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A minerals-modified glutamate medium for the enumeration of coliform organisms in water

By the Public Health Laboratory Service Standing Committee on the Bacteriological Examination of Water Supplies*

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INTRODUCTION

In a previous paper (P.H.L.S., 1968) Gray's improved modification of Folpmers' medium (Gray, 1964) was recommended as a superior alternative to MacConkey broth for the detection of coliform organisms in water by the multiple-tube method. For the experiments described in that paper an Oxoid dehydrated version of Gray's medium was used. This differed somewhat in mineral composition from the original in order to prevent the phosphates of calcium, iron and magnesium forming a precipitate which was removed by filtration in Gray's original method. It was also found on analysis that the mineral salts remaining in solution, using Gray's method, varied widely on different occasions. To avoid this variation a medium was designed at the Metropolitan Water Board Laboratories (Windle Taylor, 1965–6) with a mineral composition more nearly resembling the average composition of the medium as prepared by Gray and which could be prepared in dehydrated form. The present paper describes the results obtained in ten laboratories in which this minerals-modified dehydrated medium was compared with the original Oxoid dehydrated medium and with liquid medium prepared by Gray.

The mineral composition of the glutamate media is given in Table 1. In the original Oxoid formula as used in the previous series of experiments, the iron and calcium were retained in solution by considerably lowering the phosphate content. This was undesirable since a low phosphate concentration could greatly reduce bacterial growth as well as buffering capacity. The maximum phosphate concentration found on analysis of the medium prepared by Gray's method was therefore incorporated in the new medium. Much more calcium than magnesium was lost by precipitation and in order to retain more than minimal calcium the lowest concentration of magnesium found on analysis (0·2 g./l. of $MgSO_4 . 7H_2O$) was used. The concentration of iron was considered to be more important than that of

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calcium, especially since many water samples contain considerable amounts of calcium. In preparing the dehydrated medium the maximum amount of iron that could be used without forming a precipitate was 0·02 g./l. of ferric citrate, a figure well within the upper range found on analysis. The highest concentration of *calcium chloride that could then be* added without precipitation occurring was 0·015 g./l. of $CaCl_2$.

Table 1. Mineral composition of glutamate media (double strength, in g./l.)

	Minerals originally added by Gray (1964)	Minerals remaining in solution after precipitation by Gray's method	Minerals incorporated in the minerals-modified medium	Minerals incorporated in original Oxoid dehydrated medium
K_2HPO_4	2	1·4–1·8	1·8	0·6
$MgSO_4.7H_2O$	0·4	0·2–0·4	0·2	0·2
Ferric citrate	0·2	0·001–0·05	0·02	0·2
$CaCl_2$	0·4	0·02–0·15	0·015	0·2

MATERIALS AND METHODS

Media

The complete double-strength minerals-modified dehydrated glutamate medium used in the current trials therefore had the following formula: L(+) glutamic acid sodium salt, 12·7 g.; lactose, 20 g.; L(−) cystine, 0·04 g.; L(−) aspartic acid, 0·048 g.; L(+) arginine monohydrochloride, 0·04 g.; thiamin (aneurin hydrochloride), 2 mg.; nicotinic acid, 2 mg.; pantothenic acid, 2 mg.; K_2HPO_4, 1·8 g.; $MgSO_4.7H_2O$, 0·2 g.; ferric ammonium citrate, 0·02 g.; $CaCl_2.2H_20$, 0·02 g.; sodium formate, 0·5 g.; bromcresol purple (1 % ethanolic solution), 2 ml; NH_4Cl, 5 g.; distilled water to 1000 ml.; pH after sterilization, 6·7. Ferric ammonium citrate was used instead of ferric citrate because of its greater ease of solution, the iron content of the two compounds being approximately the same. As in previous trials, the ammonium chloride was not included in the dehydrated product but was added when making up. The medium was sterilized either at 116° C. for 10 min. or in the steamer at 100° C. for 30 min. on three successive days. The pH falls on sterilization and the extent of the fall depends on the sterilization equipment used. If the same equipment and procedure are always used the pH change should be constant, so that the pH should be adjusted, if necessary, before sterilization to achieve a pH of 6·7 after sterilization.

The original Oxoid dehydrated glutamate medium was prepared in a similar manner with pH adjustment if necessary. Medium prepared by Gray's original method was distributed in bulk to each of the ten participating laboratories (nine Public Health Laboratories and the Metropolitan Water Board). The three glutamate media were also compared with MacConkey broth made from the same batch of Oxoid dehydrated medium as was used in the previous trials.

Water samples

A wide range of samples was used, similar to that used in the previous trials, including marginally chlorinated samples, using the method previously described (P.H.L.S., 1968).

