Coliform Detection in Water By a Single-Step Technique Using the Membrane Filter

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THE SUCCESSFUL ADAPTATION of buffered desoxycholate lactose broth for use in the examination of water by the millipore filter (MF) technique and the reduction of the analytical process to a single step procedure are reported here.

In 1951, Hajna proposed a buffered desoxycholate lactose broth for use either as a presumptive or as a confirmatory medium in the examination of water (*1*). The inclusion of sodium desoxycholate in his medium was presumed to inhibit growth of spore formers and other gram-positive bacteria without affecting the growth of the coliform group organisms which were detected in the usual manner by the collection of gas in the fermentation tubes.

More recently, the MF and special indicator broth media for detection of coliform bacilli in water have been advocated by Goetz and Tsuneishi; Clark, Geldreich, Jeter, and Kabler; and Clark and Kabler (*2–4.*) In this technique, the presence of coliform organisms is detected by the production of typical sheen in the colonies growing on the filter rather than by gas formation from lactose. In the technique as proposed by the Environmental Health Center (EHC) workers, two steps are necessary: (*a*) enrichment of the membrane for 2 hours, and (*b*) transfer of the membrane from the enrichment medium to a basal medium for the development of colonies and the production of sheen. Thus the method advocated by the EHC workers requires the use of two different media and the transfer of the membranes.

Critical comparisons of quantitative results by the MF technique using desoxycholate medium and the standard methods lactose broth fermentation procedure are under study at the present time.

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The Indiana laboratory is one of the laboratories participating in the field study of the membrane filter under the auspices of the Standard Methods Committee for the Examination of Water and Sewage, American Public Health Association, and cosponsored by the American Water Works Association and the Public Health Service.

Apparatus

The filtering apparatus and the method of vacuum filtration is similar to but differs somewhat from that of the EHC workers, although the principles involved are essentially the same. The apparatus consists of a cone-shaped funnel (*A*) attached to a special filtrator (*B*) instead of the hydrosol type as used by the EHC workers. Vacuum comes from a central source.

In the study, the funnel was at first sterilized at 15 pounds steam pressure for 15 minutes, the 2 parts of the funnel being separately wrapped in kraft paper before autoclaving, according to the method advocated by the EHC workers. Subsequently, it was found that sterilizing the funnel for 30 minutes in the Arnold sterilizer (100° C.) was satisfactory.

Medium and Indicator System

Leifson (*5*) stated that in a broth or agar medium containing sodium desoxycholate below pH 7.5 none of the gram-positive bacteria tested show any appreciable growth in 24 hours, but if the pH of the desoxycholate medium is raised above 7.5, various types of gram-positive bacteria begin to grow. For example, at pH 7.6

the enterococci will generally show very tiny colonies on desoxycholate agar after 24 hours incubation. In conformity with Leifson's findings, the authors have found the formula best suited for the enumeration of coliforms in water is that given below, with a pH of 7.5.

Some peptones have been found to be unsuitable for use in this medium. Sheen was either not produced at all or, at best, poorly produced. The most satisfactory peptones were found to be:

BBL trypticase in combination with BBL thiotone; or simply the BBL polypeptone (a mixture of trypticase and thiotone).

Bacto casitone in combination with Bacto thiopeptone.

Albimi C in combination with Albimi B (or simply the Albimi "M", a mixture of the aforementioned peptones).

Although Bacto neopeptone, Bacto tryptose, and Bacto proteose peptone No. 3 may be used in the basal medium formula, they are generally unsatisfactory unless an enrichment broth is first used.

Lactose is incorporated in the formula rather than prepared as a separate solution to eliminate superfluous steps. Furthermore, heating lactose at 100° C. is preferable to autoclaving the lactose solution separately at 121° C. for 15 minutes.

Ultimate determination of the amount of sodium desoxycholate to be used per liter was based on the demonstrated sterility of the finished medium after heating for 30 minutes at 100° C. This concentration does not affect the ability of the coliform types to produce colonies with sheen, and at the same time does not support growth of the gram-positive bacteria or spore-formers, normally present in the air, which might get into the sample during filtration in the open funnel.

The desoxycholate lactose broth formula found best suited for the enumeration of coliforms in water was arrived at only after numerous trials with various brands of peptones, variations in the concentration of each ingredient, trial of different methods of sterilization and tests of the effect on the medium of storage at room and icebox temperatures.

Desoxycholate Lactose Broth

To 1,000 ml. of distilled water, add:	
Peptone (tryptic digest of casein)	10 gm.
Peptone (peptic digest of beef)	10 gm.
Yeast autolysate, or extract	3 gm.
NaCl	5 gm.
Lactose	20 gm.
K_2HPO_4	6 gm.
Sodium desoxycholate	0.2 gm.
Final pH will be 7.5	

Agitate the medium thoroughly to dissolve the ingredients. Heat to the boiling point to permit formation of precipitates; filter through coarse filter paper, and dispense with a pipetting machine in 30 ml. amounts in 25 x 200 mm. sterile cotton plugged tubes. Sterilize the tubes of measured medium in flowing steam (100° C.) for 30 minutes and then refrigerate until ready for use. Before using, the tubed medium is warmed in a beaker of hot water for a few minutes to dissolve the desoxycholate "gel."

Indicator System

The choice of the indicator system was based on the demonstrated ability of all coliform types (*Escherichia coli*, *Escherichia freundii*, *Aerobacter aerogenes*, and *Aerobacter cloacae*) to form colonies with typical sheen. The indicator solution consists of the following ingredients thoroughly mixed together in a 16 x 150 mm. tube:

Sodium sulphite (0.9 gram in 10 ml. water)	1 ml.
Basic fuchsin (3 gm. in 50 ml. water plus 50 ml. 95 percent ethyl alcohol)	1.2 ml.

To each tube of 30 ml. desoxycholate lactose broth, add 1.54 ml. of the indicator solution and agitate until mixed well. The tube of final medium should stand at room temperature for 30 minutes before the absorbent pads (50 mm. in diameter) are saturated with 2.2 ml. of the broth. After saturating the pad, the filter membrane is placed on the pad within a covered petri dish and incubated at 37° C. for 18–24 hours in an inverted position in an atmosphere saturated with moisture.

Results

Approximately 150 samples of water were examined for their coliform content using 5

portions of 10 ml. each inoculated into standard lactose broth, followed by confirmation in brilliant green lactose bile broth, in parallel with the filtration of a 50 ml. portion through the MF and observation of sheen producing colonies when desoxycholate lactose broth was used as the basal nutritive medium. The results of these comparative tests are given in the table.

Medium	*Number*
Lactose broth—Millipore filter—both showing coliforms	50
Lactose broth—Millipore filter—both negative for coliforms	72
Lactose broth—positive (by B. G. lactose bile, 2 percent)	8
Millipore filter—negative. Lactose broth—negative. Millipore filter—positive (coliforms)	17
Total water samples examined	147

It is evident that while there were 8 samples positive by the standard lactose broth fermentation procedure in which the MF tests were negative, there were twice as many in which the MF tests were positive and the lactose broth fermentation tests negative. Furthermore, 15 samples, in which positive lactose broth tubes were observed, failed to confirm in brilliant green lactose bile, and in none of these samples were sheen producing colonies observed on the filters.

Conclusions

1. A single step procedure for coliform detection in water analysis using the millipore filter has been described.

2. The formula for a desoxycholate lactose broth to be used in the MF technique is given.

3. An advantage of the desoxycholate lactose broth medium in the MF procedure for water analysis lies in the fact that enrichment of the filter is eliminated.

4. With this technique results are obtained earlier than by standard methods of water analysis.

5. Many of the false positive lactose broth tests encountered in standard methods of water analysis are eliminated.

REFERENCES

(*1*) Hajna, A. A.: A buffered desoxycholate lactose broth suitable either as a presumptive or a confirmative test for coliform bacteria. Pub. Health Lab. 9: 77–80 (1951).

(*2*) Goetz, A., and Tsuneishi, N.: Application of molecular filter membranes to the bacteriological analysis of water. J. Am. Water Works Assoc. 43: 943–984 (1951).

(*3*) Clark, H. F., Geldreich, E. E., Jeter, H. L., and Kabler, P. W.: The membrane filter in sanitary bacteriology. Pub. Health Rep. 66: 951–977 (1951).

(*4*) Clark, H. F., and Kabler, P. W.: The membrane filter in water quality tests. Am. J. Pub. Health 42: 385–388 (1952).

(*5*) Leifson, E.: New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Path. and Bact. 40: 581–599 (1935).

EQUIPMENT REFERENCES

(*A*) Funnel, S–S Coli 5, Carl Schleicher and Schuell Co., Keene, N. H.

(*B*) Filtrator, No. 9–788, Fisher Scientific Co., Chicago, Ill.