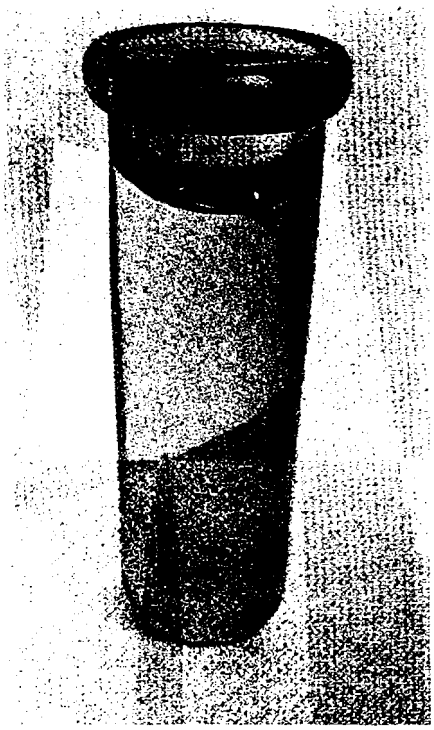

FIGURE 1
Unblown Milk Bottle Blank

Milk bottle blanks with kraft paper, metal foil, or other suitable covering sealed over the mouth, are sterilized in an autoclave. Approximately 10 ml. of melted, cooled standard agar*** are introduced into the concave cup, and the cap or closure to be tested is applied, using aseptic precautions, with sterile forceps. A sterile wooden plug of the type sometimes used for hand capping of milk bottles is useful in seating the cap firmly in position. The blank is inverted, shaken gently, and the agar allowed to harden. The blank should be incubated in an upright position, at 32° or 37° C. for 48 hours. At the end of this period, the cap or closure is removed and the colony count determined. To facilitate the removal of the agar layer from the cap or closure, the lip of the milk bottle blank may be gently heated by rotating over a Bunsen flame.

In making counts it is helpful to transfer the agar disc to a sterile Petri plate. When this is done, it is necessary to take into account the possibility of surface colonies adhering to the cap or closure.

As a supplementary procedure, a cap or closure may be rinsed with 5 ml. of sterile water placed in the concave cup. If this technic is followed, the entire amount of rinse water should be removed and distributed approximately equally between two Petri dishes. The standard agar is used for plating.

The milk bottle blank is also useful for the examination of caps and closures by broth sterility tests, for making coliform tests, and other tests with special media. Closures that require special methods for proper adjustment and sealing over the mouth of a milk bottle do not lend themselves readily to examination by this technic. However, unless these closures come in direct contact with milk or milk products, a method such as this would ordinarily not prove necessary.

b) Hoods and Closures used as Culture Dishes. Milk bottle hoods and closures may be placed, with aseptic precautions, in sterile Petri dishes and used as receptacles for agar media. For large skirted caps it may be necessary to use 150 mm. dishes. Closures may first be rinsed with 1 to 3 ml. of sterile water, plating the entire amount of water, or standard agar can be poured directly into the closure without rinsing. With the former method, standard agar may be introduced into the rinsed closure in order to determine the residual contamination.

** Unblown milk bottle blanks may be obtained from the manufacturers of glass milk bottles.

***Standard agar for milk work, used without the addition of milk.

TABLE 1

Bacteriological Analysis of Milk Bottle Caps and Paperboard Used in the Manufacture of these Products

Procedure: Disintegration methods. Results expressed in number of colonies per gram.
Medium: New Standard Agar.
Incubation: 37° C. for 48 hours.

	No. mills or plants	No. tests	Percentage of counts					Maximum counts
			Less than 10	Less than 100	Less than 250	Less than 500	More than 500	
Paperboard	8	397	31	81	87	95	5	20,400
Bottle Caps	5	1,174	45	97	99.6	99.6	0.4	1,080

With the latter method about 3 ml. of agar are used with each closure. These methods are applicable to metal hoods and closures as well as other types. Some investigators may wish to rinse the complete cap or closure. This can be done by transferring the closure, under aseptic conditions, to a sterile wide mouth Erlenmeyer flask and rinsing with 20 ml. of sterile water. After rinsing, 10 ml. of the water is removed and distributed approximately equally among three Petri dishes, followed by plating with standard agar.

