The Suitability of M-Endo-LES Agar for Total Coliform Counts by Membrane Filtration

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Abstract

The media M-Endo-LES agar (M-LES) and M-MacConkey agar (M-Mac) were compared for carrying out coliform counts by membrane filtration on potable water samples. M-LES was shown to yield higher counts than M-Mac and was adopted for use in the routine testing programme. However, difficulties were experienced with the appearance of background colonies on the M-LES plates, some of which were identified as coliforms which did not develop the typical colonial sheen which characterises coliforms on this medium. The correlation of sheen development on M-LES with lactose fermentation and organism identification was investigated as was the effect of light and storage on the M-LES.

Introduction

Johannesburg, the largest metropolis in the Republic of South Africa, does not purify its own water supply but, in common with other local authorities on the Witwatersrand, purchases treated water from a regional supplier. The quantity used is approximately 200 000 Ml per year.

The water is delivered into the Johannesburg system after breakpoint chlorination at the treatment works, followed by chloramination at an intermediate pump station, with a combined chlorine residual ranging from 0,2 to 0,8 mg/l after travelling approximately 50 km during delivery. Subsequently, approximately 0,5 mg/l of gaseous chlorine is added at the entrance to each storage reservoir. Combined chlorine residuals of 0,1 to 0,6 mg/l pertain throughout most of the system, except at some of the reticulation points furthest from the reservoirs.

In this country there are no statutory standards or methods prescribed for the analysis of potable water, therefore, in the City Health Department Laboratories, Johannesburg, the SABS Specification No 241-1971, is used as the basis for routine total coliform tests on samples from the reservoir and reticulation system of the city. Membrane filtration using Membrane MacConkey Agar (M-Mac) as the culture medium is the specified method.

Routine analyses over the last twenty years have regularly recorded an increase in the Standard Agar Plate counts with sporadic appearance of coliform organisms in the reticulated water of Johannesburg during the hotter months. In November 1979, yellow colonies similar to E.coli were seen on the M-Mac and faecal coliform plates. Most of these organisms were identified as Aeromonas hydrophila. The other belonged to the genera Klebsiella, Enterobacter, Citrobacter, Serratia, Pseudomonas and Acinetobacter, and their occasional presence persisted right through the warm season.

Grabow and du Preez (1979) compared M-Mac with M-Endo-LES (M-LES) agar as prescribed in US Standard Methods for the Examination of Water and Wastewater (1976). They concluded that the latter medium yielded higher counts and was more specific for total coliforms than M-Mac and recommended that the specification for the medium for total coliform tests of the SABS be reviewed accordingly. It was then decided to carry out a similar comparison in the Johannesburg City Health Department Laboratory.

Throughout the summer of 1980, occasional sheen colonies were seen to develop on the M-LES coliform membranes but it was observed that there were many more background colonies than would have been expected to grow on an adequately selective medium. Some of the colonies were dark red, sometimes with doubtful sheen, but others were pink and transparent with both large and small forms. It was decided to investigate these colonies.

In addition, it was noticed that M-LES develops sheen on the surface of the medium and it becomes a much deeper shade of pinkish-red when the plates are exposed to daylight for periods of 3 h or more. Also, varying instructions as to the lengths of time the medium remained usable after preparation, were advocated by different authorities as follows:

- Use of the medium on the day of preparation is recommended by Difco, the manufacturer.
- Use up to two weeks after preparation is allowed by US Standard Methods for the Examination of Water and Wastewater (1980).
- The US Environmental Protection Agency Laboratories discard M-LES plates after 48 h, although the EPA and APHA Manuals allow use up to 4 to 5 days (Power, 1977).
- Use after storage in a refrigerator up to 2 weeks is considered satisfactory by McCarty, Delaney and Grasso (1961), the originators of the medium.

A literature survey of publications dealing with coliform analysis and interpretation of the results was carried out at the same time and it was evident that many authors have recently focused attention on:

- Interference of background counts on coliform membranes with coliform enumeration (Geldreich, Allen and Taylor, 1978).
- The limitations of total coliform counts as indicators of faecal pollution (Dutka, 1973; Oger, Gavini, Delattre and Leclerc, 1981).
- Differing results obtained with various membranes and batches of media - particularly M-LES (Geldreich and Symons, 1980; Geldreich and Courchene, 1979).

Since it was felt that the above factors could be of significance in the current Johannesburg experience, it was decided to investigate the following aspects before decisions could be taken vis-à-vis revision of methodology: