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An improved formate lactose glutamate medium for the detection of *Escherichia coli* and other coliform organisms in water

By R. D. GRAY
Public Health Laboratory, Newport, Monmouthshire

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INTRODUCTION

In a previous communication (Gray, 1959) a simple formate lactose glutamate medium was described which, it was suggested, might replace MacConkey broth in the presumptive coliform examination of water. Besides being considerably cheaper, the synthetic medium yielded more isolations of true coliform organisms (and of *Escherichia coli*) with equal speed and with fewer false positive reactions. There was, however, the disadvantage that a proportion of minimally positive tubes could not be recognized until shaking had produced effervescence; and there was the further drawback that the medium was unsuitable for the 44° C. test. Since this medium contained a bare minimum of ingredients it was hoped that suitable small additions might overcome these defects without disturbing the specificity for the coliform group. The present communication describes a modification, devised after a prolonged succession of comparative trials, which surpasses standard MacConkey broth in the presumptive coliform test and is at least its equal in the 44° C. test for *Esch. coli.*

PRELIMINARY COMPARISONS

The formulae for the old and the improved formate lactose glutamate media (single strength) are as follows:

	Formate lactose glutamate (Gray, 1959) (pH 6·7)	Improved formate lactose glutamate (pH 6·7)
Sodium formate	0·25 g.	0·25 g.
Lactose	10 g.	10 g.
L(+)-glutamic acid	5 g.	5 g.
L(+)-arginine mono-hydrochloride	—	0·02 g.
L(−)-aspartic acid	—	0·024 g.
L(−)-cystine	—	0·02 g.
Potassium phosphate (K_2HPO_4)	3 g.	1 g.
Ammonium lactate (50% w/w solution)	10 ml.	—
Ammonium chloride (NH_4Cl)	—	2·5 g.
Magnesium sulphate ($MgSO_4 . 7H_2O$)	—	0·2 g.
Calcium chloride ($CaCl_2$)	—	0·2 g.
Ferric citrate scales (M.W. 335)	—	0·1 g.
Thiamin	—	1 mg.
Nicotinic acid	—	1 mg.
Pantothenic acid	—	1 mg.
Brom-cresol purple (1% alcoholic solution)	1 ml.	1 ml.
De-ionized water	1000 ml.	1000 ml.

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The objective in changing from the previous formula was to improve the *quality* of lactose fermentation by the coliform group. In this respect it was important that acid production should become more readily evident, and it would be an additional advantage if the volume of gas evolved could be increased. The preliminary comparisons, therefore, proceeded along two lines: tentative changes in the formula were first tested by minimal inocula of pure cultures of coliform organisms (principally *Esch. coli* itself), and modifications which thus proved successful were then made up in bulk and subjected to a short series of comparisons using unchlorinated water as the inoculum—on the lines of the final tests to be described. The water comparisons had the twofold object of confirming that the modification retained the numerical advantages of the original medium but did not introduce an undue liability to the production of false positive reactions.

Since publication of the previous paper (Gray, 1959) it had become clear that in the preliminary pure-culture experiments the inoculum used (one loopful of an 18 hr. culture) had been much too large. For this reason various projected additions to the original formula of Folpmers (1948) had *appeared* to be unrewarding. Further trials showed that, in order to simulate the conditions prevailing in water analysis, the inoculum should as nearly as possible approach a single viable organism, or for practical purposes three to ten organisms, as established by counts on blood agar done by the method of Miles & Misra (1938). It also became apparent that storage of the organisms in de-ionized water provided an extra stringent test of the synthetic medium and also explained its advantage over MacConkey broth: the presence of bile salt prevented the initiation of growth in a proportion of stored organisms (which grew well enough in the synthetic media) and this proportion increased with increasing time of storage.

It therefore became a regular practice to add 0·02 ml. of an 18 hr. culture to 150 ml. of sterile de-ionized water and to use as the inoculum 0·02 ml. of a suitable dilution. At first the dilution would be of the order of 1 in 100 but lower dilutions were indicated as the organisms died off until the dosage became 0·02 ml. and larger volumes (up to 1 ml.) of the undiluted fluid. To decrease the death rate (which was quite rapid with some of the cultures) sodium thiosulphate was included in the 6 oz. storage bottles used. At first the ordinary water-sampling concentration of approximately 20 p.p.m. was employed, but considerably greater benefit was later obtained from the concentration of 100 p.p.m. recommended by the American Public Health Association (1955) and by Hoather (1957).