In the testing of metal sheeting for the manufacture of hoods and closures, such as aluminum foil, two methods may be employed. Ten gram samples of foil cut into ½ inch strips in sterile Petri dishes are taken under aseptic conditions from rolls of metal at intervals of 20 to 30 feet. Before taking the samples, a margin of approximately ¼ inch is trimmed off the edges of rolls and discarded. Each 10 gram sample of aluminum foil is equivalent to approximately 3 feet. The samples are transferred to 50 ml. of sterile water in Erlenmeyer flasks of 250 ml. capacity and rinsed. Five or 10 ml. amounts of rinse water are removed and distributed among two or three Petri plates. Standard agar is used in plating. The rinses may also be used to inoculate sterile nutrient broth for sterility tests.

Metal foil may be tested by a contact culture method. Sections of foil are cut and transferred to large (150 mm.) Petri dishes. Using aseptic precautions, the sides of the sheet are raised, forming each section of foil into a rectangular dish, having an area at the base of approximately 36 sq. cm. The metal dish is made so as to fit properly in a large size Petri plate with the cover in place. Standard agar is introduced directly into the metal dish. Incubation is carried out at 32° or 37° C. for 48 hours.

EXPERIMENTAL RESULTS*

The counts obtained from analysis of bottle caps are slightly lower than those of the paperboard used in cap manufacture. This is probably largely due to the effect of paraffining the caps as they are made and possibly to a gradual decrease in count which paperboard of this grade sometimes shows on storage. These results indicate that conditions of cap manufacture at plants studied are not such as to contaminate these products during handling, fabrication, and packing. See Table 1.

Commercially pure aluminum foil used for milk bottle closures, tested according to methods described above, gives a surface count of less than 20 colonies on an area of approximately 36 sq. cm. In 116 tests of foil made at intervals of 25 feet from the start of a roll, 82 percent of the analyses yielded counts of less than 4 colonies; 31 percent of these tests showed no growth. The following table gives a summary of the bacteriological findings obtained from testing both metal and paperboard closures.

These results show that bacterial contamination of hood and closure surfaces is normally slight. Findings obtained by rinsing and contact culture methods are generally comparable.

Coliform and broth sterility tests were

* The analyses given were made by Raphael A. Gillotte.

run in connection with these experiments. Results of over 100 analyses reveal a total absence of coliform organisms. Broth sterility tests, representing 150 analyses, generally confirm the results reported in Table 2.

These studies show that, according to the methods employed, the milk bottle caps, hoods, and closures examined are in good condition bacteriologically. Further improvements can be made in the general situation. There still remains some possibility of contamination through personal handling. Cap, hood, and closure manufacturers are, however, generally appreciative of the need for proper sanitary precautions in the fabrication, handling, and packing of these products. The most important of these plants have adopted uniform sanitary codes designed to protect adequately their products from contamination and to provide clean and sanitary conditions of manufacture.

BACTERIOLOGICAL COUNT STANDARDS

It has been suggested that a reasonable bacteriological standard for paper and paperboard used in the manufacture of caps, hoods, and closures shall not exceed 500 colonies per gram of disintegrated stock. There is justification for such a standard, based upon the studies that have been made on paperboard for milk containers.

The problem of setting a satisfactory standard for fabricated closures is not so simple a matter because of the many different sizes and the low surface counts secured. One way of expressing such a count is in terms of colonies per unit surface area. It might be stated, for example, that closure surfaces which come in contact with these foods shall not exceed 2 colonies per square centimeter. This figure is comparable to a standard of not more than 1 colony per ml. capacity as determined by rinse counts of glass and paper containers for milk.

