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:

Discussion

Experiment 1

From Table 1 it may be seen that coliform counts were significantly higher on M-LES in river waters and sewage samples, when all samples, samples tested at low dilutions (1:2 to 1:10) and river samples only were considered. Potable water also yielded significantly higher counts.

When river waters and sewage samples tested at high dilutions and sewage effluents alone are considered there was no significant difference.

It is evident that overall M-LES gives significantly higher coliform counts than M-Mac and as such is a superior medium.

Table 2 shows that of all the colonies identified from the two media, M-Les selected for the typical coliforms *Escherichia*, *Enterobacter*, *Citrobacter* and *Klebsiella* and the non-coliform *Aeromonas hydrophila*, whilst M-Mac favoured *Pseudomonas* and recovered much fewer of typical coliforms.

Experiment 2

In Table 3 it is shown that *E.coli* and the typical coliforms all demonstrated varying characteristics with regard to sheen development, lactose fermentation in 24 to 48 h and lactose fermentation in 3 to 10 days with significant percentages in all groups except *Klebsiella oxytoca* and *Klebsiella pneumoniae* being anaerogenic lactose fermenters or non-lactose fermenters. Although 96,8% of the *E.coli* were sheen producers 72,6% were anaerogenic in lactose broth (Bergey, 1974) but were identified as *E.coli* by the API 20E system. *Klebsiella oxytoca* was the only species showing a typical coliform reaction including lactose fermentation. Of all the other organisms identified which included the genera *Serratia*, *Pasteurella*, *Pseudomonas*, *Aeromonas* and *Acinetobacter*, only 20% of the *Pasteurella* produced sheen and 0,5% of *Aeromonas hydrophila* fermented lactose.

From the colony descriptions it can be seen that the majority of typical coliforms appeared as small or large, dark red colonies, sometimes wet, with or without sheen, whereas the remaining isolates except for *Aeromonas hydrophila* which had dark colonies, gave pale colonies, large and small.

If the number of samples showing sheen colonies (135) is assessed for the period December 1980 to December 1981, 4,8% of the samples would have failed the coliform standard for that year. However, if the number of samples showing sheen and/or dark colonies (186) which were identified as coliforms are considered, 6,7% would have failed. This would have brought the Johannesburg water supply to non-compliance with the requirement of the SABS Specification that "not more than 5% of samples per year should contain coliforms".

From the point of view of selectivity, it may be seen that of the 186 samples from which sheen and dark colonies were isolated, only 135 contained sheen colonies so that 27,4% of the coliforms were missed by the criterion of sheen production, therefore, the dark colonies should not be ignored in coliform counting.

When lactose fermentation is considered, of the 1 183 isolates subjected to confirmatory tests, 57,5% were anaerogenic and 15,8% fermented lactose in 3 to 10 days. Therefore, only 26,7% complied with the confirmatory criterion of lactose fermentation in 24 to 48 h. It is evident that lactose fermentation which is intimately connected with sheen production was impaired in many of the isolates. This may have been due to the effects of repeated chlorination or to inadequacy of the lactose broth as a confirmatory medium, (Evans, Seidler and Le Chevalier, 1981) or to FUCHSIN-sulphite deficiency in the M-LES.

Experiments 3 and 4

From Table 4 it may be seen that there was no significant difference in the results of tests on plates which were poured in the light and in the dark.

When the effect of the age of the media is considered, the plates which were 5 days old did not give significantly better results than those which were 48 h old but they did give significantly better results than the freshly poured plates (4 h old). This would indicate that the sheen which develops on the surface of the plate and deepening of the red colour does not interfere with sheen development of the coliform colonies. There was no significant difference in the 48 h and 4 h plates.

It is interesting to note that plates dried in an incubator at 31 °C ± 1 °C gave significantly higher coliform reading than did those which were dried at room temperature.

Conclusions

From the results of Experiments 1(a) and 1(b) it may be concluded that notwithstanding the difficulty experienced with coliform differentiation on M-LES, it is still a more suitable medium than M-Mac and as such, should replace the latter in the Standard Specification (1971).

It is evident that the dark red colonies appearing on the M-LES plates should not be ignored but should be subjected to confirmation of lactose fermentation on a medium such as M-LAC broth (Seidler, Evans, Kaufman, Warwick and Le Chevalier, 1981),

Also, *Aeromonas hydrophila* appears as a dark red colony on M-LES, similar in appearance to the coliforms which have not developed sheen. In this way it interferes with coliform identification. It is suggested that an oxidase test be carried out at the same time as the suspect colony is transferred to the lactose medium for the fermentation test to eliminate *Aeromonas* and other oxidase positive organisms.

M-LES plates for best performance should be poured under daylight conditions and dried in an incubator at 31 °C ± 1 °C. The plates should not be used immediately on preparation but between 48 h and 5 days after pouring.

Since regrowth of some coliforms, particularly *Klebsiella spp* takes place in the reticulation systems and preliminary experiments have shown this to be the case in Johannesburg, the suitability of the total coliform test as an indicator of faecal pollution is queried.

The total coliform test has value as an indicator of the adequacy of water treatment processes but the detection of *E.coli 1* is the only real indication of faecal contamination.