The bacterial suspension having been thus prepared and checked, the inoculum was added by dropper to replicate tubes (usually five but sometimes three) of single-strength (*a*) MacConkey broth, (*b*) original formate lactose glutamate medium, and (*c*) one or more of the modifications under test, and also to duplicate plates of blood agar for concurrent viable counts. MacConkey broth was retained throughout as the standard control, but in due course the original formate lactose glutamate medium was replaced in the series by the interim modification so far approved.

By this means it was possible to conduct a very large number of experiments, and to find (*a*) whether any additional substance improved the medium's performance,

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and (*b*), if so, the optimal concentration of the substance. In certain instances, as with the more expensive amino acids, we sought not the optimum but the minimum effective concentration.

One of the earliest changes made was to replace ammonium lactate by an equivalent amount of ammonium chloride. The result was a doubling of the gas evolved, suggesting, as had been conjectured, that the lactate by competing with the lactose had interfered with the fermentation. On the suggestion of Burman (1960, pers. comm.) the concentration of potassium phosphate was reduced from 0·3 to 0·1 %. This change did not alter the speed of fermentation but, by removing much of the buffer effect, allowed acid production to become more readily visible. It was found that acid production was further enhanced by the addition of magnesium sulphate. The range of effective concentration was quite wide but after a number of trials it appeared that 0·2 g./l. was optimal. Later on in the trials significant improvements in fermentation were obtained by the addition of calcium and iron. The addition of iron introduced solubility problems. Ferrous sulphate, although beneficial, had to be abandoned since it produced a precipitate either immediately or during incubation. Similar difficulties occurred with ferric chloride. The more soluble ferric citrate provided equal benefit without these difficulties. The addition of manganese did not produce any detectable advantage.

On the question of additional amino acids the observations of previous workers, principally Pinsky & Stokes (1952) and Gest (1954) led us to expect, despite our earlier failure with large inocula, that there should be some advantage obtainable. With the minimal inocula we could detect an immediate benefit with aspartic acid and arginine added singly, but a considerably greater benefit when they were added together. The effect seemed to be greatest when they were added in equimolecular proportions. The original mixture tried was aspartic acid 0·24 g./l. and arginine 0·2 g./l., but for reasons of expense these amounts were successively reduced and an equally good effect was obtainable with one tenth of these quantities. At this stage, a further suggestion from Burman (1960, pers. comm.) was tried, that of doubling the glutamic acid concentration (i.e. to 10 g./l.). In the presence of arginine (0·02 g./l.) and aspartic acid (0·024 g./l.) the extra glutamic acid produced no effect. On the other hand, a halving of the glutamic acid concentration (i.e. to 2·5 g./l.) dramatically impaired the medium; normal function could be restored by greatly increasing the amounts of arginine and aspartic acid, but it was, of course, much more economical to revert to the original concentration of glutamic acid.

Most of the experiments with added minerals were, in fact, conducted with the medium already containing these three amino acids in the amounts listed in the formula. Once the mineral basis had been adjusted, further amino acids were tried. The choice was restricted to the cheaper ones (e.g. cystine, glycine, histidine, leucine, lysine and tyrosine). The only discernible benefit was obtained from cystine alone and it became evident that above a certain concentration (approx. 0·1 g./l.) cystine exerted a strongly inhibitory action. The optimum level to match the other amino acids present appeared to be 0·02 g./l. At this stage the purine bases had also been tested and an almost similar benefit was detected with guanine. Since, how-

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ever, the presence of cystine and guanine together conferred no advantage over that of cystine alone, guanine was excluded.

Finally, numerous growth factors were tried including most of the known factors of the vitamin B complex. The effect of thiamin was a noticeable speeding of fermentation; nicotinic acid and pantothenic acid also seemed to confer some benefit. Other factors tested and discarded were nicotinamide, riboflavine, pyridoxine, cyanocobalamin, and para-aminobenzoic acid. The inclusion of nicotinic acid in the formula resulted less from benefits observed at 37° C. than from the observations of Ware (1951) that nicotinamide (or nicotinic acid) was an essential nutrient for some strains of *Esch. coli* to produce fermentation at 44° C.

When the finally developed medium came to be prepared at double strength, problems of insolubility recurred. It was impossible to avoid the formation of a precipitate on heating when the medium contained lactose, cystine, ferric citrate and potassium phosphate in the concentrations listed. The presence of lactose and of phosphate was, of course, obligatory. The precipitate could be avoided by omitting from the formula either cystine or iron, both of which had significantly added to the medium's performance in the single-strength tests. It was eventually found quite practicable to include a precipitation and filtration process during the preparation of the double-strength stock (see Appendix): the optimum performance of the medium was thereby maintained and no further precipitate occurred during sterilization or subsequent incubation.

MATERIALS AND METHODS

These refer to the comparative trials made between standard MacConkey broth and the finally developed medium.

Media used

MacConkey broth (double strength and single strength) was prepared as officially recommended (Ministry of Health, 1956). The peptone (Evans's) was adopted for optimal gas production and the bile salt (Hopkin and Williams's sodium taurocholate) for minimal inhibition. The double-strength medium was distributed in 10 ml. volumes in $6 \times \frac{3}{4}$ in. test tubes and the single-strength medium in 5 ml. volumes in $6 \times \frac{5}{8}$ in. test tubes, all containing Durham tubes.

The improved formate lactose glutamate medium (double and single strength) was prepared as described in the Appendix. Distribution was the same as for MacConkey broth except that for the single-strength medium $6 \times \frac{1}{2}$ in. test tubes were used. Burman & Oliver (1952) recommended this procedure for the original glutamic acid medium but experience at this laboratory suggests that with the new medium the $6 \times \frac{5}{8}$ in. tubes are equally suitable.

Sterilization of media

Both media were autoclaved at 10 lb. for 10 min. Previously, to minimize hydrolysis of the lactose, the synthetic medium had been sterilized by steaming for 45 min. on each of 2 successive days. In the course of this investigation, however,

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it was discovered that the lactose in the improved synthetic medium could withstand one autoclaving as well as in MacConkey broth.

Water samples and procedure

(a) Presumptive coliform trial

All samples of unchlorinated water arriving at the laboratory for routine examination were inoculated into each medium as follows: 10 ml. into each of five tubes of double-strength medium; and 1 ml. into each of five tubes of single-strength medium. The tubes were incubated at 37° C. and inspected after approximately 18, 24 and 48 hr. Those showing production of acid and gas were recorded as presumptive positives.* These were at once further tested by subculture in MacConkey broth for incubation at 44° C. for 24 hr. for gas production, and plating on to MacConkey agar for incubation at 37° C. for 24 hr. in order to provide colonies for any subsequent testing.

Any presumptive positive tube giving a positive fermentation test at 44° C. was regarded as containing *Esch. coli*. When no fermentation occurred at 44° C., several colonies (if there were any) from the MacConkey plate were subcultivated into lactose peptone water. If, after 48 hr. incubation at 37° C., the lactose peptone water showed acid and gas, the presumptive tube was considered to contain true coliform organisms other than *Esch. coli*. If there were no colonies on the MacConkey plate or if, after 48 hr. incubation, the lactose peptone water failed to show acid and gas, the presumptive positive reaction was classified as a false positive reaction, i.e. a presumptive positive tube from which no lactose-fermenting coliform organisms could be isolated.

(b) Comparative trial of 44° C. *test for* Esch. coli

Since the presumptive coliform trial showed the synthetic medium to produce at all periods of incubation a greater yield both of coliform organisms and of *Esch. coli*, it was decided, in this second comparison, to perform the preliminary presumptive test only in the synthetic medium. During this trial all samples of unchlorinated water arriving at the laboratory for routine examination were inoculated into the synthetic medium in the ordinary way (50 ml. and 5 × 10 ml. volumes into double-strength and 5 × 1 ml. volumes into single-strength medium). The bottles and tubes were incubated at 37° C. and inspected after approximately 18, 24 and 42 hr. The routine tests were usually started at or about 4 p.m.; the 18 hr. and 42 hr. periods occurred therefore at or about 10 a.m. Tubes showing production of acid and gas were recorded as presumptive positives and those occurring at 18 and 42 hr. were subcultured for the 44° C. tests at 10 a.m. Those showing acid and gas first at 24 hr. were subcultured at 4 p.m. and then the primary tubes were placed in the refrigerator until the following morning. From these a second sub-

* In accordance with standard practice the minimum criterion for a 'positive' MacConkey tube was the presence of acid, together with enough gas to fill the concavity of the Durham tube. With the synthetic medium, however, a reliable minimal criterion was clearly detectable acid together with any gas (produced, if necessary, as effervescence after shaking).

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culturing was then made at 10 a.m. in order to provide for a 6 hr. reading during working hours. Preliminary experiments suggested that this period of refrigeration did not affect the subsequent 6 hr. result.