Methods of recording results

All samples were set up using one 50 ml., five 10 ml., and five 1 ml. volumes with each of the four media. When necessary, samples were diluted in order to give some negative tubes in such a series. The statistical significance of differences in numbers of positive tubes is much more easily assessed using these quantities throughout. The tubes were incubated at 37° C. without prior warming. A simplified system of recording the amount of acid and gas after 18, 24 and 48 hr. was adopted; otherwise the procedure was as in the previous trial. At 18 and 24 hr., cultures were regarded as positive if they produced any amount of acid and gas, however small. At 48 hr. cultures were not regarded as positive unless there was sufficient gas to fill the concavity of the Durham tube.

RESULTS

Methods of comparison

Comparisons based on the numbers of positive tubes obtained in all laboratories are given in Table 2. This involves a bias in favour of laboratories which examined the greatest number of samples. Results from individual laboratories have therefore been discussed separately but detailed results have not been presented. As all positive results have been counted there is also a bias in favour of samples giving large numbers of positive tubes. Furthermore, although differences between the media are revealed there is no indication of the magnitude of these differences. To overcome these two factors, some results have also been compared in Table 3 on the basis of mean and median ratios. As it is important for the water bacteriologist to know the effect of a change of method on existing standards of bacterial quality and as these are normally applied to 48 hr. results, it is only the 48 hr. results that have been compared by mean and median ratios.

Comparison by numbers of tubes

From Table 2 it can readily be seen that the differences between the three glutamate media were much less than the differences between the glutamate media and MacConkey broth. Comparison of each pair of corresponding figures for coliform organisms and *Esch. coli* shows that at 18 hr. two of the glutamate media were superior to MacConkey broth for *Esch. coli* in unchlorinated water but all were inferior in the other comparisons. At 24 hr. all the glutamate media were superior to MacConkey broth in all comparisons, except for Gray's laboratory-prepared medium for coliform organisms in chlorinated samples. At 48 hr. all the glutamate media were superior to MacConkey broth in all comparisons.

Comparing the results given by glutamate media, one or other of the dehydrated media was superior to Gray's laboratory-prepared glutamate medium in all the

Table 2. *Comparison of glutamate media and MacConkey broth by numbers of positive tubes*

	Number of tubes yielding								
	False positive reactions			Coliform organisms			*Esch. coli*		
	18 hr.	24 hr.	48 hr.	18 hr.	24 hr.	48 hr.	18 hr.	24 hr.	48 hr.
Unchlorinated samples (no. of tubes examined 2079)									
Oxoid MacConkey broth	17	37	100	625	806	1060	467	528	582
Original dehydrated glutamate medium	1	11	87	459	810	1180	408	686	791
Modified dehydrated glutamate medium	2	20	97	557	858	1175	503	707	764
Gray glutamate medium	2	17	81	538	848	1180	488	670	761
Chlorinated samples (no. of tubes examined 715)									
Oxoid MacConkey broth	4	19	49	125	216	315	77	121	128
Original dehydrated glutamate medium	1	5	31	49	220	389	40	148	211
Modified dehydrated glutamate medium	0	1	37	59	223	395	39	144	203
Gray glutamate medium	0	3	44	44	202	361	22	127	165

comparisons, except one where results were equal. These results indicate that Gray's laboratory-prepared medium has no advantage over the dehydrated media.

Comparing the two dehydrated glutamate media, the modified medium was superior to the original dehydrated medium at 18 and 24 hr. in six out of eight comparisons, and was slightly inferior at 48 hr. in three out of four comparisons. The modified medium therefore gave earlier positive results and thus overcame the major disadvantage in the original dehydrated medium.

Variations between individual laboratories

The results so far quoted are from the sum of results for all ten laboratories. There was some variation between individual laboratories but detailed figures are not shown. The superior results with glutamate media in 18 hr. for *Esch. coli* in unchlorinated waters were obtained in four laboratories, and one laboratory obtained higher 18 hr. results for *Esch. coli* with all glutamate media than with MacConkey broth also in chlorinated waters. At only one laboratory was MacConkey broth at 24 hr. superior to all the glutamate media in all comparisons. In all laboratories one or more of the glutamate media were superior at 48 hr. to MacConkey broth in all comparisons.

The only laboratory in which Gray's laboratory-prepared medium differed markedly in performance from the general pattern was in Gray's own laboratory. He obtained better results with his medium than with the other glutamate media in all comparisons. He did not, however, examine any chlorinated samples.

Comparing the two dehydrated media, the 18 and 24 hr. results showed a remarkably similar pattern in all laboratories. One laboratory showed lower and one showed higher results at 18 hr. with the modified medium in all comparisons. All other laboratories had some results higher and some lower. The inter-laboratory variation was therefore not very great.

Comparison by ratios

In order to determine what difference in results may be expected from the use of the modified dehydrated glutamate medium, the mean and median ratios of the most probable number (MPN) results obtained with this medium to those obtained with MacConkey broth were determined (Table 3). These ratios confirm what has always been found with glutamate media, that the increased counts are due mainly to improved detection of *Esch. coli*.

Whether the difference is considered to be greater with chlorinated than with unchlorinated water depends on whether mean or median ratios are taken as the criterion. It must be emphasized, however, that these ratios indicate only the sort of results which may be expected. Individual ratios varied widely around these figures, and it must be borne in mind that when calculating MPN ratios, duplicate samples examined by identical methods could give a ratio of approximately 9:1 within the 95% confidence limits.

It is of interest to note, however, that the highest single ratio obtained in the whole series was 50:1 for an *Esch. coli* result on an unchlorinated sample, and the

highest count obtained with a corresponding negative result with MacConkey broth was 25/100 ml., again for an *Esch. coli* result on an unchlorinated sample.

The highest single inverse ratio was 1:14, also for an *Esch. coli* result on an unchlorinated water. The highest count given by MacConkey broth with a negative result in the corresponding modified glutamate medium was 5 *Esch. coli* per 100 ml. in a chlorinated sample.

Table 3. *Ratios of modified dehydrated glutamate results to MacConkey broth results in 48 hr.*

	Unchlorinated		Chlorinated	
	Coliform organisms	*Esch. coli*	Coliform organisms	*Esch. coli*
Mean ratio	2·0	3·2	1·5	2·9
Median ratio	1·0	2·7	1·2	5·0

Comparison between dehydrated and laboratory-prepared media of the same composition

At the Metropolitan Water Board Laboratories where the mineral modifications were formulated in conjunction with Oxoid Ltd., an additional series of comparisons was made with medium prepared from separate ingredients in the laboratory in the normal manner, having the same composition as the dehydrated minerals-modified glutamate medium. The results, based on numbers of positive tubes, are presented in Table 4. This shows that the general pattern of results compared with MacConkey broth was similar to the complete series for all laboratories given in Table 2. It also shows that there was very little difference between the results with the dehydrated and laboratory-prepared media. This confirms that the process of manufacturing the dehydrated medium did not produce any adverse effect.

DISCUSSION

These trials confirm the conclusions reached in the previous report (P.H.L.S., 1968) that Gray's glutamate medium is superior to MacConkey broth for the detection of coliform organisms in water, and that the difference between them increases with incubation time up to 48 hr. One disadvantage of glutamate media has been that growth is sometimes slower than in MacConkey broth, so that incubation for 24 hr. is required to achieve equivalent or better results. Although the 48 hr. results with the minerals-modified medium were slightly lower than with the original dehydrated medium, they were still significantly higher than results with MacConkey broth, which was the previously accepted standard. The differences in favour of the minerals-modified medium at 18 and 24 hr. were generally greater than those in favour of the original dehydrated medium at 48 hr. As under most circumstances early results are important so that remedial action can be taken quickly, the minerals-modified glutamate medium described in this paper is recommended. The results show that the differences between these

Table 4. *Comparison of dehydrated and laboratory-prepared minerals-modified glutamate*

	Numbers of tubes yielding								
	False positive reactions			Coliform organisms			*Esch. coli*		
	18 hr.	24 hr.	48 hr.	18 hr.	24 hr.	24 hr.	18 hr.	24 hr.	48 hr.
	Unchlorinated samples (*no. of tubes examined* 187)								
Oxoid MacConkey broth	1	3	13	107	124	138	85	87	87
Modified dehydrated glutamate medium	0	1	2	99	127	141	97	106	107
Modified laboratory-prepared glutamate medium	0	0	0	102	124	146	94	102	105
	Chlorinated samples (no. of tubes examined 187)								
Oxoid MacConkey broth	1	6	12	8	24	42	7	13	15
Modified dehydrated glutamate medium	0	0	5	0	23	61	0	20	39
Modified laboratory-prepared glutamate medium	0	0	1	0	29	61	0	27	42

modifications in glutamate media are very small compared with the differences between any one of them and MacConkey broth. It is interesting to note that the minerals-modified medium is closer in composition to the medium originally described by Gray.

The recommendation in this paper applies solely to the use of glutamate medium for enumeration of coliform organisms in water. The conclusions reached must not be interpreted as being applicable to milk or foods. The selectivity of the medium depends on the use of nutrients with a limited availability. It contains no inhibitory substances. The introduction of additional non-selective nutrients with the sample could be expected to change its growth characteristics.

SUMMARY

Oxoid dehydrated MacConkey broth was compared with laboratory-prepared Gray's improved formate lactose glutamate medium and with two dehydrated versions of it, by participants in ten laboratories. A variety of chlorinated and unchlorinated water samples was used. The superiority of the glutamate media over MacConkey broth for the detection of coliform organisms in water was again confirmed. The minerals-modified version, either dehydrated or laboratory-prepared, gave the best results and is the medium recommended for use in water examination. Care should be taken to ensure that the final pH after sterilization is 6·7.

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