Another way of expressing a count standard would be to state arbitrarily, based upon results such as those reported above, that counts shall be less than 10 or less than 25 colonies per cap or closure. Either count appears to be reasonable. There is some feeling, however, that it might be better at first to fix a more lenient standard until the problem has been given more study.

BIBLIOGRAPHY

1. Jackley, J. G., The Danger of Improperly Capped Milk Bottles. *J. Dairy Science,* **5**, 406-411, (1922).
2. Isaacs, M. L. and Zeiber, I. A Comparative Study of Milk in Bottles with Single and Double Caps. *Amer. J. Hygiene,* **16**, 806-822 (1932).
3. McFarlane, V. H. and Weinzirl, J. The Protective Value of the Single and the Double Cap for Bottled Milk. *Amer. J. Hygiene,* **19**, 521-531 (1934).
4. Arnold, Lloyd. The Pouring Lip and Cap as Sanitary Factors in Bottled Milk. *J. Milk Technol.,* 2, 41-43 (1939).

TABLE 2

Bacteriological Analysis of Hoods and Closures by Surface Rinsing and Contact Culture Methods

Medium: New Standard Agar
Incubation: 37° C. for 48 hours

Method	No. closures tested	Percentages of closures showing no growth	Percentages of closures having count of			Maximum counts
			2 colonies or less	4 colonies or less	More than 4 colonies	
Rinse	260	25	81	94	6	502**
Contact	366	42	96	98	2	9

** A count of this magnitude was obtained in only a single case. Results such as these are unusual and unexplainable with present information. The maximum count secured in all of the other tests was 34 colonies per closure surface which is probably a fairer indication of the highest counts obtained by these methods.

5. Rice, John W. Bottle Caps as a Source of Contamination of Milk. *Sixth Annual Report, Pennsylvania Association of Dairy and Milk Inspectors,* 1930, 96-107.
Controlling the Bacterial Content of the Fibre Bottle Cap. *Fifteenth Annual Report, Pennsylvania Association of Dairy Sanitarians,* 1939, 98-105.
6. Rice, J. L., Van Saun, A. I. and Haywood, K. E. Bacterial Counts on Milk Bottle Caps with a Comparison of the Effect of Different Methods of Capping on Such Bacterial Counts. Abstract in *Abst. Bact.,* **8,** 15, (1924).
7. Pizzoferrato, J. A. Origin, Development and Extent of Use of Caps Covering the Pouring Lip of Milk Bottles. *The Milk Sanitarian,* **6,** 20-21 (1937).

The Grading of Food Establishments. Kentucky Bulletin of the Dept. of Health, Vol. 12, No. 6, January 1940, pp. 126-128. *Pub Health Engin. Abs.* xx, Mi, 43.

There are 455 food establishments in Jefferson County, Kentucky, and the County Health Department began regrading these on January 1, 1939, using the U.S.P.H.S. tentative code. A preliminary survey was made and an intensive educational program was carried out by the inspectors and public health nurses. The grading system was explained to Parent-Teacher Associations and other civic groups. The results of this program have been very gratifying. In December 1938, 22% of the food establishments held Grade A certificates; in November 1939, 38.8% were Grade A establishments. The number of Grade B establishments decreased from 49% in December 1938 to 6% in November 1939. The most prevailing defects found in 1241 individual inspections were in walls and ceilings, toilet facilities, water supplies, lavatory facilities, cleaning and disinfection of equipment and in the disposal of garbage.

"The experience of Jefferson County Health Department may be matched by any county that makes and carries out an educational plan before and during the inspection work."

Edwin F. Franz.

Perfuming the Toilet Room. New Hampshire Health News, Vol. 18, No. 5, May 1940, pp. 14-15. *Pub. Health Engin. Abs.* xx, PHA, 11.

An interesting little article on the use of various "drip" methods for the disinfection of urinals and toilet bowls. The writer, in response to an opinion from the manufacturer, gives the department's viewpoint on the use of such commercial products and comes to the conclusion that they merely substitute one odor for another. A very handy letter to have around in order to answer such inquiries from other manufacturers.

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