From each presumptive positive tube a loopful of medium was transferred into single-strength (*a*) MacConkey broth and (*b*) improved formate lactose glutamate medium. The duplicate tubes were then placed in the 44° C. water bath and inspected (for gas production) (1) after 6 hr. and (2) after 24 hr.

(*c*) *General P.H.L.S. trial*

It was considered advisable to check the Newport results, and 27 other P.H.L.S. laboratories undertook to participate in a comparative trial between their regular standard water medium (MacConkey broth or Teepol broth) and the synthetic medium (which was prepared in Newport and distributed to the other laboratories). Each participating laboratory agreed to examine approximately 30 unchlorinated water samples as follows: 5×10 ml. volumes and 5×1 ml. volumes were inoculated into appropriate tubes of both media which were incubated at 37° C. and inspected at 18 and/or 24 hr. and 48 hr. Each presumptive positive tube (without necessarily testing for false positive results) was then subcultured into fresh tubes of both media which were held at 44° C. for 24 hr. and then inspected for gas production.

RESULTS

Presumptive coliform trial at Newport

Over a period of 4 months 100 routine samples of unchlorinated water were examined in the manner described. The cumulative results are shown in Table 1, where it can be seen that, as regards both true coliform organisms and those subsequently identified as *Esch. coli*, the numbers of isolations appearing by 18, 24 and 48 hr. were all noticeably greater in the synthetic medium than in MacConkey broth. In comparing these figures it must be borne in mind: (*a*) that in order to minimize the suppressive effect of bile salt on the coliform group (see P.H.L.S. Water Sub-committee, 1958; Gray, 1959) a relatively non-inhibitory brand of sodium taurocholate was specially chosen; and (*b*) the higher figures obtained in the synthetic medium were virtually unassociated with false positive results.

Table 1. *Reactions produced by* 100 *samples of unchlorinated water examined at Newport, Monmouthshire*

Medium	No. of false positive reactions appearing by			No. of true coliform reactions appearing by			No. of *Esch. coli* producing a presumptive positive reaction by		
	18 hr.	24 hr.	48 hr.	18 hr.	24 hr.	48 hr.	18 hr.	24 hr.	48 hr.
MacConkey broth	0	1	18	128	151	517	115	123	127
Improved formate lactose glutamate medium	0	0	1	140	189	651	136	178	199

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Newport comparative trial of 44° C. *test for* Esch. coli

Over a period of 7 months 1099 presumptive positive tubes arising in the synthetic medium from 156 samples of unchlorinated water were tested in duplicate as already described. The results are shown in Table 2, where it can be seen that in the 44° C. test both media behaved almost identically. It is noteworthy that from the three different types of presumptive positive tubes (18, 24 and 48 hr.) the 6 hr. 44° C.-positive tubes are as numerous in the synthetic as in the standard medium.

Table 2. 44° C. *test for* Escherichia coli

(Comparison between MacConkey broth and improved F.L.G. medium—results arising from 156 samples of unchlorinated water examined at Newport, Mon.)

Presumptive positive reactions detected in improved F.L.G. medium		No. producing fermentation at 44° C. in			
		MacConkey broth by		Improved F.L.G. medium by	
Time of appearance (hr.)	No.	6 hr.	24 hr.	6 hr.	24 hr.
18	536	532	535	533	536
24	121	70	79	70	82
42	442	17	35	18	42
Totals	1099	619	649	621	660

General P.H.L.S. trial

The results of this trial are shown in Table 3. Although there are undoubted fluctuations from laboratory to laboratory, the aggregated totals confirm the trend shown in Newport.

First, with regard only to the presumptive positive results, the totals by 18 and 24 hr. were slightly greater and by 48 hr. considerably greater in the synthetic than in the standard medium. The final increase (3054 − 2830 = 224) represents an 8% improvement upon the performance of the standard medium. Since false positive results are much less common in the synthetic than in the standard media it is to be expected that full elimination of these would have shown an even greater improvement.

