

An Improved Stain for the Direct Microscopic Examination of Milk

MANLEY MANDEL¹

*Department of Bacteriology and Public Health, Michigan State College,
East Lansing, Michigan.*

In the course of an investigation of the failure of the Breed direct microscopic examination to disclose the presence of "physiologically old" coliform organisms seeded in milk, an improved milk stain containing the cationic surface-active agent, cetyl pyridinium chloride, was developed. The results of this investigation of the phenomenon first reported by Whitehead (1) will be published at a later date. It is the purpose of this note to present the advantages and the preparation of the aforementioned stain.

When replicate slides of milk samples seeded with 24 hour cultures of coliform organisms were stained with Breed's stain, Mallman's acid stain, and the new stain, the slides stained with the latter stain yielded significantly higher counts, in closer correlation with the standard plate counts, than the direct counts where the former stains were employed. In addition to yielding higher counts on seeded milk samples, the procedure was especially advantageous in the examination of raw milk samples where rod form organisms constituted an appreciable proportion of the population. Further advantages of the staining procedure were the decolorization of the background, providing heightened contrast between bacteria and background, and preparations uniformly free of surface debris even when month-old preparations of the staining solution were used.

Preparation of milk films. Milk

films may be prepared either by the methods appearing in *Standard Methods for the Examination of Dairy Products*, 8th edition, or by the method of Bryan, Mallmann, and Turney (2). Slides are air dried and fixed in heat.

Staining procedures. The milk film is defatted by one minute immersion in xylene. The excess xylene is drained from the slide and the slide is rinsed one minute in 95 percent ethanol, drained and immersed one minute in the staining solution. The reverse side of the slide is briefly immersed in a gentle stream of water to remove the excess stain, drained, and dried.

Preparation of the staining solution. To 10 ml. of saturated alcoholic methylene blue chloride (certified for bacteriological use), add 0.034 gm. cetyl pyridinium chloride and make up to 100 ml. with 30 percent ethanol. The cetyl pyridinium chloride may be conveniently added as 3.4 ml. of a 10 percent aqueous solution.

Microscopic appearance of the stained milk films. The bacteria appear dark blue or black and the leucocytes and epithelial cells appear blue against a colorless or very pale blue background. The crest of the smear retains the stain more heavily than the remainder of the smear, permitting ease in focusing the microscope. No destaining of the milk films has been found necessary in the author's experience.

The staining procedure described above has been used by the author with good results. Extensive comparisons of the procedure with stains other than

¹ Present address: Division of Bacteriology, University of California, Davis, California.

the Breed stain have not been completed at this writing. Presentation of the technique at this time, however, is made so that others may avail themselves of the method.

REFERENCES

1. Whitehead, H. R. A Note on the Direct Microscopic Count of Bacteria in Milk. *J. Dairy Res.*, 2, 80 (1931).
2. Bryan, C. S., Mallmann, W. L. and Turney, G. J. Some Microscopic Technics for Determining the Bacteriological Quality of Milk. *Mich. Agr. Exper. Sta., Circ. Bull.* 186 (1944).

THE ORANGE COUNTY MILK SANITARIANS ASSOCIATION

The Orange County (N. Y.) Milk Sanitarians Association, entering its fourth year, is a large and active infant in the family of milk sanitarians organizations. Started in 1944, at the time of writing it has a membership of 124. Its present officers are George H. Decker, Liberty, N. Y., president; Andrew J. Tompkins, Middletown, N. Y., vice-president; Howard B. Marlatt, Middletown, N. Y., secretary; and Dr. Harry C. Temple, Pine Bush, N. Y., treasurer. Executive Committee members, other than the officers, are Paul L. Brooks, Middletown, Erik Lundstedt, Goshen, and Samuel Abraham, Middletown.

The scope of the organization is somewhat broader than its name would indicate. The membership, according to its constitution, is made up "of persons actively engaged in sanitary milk control or in some phase of the dairy industry." While a majority of its members are Orange County residents, it has members in New Jersey, Pennsylvania, and New York City.

Its object, which is being effectively

accomplished, is "to assist its members to more efficiently discharge their responsibilities, to bring them together for discussion of recent information revealed in the fields of practice, to develop more uniform and efficient supervision of milk and its products, and to promote good fellowship."

Meetings are held four times a year. At a meeting in February, 1947, at which your reporter had the privilege of being a speaker, Dr. H. W. Sherman and Dr. William A. Hagen, of Cornell University, were among the other speakers. The fact that the meeting was held in a church hall and during a blizzard had no apparent effect on either attendance or enthusiasm.

The last meeting was held in Middletown on December 12, 1947. The subject discussed was "Specification and Operation of Can Washers," the speakers being Clarence W. Weber, New York State Department of Health, Albany, and R. L. Batchelor, The Lathrop-Paulson Company, New York City.

P. B. B.