A COMPARISON OF MEDIA FOR THE DETECTION OF COLIFORM ORGANISMS IN WATER

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INTRODUCTION

MacConkey broth has been used in this country as the standard medium for the primary isolation of coliform organisms from water since the early part of this century, and a comparison of this medium and two media containing glutamic acid has recently been carried out by the Public Health Laboratory Service Water Subcommittee (Report, 1958*a*). The results of these comparative trials led the committee, of which I was an active member, to conclude that neither the lactose nor the glucose glutamic acid medium described could be recommended unreservedly as satisfactory substitutes for MacConkey broth and that further work was indicated. It was therefore decided to undertake a new series of comparative trials, on a relatively small scale in this laboratory, with a different range of media in the hope of finding a more satisfactory substitute for MacConkey broth and this communication reports the results of the trial.

The media chosen for this work were: lactose broth, lauryl sulphate broth (both these media are commonly used in the U.S.A., but not commonly in this country), Teepol broth (Jameson & Emberley, 1956), glucose glutamic acid medium (Folpmers, 1948; Burman & Oliver, 1952) and MacConkey broth. Glucose glutamic acid was chosen for this trial rather than the lactose preparation of Burman & Oliver because the P.H.L.S. Water Sub-Committee's trial has shown that the former medium gave a higher yield of *Escherichia coli* (*Bacterium coli* 1) and of other coliform organisms than the lactose glutamic acid medium.

MATERIALS AND METHODS

Culture media used and method of preparation

MacConkey broth, lactose broth, Teepol broth, lauryl sulphate broth and glucose glutamic acid media were made up double-strength and distributed in 10 ml. quantities in $6 \times \frac{3}{4}$ in. test-tubes each containing a Durham tube.

MacConkey broth was made according to the formula given in the Ministry of Health and Ministry of Housing and Local Government's report on *The Bacteriological Examination of Water Supplies* (Report, 1956).

Lactose broth was made according to the formula given in *Standard Methods for the Examination of Water, Sewage and Industrial Wastes* (American Public Health Association, 1955), save that Lab Lemco was used in place of beef extract and Evans's peptone in place of Bacto-peptone.

Teepol broth was made according to the formula given by Jameson & Emberley (1956), save that Evans's peptone was used in place of Eupeptone No. 2 and the final concentration of brom-cresol-purple—after the addition of the water under examination—was 1 in 100,000.

https://doi.org/10.1017/S0022172400020015 Published online by Cambridge University Press

Lauryl sulphate broth was made according to the method given in *Standard Methods for the Examination of Water, Sewage and Industrial Wastes* (American Public Health Association, 1955) save that Evans's peptone was used in place of tryptose and sodium lauryl sulphate (Hopkin & Williams) in place of sodium lauryl sulfate, Duponol (W. A. Flakes).

Glucose glutamic acid medium was prepared by the method described by the Public Health Laboratory Service Water Subcommittee (Report, 1958*a*).

Brilliant green lactose bile broth was prepared from Oxoid dehydrated brilliant green bile (2%) broth (Code No. CM 31) following the instructions given by the manufacturers. The medium was distributed in 5 ml. volumes in $6 \times \frac{1}{2}$ in. test-tubes each containing a Durham tube.

Methods employed for examination of water samples

The standard method of examining water for coliform organisms in this laboratory is to inoculate 50 ml. of double-strength MacConkey broth in a 6 oz. medical flat bottle with 50 ml. of water, five $6 \times \frac{3}{4}$ in. test-tubes each containing 10 ml. of double-strength MacConkey broth with 10 ml. of water, and five $6 \times \frac{1}{2}$ in. test-tubes each containing 5 ml. of single-strength MacConkey broth with 1 ml. of water. For waters expected to contain larger numbers of coliform organisms, smaller volumes of water are inoculated into single-strength MacConkey broth in addition to, or in place of, some or all of the volumes mentioned.

In the present investigation four of the tubes containing 10 ml. double-strength MacConkey broth were replaced by four $6 \times \frac{3}{4}$ in. test-tubes, each containing 10 ml. of a different one of the four other media under test at the appropriate concentration. 10 ml. of each water used in the comparison were then inoculated into 10 ml. of MacConkey broth, 10 ml. of lactose broth, 10 ml. of Teepol broth, 10 ml. of lauryl sulphate broth and 10 ml. of glucose glutamic acid medium, each at the appropriate strength.

After inoculation the tubes were incubated at 37° C. and inspected after 18, 24 and 42 hr. Each tube showing the production of gas or acid and gas (presumptive positive reaction) was subcultured into two tubes of brilliant green lactose bile broth (Report, 1958*b*). One of the tubes of brilliant green lactose bile broth was incubated at 37° C. for up to 48 hr.; the other at 44° C. for up to 24 hr.

The production of gas within 48 hr. in a tube of brilliant green lactose bile broth incubated at 37° C. was regarded as confirming the presence of coliform organisms in the corresponding primary tube (American Public Health Association, 1955). The production of gas within 24 hr. in a tube of the same medium incubated at 44° C. was regarded as demonstrating the presence of *Esch. coli* in the corresponding primary tube (Mackenzie, Taylor & Gilbert, 1948).

EXPERIMENTAL RESULTS

In all, 1691 samples of water were examined by this method over a period of 18 months. The distribution of these samples between unchlorinated, prechlorinated and chlorinated samples is shown in Table 1.

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Table 1. *Behaviour of* 1691 *specimens of water of all types in different media*

Type of water	No. of specimens which did not give presumptive positive reactions in any of the five media	No. of specimens which gave the same (positive or presumptive positive) reaction in all five media after the same period of incubation	No. of specimens which did not give the same results in all five media after the same period of incubation	Total
Unchlorinated	306 (37·0 %)	91 (11·0 %)	430 (52·0 %)	827 (100 %)
Prechlorinated	32 (30·2 %)	0 (0·0 %)	74 (69·8 %)	106 (100 %)
Chlorinated	611 (80·6 %)	3 (0·4 %)	144 (19·0 %)	758 (100 %)
Total	949 (56·1 %)	94 (5·6 %)	648 (38·3 %)	1691 (100 %)
	1043* (61·7 %)		742 (43·9 %)†	

* The results obtained with the 1043 specimens which gave the same results in all five media are set out in Table 2.

† The results obtained with the 742 specimens in which presumptive positive reactions occurred in one or more of the media are set out in Table 3.

Table 2. *Results obtained with the* 1043 *specimens in which the results were the same in all five media*

Type of water	No. of specimens examined	No. of specimens which gave coliform organisms from all media: After 18 hr.	Between 18 and 24 hr.	Between 24 and 42 hr.	No. of specimens which gave *Esch. coli* from all media: After 18 hr.	Between 18 and 24 hr.	Between 24 and 42 hr.	No. of specimens which gave false presumptive positive reactions in all media	No. of specimens in which no coliform organisms were isolated from any medium after 42 hr. incubation
Unchlorinated	397	80	2	9	79	—	—	—	306
Prechlorinated	32	—	—	—	—	—	—	—	32
Chlorinated	614	2	—	1	2	—	—	—	611
Total	1043	82	2	10	81	—	—	—	949

Table 1 also shows that 1043 of the 1691 samples gave the same result in all five media; the behaviour of these 1043 samples is analysed further in Table 2.

From Table 2 it can be seen that no specimen gave false presumptive positive reactions in all five media, and that none of the thirty-two prechlorinated samples and only three of the 614 chlorinated samples yielded *Esch. coli* (*Bact. coli* I) or other coliform organisms in all five media after the same period of incubation.

Table 3 sets out the results obtained in the various media in the 742 samples in which presumptive positive reactions were obtained in one or more of the media.

The samples which gave identical results in all five media provide no information on the relative merits of the media, so these 1043 specimens have been omitted in Table 4. The comparisons which follow are based on the results obtained with the remaining 648 samples.

https://doi.org/10.1017/S0022172400020015 Published online by Cambridge University Press

The behaviour of the 648 samples which did not give the same result in all five media (see third column of Table 1) was very much the same whether the water was unchlorinated, prechlorinated or chlorinated, and the results obtained with these samples have therefore been combined in Table 4 irrespective of the source of the water. Of these 648 samples 150 either yielded coliform organisms in all five media after differing periods of incubation, or yielded *Esch. coli* in some of the media and coliform organisms but not *Esch. coli* in the others. 182 samples gave rise to false presumptive positive reactions only, 138 in one medium only, 44 in two or more media. In the remaining 316 samples, some of the media yielded coliform organisms and the others did not.

Table 3. *Results obtained in the various media from the* 742 *specimens in which presumptive coliform reactions appeared in one or more media*

Medium	No. of presumptive positive reactions after 42 hr.	No. of isolations of coliform organisms—including *Esch. coli*—after 18 hr.	24 hr.	42 hr.	No. of isolations of *Esch. coli* after 18 hr.	24 hr.	42 hr.	No. of false presumptive positive reactions after 42 hr.
MacConkey broth	424	229 (40·9 %)	313 (55·9 %)	392 (70·0 %)	179 (59·5 %)	187 (62·1 %)	188 (62·5 %)	32
Lactose broth	573	205 (36·6 %)	284 (50·7 %)	382 (68·2 %)	164 (54·5 %)	185 (61·5 %)	189 (62·8 %)	191
Teepol broth	451	202 (36·1 %)	295 (52·7 %)	396 (70·7 %)	164 (54·5 %)	178 (59·1 %)	184 (61·1 %)	55
Lauryl sulphate broth	466	195 (34·8 %)	284 (50·7 %)	396 (70·7 %)	165 (54·8 %)	180 (59·8 %)	187 (62·1 %)	70
Glucose glutamic acid medium	433	119 (21·3 %)	255 (45·5 %)	383 (68·4 %)	108 (35·9 %)	173 (57·5 %)	203 (67·4 %)	50
Total number of samples from which result was obtained	742*			560 (100 %)			301 (100 %)	

* 182 specimens gave rise to only false presumptive positive reactions. From a number of other specimens, some media gave rise to false presumptive positive reactions whereas other media yielded coliform organisms.

From Table 4 it can be seen that the total yield of coliform organisms and of *Esch. coli* after 42 hr. incubation is very much the same for all five media. There are differences in the numbers of samples from which these organisms were isolated by the different media but these differences are not significant. There are, however, large differences in the numbers of false presumptive positive reactions given by the five media, lactose broth giving rise to more than two and a half times as many false presumptive positive reactions as the medium giving the next highest number (lauryl sulphate broth) and nearly six times as many as the medium giving the smallest number of false presumptive positive reactions (MacConkey broth).

From Table 4 it can also be seen that for each medium, except glucose glutamic acid medium, at least three-quarters of the samples which yielded *Esch. coli* gave a positive reaction within 18 hr. About half of the samples which yielded *Esch. coli* in glucose glutamic acid medium gave a positive reaction between 18 and 24 hr., but about one-quarter of the samples gave positive reactions within 18 hr. and the

remaining quarter between 24 and 42 hr. incubation. The total yields of coliform organisms are more evenly spread over the 42 hr. of incubation. It is not, however, possible to compare the results for coliform organisms other than *Esch. coli* in the different media, since some of the tubes from which *Esch. coli* were isolated may also have contained other coliform organisms. The great majority of the false presumptive positive reactions in each of the five media only appeared after more than 24 hr. incubation.

Table 4. *Results with* 648 *samples not giving the same results in all five media*

Medium	No. of presumptive positive reactions appearing: After 18 hr.	Between 18 and 24 hr.	Between 24 and 42 hr.	Total	No. of isolations of coliform organisms—including *Esch. coli*: After 18 hr.	Between 18 and 24 hr.	Between 24 and 42 hr.	Total
MacConkey broth	148	92	90	330	147	82	69	298
Lactose broth	137	114	228	479	123	77	88	288
Teepol broth	123	97	137	357	120	91	91	302
Lauryl sulphate broth	113	96	163	372	113	87	102	302
Glucose glutamic acid medium	37	138	164	339	37	134	118	289

Medium	No. of isolations of *Esch. coli*: After 18 hr.	Between 18 and 24 hr.	Between 24 and 42 hr.	Total	No. of false presumptive positive reactions appearing: After 18 hr.	Between 18 and 24 hr.	Between 24 and 42 hr.	Total
MacConkey broth	98	8	1	107	1	10	21	32
Lactose broth	83	21	4	108	14	37	140	191
Teepol broth	83	14	6	103	3	6	46	55
Lauryl sulphate broth	84	15	7	106	—	9	61	70
Glucose glutamic acid medium	27	65	30	122	—	4	46	50

Table 5 sets out the results given by the 341 specimens in which the results in one medium differed from the results in the other four media. In this table the successes of each medium in isolating coliform organisms when all the other media have failed, are contrasted with its failures to isolate coliform organisms when all the other media have succeeded. A good medium should give a large number of successes and a small number of failures, and the greater the contrast the better the medium. The number of successes in MacConkey broth is virtually the same as the number of failures in this medium. The number of failures in lactose broth also equals the number of successes in this medium. Teepol broth, lauryl sulphate broth and glucose glutamic acid medium each have more successes than failures. On this basis alone these three media would all be somewhat better than MacConkey broth or lactose broth.

Table 5 also shows the number of instances in which a false presumptive positive reaction in one medium was the only reaction obtained. This gives another indication of the relative merits of the media in terms of the amount of work required to obtain the final answers. MacConkey broth gave by far the smallest number of single false presumptive positive reactions followed by glucose glutamic acid medium, Teepol broth and lauryl sulphate broth with lactose broth a long way behind.

Table 5. *Results with* 341 *specimens in which the results in one medium differed from the results in the other four media*

Medium	No. of specimens in which one medium did not yield coliform organisms whereas the other four media did	No. of specimens in which one medium yielded coliform organisms whereas the other four media did not	No. of specimens in which one medium gave a false presumptive positive reaction when the other four media gave negative reactions
MacConkey broth	16	17	2
Lactose broth	31	31	96
Teepol broth	11	22	13
Lauryl sulphate broth	14	27	18
Glucose glutamic acid medium	14	20	9

DISCUSSION

This investigation has been carried out on a relatively small number of samples of water which were obtained from a comparatively small area of the country and which were examined in one laboratory. The results therefore can give no more than an indication of the relative merits of the media used and an experiment would have to be conducted on a much larger scale in a number of laboratories before any more general conclusions could be drawn.

In 1043 (61·7 %) of the 1691 samples examined the results obtained with all five media were the same. In the 648 (38·3 %) of the samples in which there was some difference between the results obtained in the five media, the total yield of *Esch. coli* and other coliform organisms was very much the same in all five media. There are, however, very considerable differences in the number of false presumptive positive reactions given by the five media (Tables 4, 5) and consequently in the amount of work that has to be done with specimens inoculated into the different media in order to obtain the same result. There are also differences in the rapidity with which the reactions appear in the various media (Table 4).

The World Health Organization booklet *International Standards for Drinking-Water* (Report, 1958*b*) states 'The first essential for the detection and estimation of coliform organisms in water is that every coliform organism should have the maximum possibility of developing in the medium into which it is inoculated. It is apparent, therefore, that if selective media are used for the detection of coliform organisms, care should be taken to ensure that they are not inhibitory, but encourage the growth of these organisms.' All of the media used in this investigation are in a sense selective, in that they are designed to encourage the growth of the

organisms for which a search is being made, and this applies particularly to the media containing lactose. The media containing bile salt, Teepol and lauryl sulphate are open to the criticism that they contain substances which may be inhibitory to some coliform organisms, though there is no evidence in this investigation that such media did suppress the growth of any coliform organisms. Bile salts may vary in their effect from one batch to another (Burman, 1955) and Teepol is a commercial detergent which may vary in its composition from one time to another. All of the media—except glucose glutamic acid medium—contain peptone, which again is not a defined chemical substance and is known to vary from one brand to another (Burman, 1955), and also to vary from batch to batch of the same brand. These three ingredients are thus open to criticism on this account. In addition lactose broth contains meat extract which is another substance whose exact composition is not standardized. There would therefore appear to be good grounds for using a chemically defined medium—such as glucose glutamic acid medium—for the isolation of coliform organisms from water and in this investigation this medium has given as good results as the other four media, except that the reactions have taken rather longer to appear than in the other media.

A glutamic acid medium containing lactose might be expected to be a better medium for detecting the presence of lactose-fermenting organisms than one containing glucose. In the comparison carried out by the Public Health Laboratory Service Water Sub-Committee (Report, 1958*a*) it was, however, shown that a significantly smaller number of coliform organisms were detected by the use of lactose glutamic acid medium than by the use of glucose glutamic acid medium. The lactase of *Esch. coli* and of *Aerobacter aerogenes* has been shown to be adaptive (Cohn, 1957) and it may be that this is true of the lactase of other coliform organisms also. A simple medium such as a glutamic acid medium may not allow adaptation to take place sufficiently rapidly to confer any advantage on lactose-fermenting cells.

In this investigation glucose glutamic acid medium did not give rise to an unduly large number of false presumptive positive reactions. Indeed, there were fewer than were found with lactose broth, Teepol broth or lauryl sulphate broth and only one and a half times as many as MacConkey broth. In the larger trial carried out by the Public Health Laboratory Service Water Sub-Committee (Report, 1958*a*), however, glucose glutamic acid medium gave rise to many more false presumptive positive reactions than MacConkey broth, but these were largely confined to two of the six laboratories taking part in the investigation. This may well have been due to the differing bacterial flora in the samples examined in the different laboratories. If most of the samples examined did not contain many organisms which were capable of producing gas from glucose but not from lactose, then the number of false presumptive reactions with glucose glutamic acid would be small.

Each of the five media used in this investigation thus has its theoretical disadvantages. In this investigation, however, the yield of coliform organisms and of *Esch. coli* from the five media has been very similar. Lactose broth, however, gave rise to a much higher proportion of false presumptive positive reactions than any

of the other four media tested, and all the reactions in glucose glutamic acid medium tended to appear rather more slowly than in the other media.

Glucose glutamic acid medium has the great advantage of being a chemically defined medium, and therefore not being so likely to vary from batch to batch or from one laboratory to another, and of also not containing any deliberately added substances which might be inhibitory to coliform organisms.

It may well be that other glutamic acid media—such as that being investigated by Gray (personal communication)—will, in wider use, be found to be more satisfactory than the present glucose glutamic acid medium. Although the results with glucose glutamic acid medium in the present investigation have been satisfactory, two of the laboratories which used this medium in an earlier investigation (Report, 1958*a*) found that it gave a large number of false presumptive positive reactions. It would appear, however, that a glutamic acid medium offers the hope of a chemically defined medium which would not be inhibitory to coliform organisms, and which would not give rise to an unduly large number of false presumptive positive reactions, and that such media are therefore worthy of further investigation.

SUMMARY

Five media—MacConkey broth, lactose broth, Teepol broth, lauryl sulphate broth and glucose glutamic acid medium—have been compared as primary media for the isolation of coliform organisms from 1691 samples of water received for examination during a period of 18 months.

Of these samples 1043 (61·7 %) gave an identical result in all five media. In the other 648 (38·3 %) samples there were differences either in the types of coliform organism isolated or in the rapidity with which the reactions appeared in the different media. The differences in the final yields of coliform organisms and *Escherichia coli* in the five media are not statistically significant.

There were differences in the number of false presumptive positive reactions given by the different media—lactose broth giving many more false presumptive positive reactions than any of the other media and nearly six times as many as MacConkey broth, which gave the smallest number.

All the reactions tended to appear rather more slowly in glucose glutamic acid medium than in the other media.

Glucose glutamic acid medium has the advantages of being a chemically defined medium and of not containing any substances which are likely to be inhibitory to coliform organisms. Other glutamic acid media may be found on further investigation to give better results than the present glucose glutamic acid medium, but those presented here suggest that media containing glutamic acid are worthy of further investigation.

I am indebted to Dr Ian Sutherland of the M.R.C. Statistical Research Unit for statistical advice and to Dr R. D. Gray of the Public Health Laboratory, Newport (Mon.) and to Dr A. H. Tomlinson of the Public Health Laboratory, Oxford, for much helpful criticism.

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(*MS. received for publication* 3. I. 59)