Secondly, with regard to the presumptive positive results which were shown by the 44° C. test to contain *Esch. coli*, the positive 37° C. tubes in each medium were tested in both media at 44° C.; there were therefore 4 ways in which positive 44° C. tests could be obtained:

(1) presumptive positives in standard water medium producing positive 44° C. results in standard water medium 'S.W.M./S.W.M.';

(2) presumptive positives in standard water medium producing positive 44° C. results in improved F.L.G. medium 'S.W.M./I.F.L.G.';

(3) presumptive positives in improved F.L.G. medium producing positive 44° C. results in standard water medium 'I.F.L.G./S.W.M.';

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Table 3. *Reactions produced by 785 samples of unchlorinated water examined in 27 P.H.L.S. laboratories*

Laboratory	No. of samples examined in duplicate	No. of samples giving positive results in at least one medium	No. of presumptive coliform reactions appearing in: Standard water medium by			Improved F.L.G. medium by			No. of *Esch. coli* producing a presumptive positive reaction in: S.W.M./S.W.M. by			S.W.M./I.F.L.G. by			I.F.L.G./S.W.M. by			I.F.L.G./I.F.L.G. by		
			18 hr.	24 hr.	48 hr.	18 hr.	24 hr.	48 hr.	18 hr.	24 hr.	48 hr.	18 hr.	24 hr.	48 hr.	18 hr.	24 hr.	48 hr.	18 hr.	24 hr.	48 hr.
Bath	30	18	28	28	64	27	27	65	27	27	28	27	27	29	27	27	30	27	27	29
Birmingham	38	21	—	66	74	—	92	109	—	66	68	—	66	68	—	91	96	—	92	97
Bradford	32	22	—	94	112	—	101	116	—	92	98	—	92	98	—	97	104	—	97	104
Brighton (T)	30	25	—	110	137	—	120	144	—	89	89	—	88	88	—	96	97	—	95	96
Bristol	30	24	56	60	138	51	57	127	49	52	58	49	52	58	45	49	60	44	48	59
Cambridge (T)	26	10	10	16	48	7	15	46	9	11	11	9	11	11	5	7	7	5	7	7
Cardiff	23	22	—	104	138	—	109	144	—	102	123	—	97	106	—	98	103	—	92	98
Carlisle	30	19	—	67	87	—	77	105	—	67	68	—	67	68	—	76	76	—	76	76
Carmarthen	21	17	—	62	101	—	58	135	—	31	33	—	28	30	—	30	33	—	28	31
Conway	34	29	85	85	198	81	81	204	66	66	75	65	65	72	73	73	93	73	73	91
Dorchester	29	20	—	98	132	—	100	129	—	60	85	—	60	85	—	64	82	—	61	79
Hull	14	14	—	79	119	—	43	116	—	75	88	—	76	90	—	42	94	—	42	96
Leicester	23	19	—	76	136	—	71	134	—	66	68	—	66	68	—	62	69	—	62	69
*Manchester (T)	35	29	134	155	164	126	152	191	130	136	136	130	136	136	125	145	146	125	145	146
*Northallerton	29	23	40	66	101	65	83	122	25	31	34	33	42	45	39	45	47	40	46	48
Norwich (T)	30	19	30	51	122	29	67	135	23	25	28	23	25	30	23	31	33	20	29	33
Nottingham	59	9	10	22	29	10	28	44	10	21	21	10	21	21	10	26	27	10	26	27
Plymouth	30	25	—	88	138	—	80	149	—	86	98	—	86	98	—	77	101	—	77	101
Portsmouth (T)	13	13	35	36	52	35	35	47	32	32	32	32	32	32	31	31	32	32	32	34
Preston	28	18	32	32	57	57	57	84	27	27	36	30	30	39	48	48	55	52	52	61
Salisbury	27	9	3	5	67	1	7	81	—	—	39	—	—	45	—	—	42	—	—	50
Shrewsbury (T)	30	19	—	35	108	—	29	95	—	24	26	—	24	26	—	19	26	—	19	26
Southend	30	27	53	91	130	45	73	151	38	42	48	38	42	48	40	42	52	39	41	51
Stafford	30	17	21	34	84	10	17	64	9	9	9	9	9	9	9	9	9	9	9	9
Taunton	30	19	—	8	88	—	11	99	—	—	17	—	—	32	—	—	20	—	—	30
*Wakefield	28	20	52	72	99	51	64	114	42	55	57	42	55	57	41	51	51	41	51	51
Worcester	26	17	—	47	107	—	47	104	—	37	38	—	37	38	—	41	42	—	41	42
Totals	785	524	589	1687	2830	595	1701	3054	487	1329	1511	497	1334	1527	516	1377	1627	517	1368	1641

* False positive reactions checked and excluded.
Most laboratories used MacConkey broth as their standard water medium; those using Teepol broth are marked (T).

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(4) presumptive positives in improved F.L.G. medium producing positive 44° C. results in improved F.L.G. medium 'I.F.L.G./I.F.L.G.'.

If the aggregated totals are compared, it will be seen that method (1) (S.W.M./S.W.M.) yields the lowest totals of *Esch. coli* at all three periods; that method (2) (S.W.M./I.F.L.G.) shows a slight increase, method (3) (I.F.L.G./S.W.M.) a still greater increase and method (4) (I.F.L.G./I.F.L.G.) the highest figures of all at 18 and 48 hr. As was to be expected from the greater presumptive yield in the synthetic medium, the most noticeable increases in the *Esch. coli* totals occur where the synthetic medium was used for the presumptive test: 1627 and 1641 as against 1511 and 1527.

DISCUSSION

The results in Newport (Tables 1 and 2) and in the other 27 areas, considered as one whole (Table 3, column totals), suggest that from non-chlorinated water (1) improved formate lactose glutamate (I.F.L.G.) produces more isolations both of presumptive coliform organisms and of *Esch. coli* than does standard MacConkey broth (or its derivative Teepol broth) and (2) the increased isolations are obtained without loss of speed: only slight increases are visible by 18 and 24 hr. but noticeable increases are obtained by 42–48 hr.

False positive reactions

The work at Newport additionally suggests that, so far as non-chlorinated water is concerned, the synthetic medium produces so small a proportion of false positive reactions that presumptive positive reactions, even at 48 hr., can be reliably considered to contain true coliform organisms. It is, perhaps, unfortunate that in the wider trial only three of the twenty-seven laboratories tested for false positive reactions; but they were not asked to do so, as this is not customarily done in the routine examination of non-chlorinated water, and the object of the trial was to simulate standard routine as closely as possible. The three laboratories which included the extra tests produced the following totals of false positives: (*a*) from MacConkey (or Teepol) broth 5, and (*b*) from the synthetic medium 7 (all obtained at Manchester). As compared with their total isolations of true coliform organisms, (*a*) 364 and (*b*) 427, the figures for false positives are too small to invalidate the usual practice of concluding that, with non-chlorinated water, presumptive coliform reactions can be taken to represent the presence of coliform organisms.

The problem of false positive reactions, however, has always been principally associated with chlorinated waters, and the chief reason for this is the survival (after chlorination) of *Cl. welchii* and similar sporing anaerobes which readily ferment lactose in MacConkey broth (Taylor, 1949; P.H.L.S. Water Sub-committee, 1953) and in other conventional water media incorporating peptone (Teepol broth, American lactose broth, and lauryl tryptose broth—see Jebb, 1959). The original formate lactose glutamate medium (Gray, 1959), like its predecessor lactose glutamic acid (Burman & Oliver, 1952; P.H.L.S. Water Sub-committee, 1958), produced an almost negligible proportion of false positive reactions even from chlorinated waters. It had been found in Newport that neither of these media

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would support the growth of *Cl. welchii* even after heavy inocula. In the course of modifying the 1959 medium we repeatedly checked that the extra nutrients added did not destroy this advantage; no strain of *Cl. welchii* has been found to ferment or even grow in the final medium. It was, therefore, hoped that with this medium the problem of false positives would become virtually eliminated, and this hope is encouraged by recent experience at Newport. Here, since 1 May 1963, improved formate lactose glutamate has been exclusively used for routine water analysis and every one of the few presumptive positive reactions arising from chlorinated water has been fully tested. So far, after 12 months, 1224 chlorinated samples have been examined of which 1111 were completely negative. From the remaining 113 samples, 396 presumptive positive tubes were obtained. Each of these proved to contain true coliform organisms (and 170 *Esch. coli* itself). In other words, not one false positive reaction has yet been discovered from chlorinated water samples examined in I.F.L.G. at Newport.

In the Metropolitan Water Board Laboratories, however, where a different modification of formate lactose glutamate has been in routine use since January 1962 (Taylor, 1964), certain chlorinated waters—principally river-derived supplies—have been found to yield, particularly in the winter months, a substantial proportion of false positive reactions after 48 hr. incubation. With experience these became readily recognizable from true reactions by the poor degree of acid and the small quantity of gas evolved, and are now being discarded on sight. The causative organisms are *aerobic* spore-bearers, and five representative cultures were recently sent to Newport (Burman, 1964, pers. comm.). Heavy inoculation of each culture into I.F.L.G. produced by 48 hr. (and sometimes sooner) indisputable fermentation, i.e. detectable acid associated with adequate gas. This is not to say that similar appearances would have been produced in the I.F.L.G. medium by the smaller numbers to be expected in the natural conditions of water examination; the Newport medium is now being tested by the M.W.B. laboratories. Only further and much more general experience will determine whether this is a problem peculiar to London (or the M.W.B. version of F.L.G.) or whether it may be met in other parts of the country such as, perhaps, Manchester. It is certainly no problem at present in Monmouthshire, where most of the chlorinated supplies come from surface waters.

Interpretation of routine water analysis

From the work at Newport and elsewhere it would seem reasonable to suggest that presumptive positive reactions from non-chlorinated water appearing in I.F.L.G. by 18 hr. might be deemed without further testing to contain *Esch. coli*. In this way, without serious error, the 44° C. test could be reserved for the many fewer presumptive positive reactions arising at 24 and 48 hr. The saving in labour would be worth considering.

In the case of chlorinated waters, which should, of course, always ideally be totally negative, any presumptive positive reaction would continue to require (1) checking to eliminate false positives and (2) differentiation into *Esch. coli* or other true coliform organisms.

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Observations regarding the new medium

It has been the intention in Newport to improve the original F.L.G. medium so as to provide the coliform group, and *Esch. coli* particularly, with the optimum ingredients within the limits of expense and practicability for maximum growth and lactose-fermentation, while as far as possible limiting the available carbon and energy source to lactose. Having been thus 'tailor-made' for the coliform group, the medium requires no inhibitory substance. Non-lactose-fermenters are substantially inhibited by the lack of a suitable energy source: herein lies the advantage over glucose glutamic acid. On plates of solid I.F.L.G., containing ten times the quoted concentration of brom-cresol purple indicator, this effect can be seen. Yellow coliform colonies attain after overnight incubation something approaching the normal size of 2 mm. diam., usually with a surrounding yellow zone of diffused acid; non-lactose-fermenters (e.g. salmonellae) under the same conditions appear as purple pin-point colonies clearly distinguishable by colour and size, so long as colonies of both types are reasonably discrete. Proteus can produce purple pin-point colonies but is unable to swarm. Enterocococci are unable to grow at all.

Whilst it is not suggested that I.F.L.G. has any claim to replace MacConkey agar or similar media in general bacteriology, as for example routine examination for faecal pathogens, it is hoped that I.F.L.G. agar may serve as a basis for the coming method of routine water examination—membrane filtration. So far, only a few pure-culture experiments have been made but these suggest that the membrane should be incubated over a plate of solid medium rather than over the usual pad impregnated with liquid medium. By the former method *Esch. coli* colonies, after overnight incubation at 37° C., reach full normal size (2–3 mm. diam.) and present a bright yellow colour, whereas non-lactose-fermenters such as Proteus produce only pin-point colonies, either colourless or pale violet, which are almost invisible to the naked eye. There is, therefore, reason to hope that the coliform count would become much easier to read than with MacConkey agar and neutral red (or phenol red), where pink colonies have to be distinguished from yellow.

Finally, as regards expense, the original F.L.G. medium was found in 1959 to be 2·5 times cheaper than MacConkey broth. The I.F.L.G. medium has small amounts of extra ingredients but is not significantly more expensive than F.L.G. Meanwhile, however, the prices of the biological ingredients have so increased that the cost of the cheapest MacConkey broth is now more than four times, and the cost of Teepol broth made with a good peptone is more than three times, that of I.F.L.G. The increasing use made of bile salt for pharmaceutical purposes will almost certainly lead to further scarcity and higher prices, so that the saving offered by the synthetic medium should increase still further. If, in addition, the use of membrane filtration permits 100 ml. of water to be examined by 10 ml. of single-strength agar medium instead of 100 ml. of double-strength fluid medium, the economies would be very considerable.

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SUMMARY

A chemically defined fluid medium—improved formate lactose glutamate—is described of which the cost is less than a quarter that of standard MacConkey broth and which is at least equally suitable for routine bacteriological examination of water. It has actual advantages in that the medium after fermentation is clearer and that, therefore, acid and gas production is more easily seen; moreover more isolations of both coliform bacteria and of *Esch. coli* are obtained with fewer false positive reactions. Trials with a solid version of the medium suggest that the relative freedom from false positive reactions arises from the failure of the medium to grow *Cl. welchii*—one of the commonest causes—and enterococci, another suspected participant. In fact, without any inhibitory agent the medium appears to be virtually specific for the coliform group and particularly favours *Esch. coli*. It remains to be seen whether certain aerobic spore-bearers found from time to time in surface waters may, to some slight extent, interfere with the almost complete specificity so far discovered.