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GELDREICH, E.E., ALLEN, M.J. and TAYLOR, R.H. (1978) In-

TABLE 3:
ORGANISMS ISOLATED FROM THE POTABLE WATER SUPPLY ON M-ENDO-LES AGAR

Organism	Number of Isolates	Sheen Producers	%	Non-Sheen Producers	%	Lactose Fermenters 24-48 h	%	Lactose Fermenters 3-10 d	%	Non-Lactose Fermenters	%	Colonial Appearance on M-LES
E. coli	95	92	96,8	3	3,2	25	26,3	1	1,1	69	72,6	large wet sheen
Citrobacter freundii	96	30	31,3	66	68,8	33	34,3	24	25,0	39	40,6	small, dark with or without sheen
Enterobacter agglomerans	397	39	9,8	358	90,2	50	12,6	60	15,1	287	72,3	small, dark sometimes wet
Enterobacter aerogenes	12	6	50,0	6	50,0	nil	—	nil	—	12	100	– do –
Enterobacter cloacae	226	53	23,5	173	76,5	58	25,7	57	25,2	111	49,1	small, dark
Enterobacter spp	201	12	5,9	189	94,0	45	22,4	42	20,9	114	56,7	dark to pale, various sizes
Klebsiella pneumoniae	25	19	76,0	6	24,0	22	88,0	3	12,0	nil	—	large, wet
Klebsiella oxytoca	83	78	93,9	5	6,0	83	100	nil	—	nil	—	mostly small sheen
Klebsiella spp	48	nil	—	48	100	1	2,1	nil	—	47	97,9	dark and light
Sub-total	1 183	329	27,8	854	72,2	317	26,7	187	15,8	679	57,5	
Serratia liquefaciens	11	nil	—	11	100	nil	—	nil	—	11	100	pale
Serratia marcescens	66	nil	—	66	100	nil	—	nil	—	66	100	pale
Pasteurella spp	29	20	68,9	9	31,0	nil	—	nil	—	29	100	small, pale
Pseudomonas aeruginosa	3	nil	—	3	100	nil	—	nil	—	3	100	small, pale
Pseudomonas fluorescens	12	nil	—	12	100	nil	—	nil	—	12	100	small, pale
Pseudomonas maltophilia	7	nil	—	7	100	nil	—	nil	—	7	100	small, light
Pseudomonas spp	76	nil	—	76	100	nil	—	nil	—	76	100	small, pale
Aeromonas hydrophila	8 349	nil	—	8 349	100	nil	—	38	0,5	8 311	99,5	dark & light, sometimes wet
Acinetobacter spp	307	nil	—	307	100	nil	—	nil	—	307	100	small, pale
Sub-total	8 860	20	0,2	8 840	99,7	nil	nil	38	0,4	8 822	99,6	
Total	10 043	349	3,5	9 694	96,5	317	3,2	225	2,2	9 501	94,6	

development of sheen and/or non-sheen colonies. Of these, 62 contained sheen colonies only; 51 dark colonies only and 74 a mixture of sheen and non-sheen colonies. Representative colonies, 1 to 3 of each type which grew, according to colour, texture and size, were transferred to red MacConkey agar plates.

At first, lactose broth tubes were inoculated at the same time as the red MacConkey plates to check lactose fermentation. However, some factor, possibly the repeated exposure to chlorination to which these bacteria had been subjected, appeared to have impaired their lactose fermentation systems and lactose was frequently not fermented at all or took longer than 48 h which is the maximum period prescribed for lactose fermentation by typical coliforms. Subsequently, lactose broth tubes were inoculated from single colonies which were picked from the red MacConkey plates for identification on API 20E strips.

Ten thousand and forty three isolates were categorised according to sheen development, length of time taken for lactose fermentation to become evident and identification by the API 20E system. The results are shown in Table 3.

Experiments 3 and 4

The Effect of Light and Storage on Coliform Colony Appearance using M-LES

M-LES was prepared and exposed to the following conditions before use:-

Plates were poured in the laboratory under daylight conditions at intervals of 5 days, 48 h and 4 h, prior to testing. At the same time, plates were similarly poured in a darkened room. The plates were stored in the refrigerator until the day of use.

On the day of the experiment the plates were divided and dried for one hour to prevent confluent growth on the membranes due to condensed moisture on the surface of the agar: in the laboratory, as above; in a dark cupboard; and in an incubator at 31 °C ± 1 °C.

The plates which were poured in the darkened room were kept in a closed box in the refrigerator until used.

Samples

A river water was diluted 1:10 for Sample 1 and 1:20 for Samples 2 to 4. Five x 100 mℓ aliquots of the diluted sample were filtered for each combination of conditions. The results are shown in Table 4; each result is the average of 5 counts.

TABLE 4:
THE EFFECT OF LIGHT, AGE AND TEMPERATURE ON SHEEN COLONIES DEVELOPING ON M-ENDO LES AGAR

Samples	Mean Coliform Count /100mℓ	t-Test*	t-Test Assessment
Effect of light			
Poured in the light	4,6	0,8	not significant
Poured in the dark	2,8		
Effect of age			
5 days	9,4	1,0	not significant
48 hours	23,5		
5 days	9,4	1,8	significant
4 hours	2,4		
Effect of temperature			
31 °C	8,5	2,9	significant
20 °C	0,7		

*The t-Test with a 95% confidence limit at 1,7

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