The medium is recommended in place of MacConkey broth and other peptone-containing media, not only for the prospect of better results, in that suppression of the coliform group does not occur, but also for cheapness and for freedom from the inevitable variability associated with media incorporating peptone and, particularly, bile salt. It is hoped that this medium will shortly become available commercially in dehydrated form at a price still considerably less than that required to make up MacConkey broth from its constituents.

Some labour-saving curtailments in the routine of water examination are also suggested.

It is hoped not only that the fluid medium will supplant MacConkey broth for the dilution method of water analysis but that the solid medium, without material alteration, will also prove suitable for water examination by membrane filtration.

I am deeply grateful to my laboratory staff, particularly Mr J. H. Evans, F.I.M.L.T., and Mr G. H. Lowe, F.I.M.L.T., for undertaking so much of the work when routine work was particularly heavy, and for many valuable suggestions. My thanks in addition are due to Mrs Peggy Johnson and Mrs Marilyn Childs, who made up the medium, and also to the directors of the participating P.H.L.S. laboratories not only for having the work carried out but for helpful comments.

APPENDIX

Preparation of improved formate lactose glutamate medium

The formula for the single-strength medium has already been given. In practice it is convenient to make 10 l. of double-strength stock solution containing all the ingredients except the lactose and thiamin (to minimize loss by heat) and indicator. It is advisable first of all, however, to make the following stock solutions:

(*a*) Calcium chloride ($CaCl_2$), 25 g.; de-ionized water, 500 ml. Clarify by adding 0·4 ml. of concentrated hydrochloric acid and sterilize by autoclaving at 10 lb. for 10 min.

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(*b*) Thiamin, 0·1 g.; de-ionized water, 100 ml. The contents of 1 ampoule (100 mg.) are added aseptically to 99 ml. of *sterile* de-ionized water. (Do not heat.) This solution should be kept in the refrigerator and should be discarded after 6 weeks.

(*c*) Brom-cresol purple, 1 g.; absolute alcohol, 100 ml.

Stock double-strength solution

Place 9 l. of de-ionized water in a stainless-steel bucket and heat. Whilst this is heating, weigh out into 4 separate flasks:

(1)	L(+)-arginine mono-hydrochloride	0·4 g.
	L(−)-aspartic acid	0·48 g.
(2)	L(−)-cystine	0·4 g.
(3)	Ferric citrate scales (M.W. 335)	2 g.
(4)	Nicotinic acid	0·02 g.
	Pantothenic acid	0·02 g.

From a separate litre of de-ionized water are removed measured volumes for dissolving the contents of each flask. In the case of flask (4) the contents are readily dissolved by 5 ml. of cold water. For flasks (1) and (3) 50 and 100 ml. volumes, respectively, together with heating are required. In order to dissolve the cystine in flask (2) it is necessary to use 100 ml. of water, together with heat and the addition of 10 ml. of 5N-sodium hydroxide.

To the 9 l. of water (kept hot in the steamer) add the L(+)-glutamic acid (100 g.) and approximately 140 ml. of 5N-sodium hydroxide. While the bulk is cooling add:

Potassium phosphate (K_2HPO_4)	20 g.
Ammonium chloride (NH_4Cl)	50 g.
Magnesium sulphate ($MgSO_4 . 7H_2O$)	4 g.
Sodium formate	5 g.

Now add the contents of the four flasks and rinse each flask (with water from the spare litre) into the bucket. Add 80 ml. of the 5 % solution of calcium chloride. Finally, discard from the spare litre flask 230 ml. (to compensate for the 80 ml. of calcium chloride solution and the 150 ml. of sodium hydroxide added) and add the remaining water to the bulk.

Allow the bulk to cool and bring the pH to 7·5. Place the bucket on a gas ring and boil for at least 3 min. Filter while hot through Green's 904½ filter-paper. Adjust the pH to 6·8, bottle in 500 ml. amounts and autoclave at 10 lb. for 10 min.

For use

To each 500 ml. as required add lactose (10 g.) and (with aseptic precautions) 1 ml. of the 0·1 % solution of thiamin. (For single-strength medium now dilute with an equal volume of de-ionized water.) Check that the pH is 6·8, add the brom-cresol purple solution (1 ml. for each 500 ml. of double-strength or 1000 ml. of single-strength medium), distribute into bottles and tubes, and sterilize by autoclaving at 10 lb. for 10 min. only. The final pH after autoclaving is 6·7.

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