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Quality control tests of two salmonella enrichment media using different inocula

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SUMMARY

Inocula of polluted water naturally infected with salmonellas effectively distinguished six brands of selenite and six brands of tetrathionate enrichment media into satisfactory and unsatisfactory categories. Minimal inocula of pure cultures differentiated the tetrathionates, but not the selenites. Inocula of naturally infected chicken giblets suggested that there was a difference between two comparable brands of tetrathionate, but this was not statistically significant. The difference was, however, clearly demonstrated by minimal inocula of pure cultures.

Intensive investigation of two inferior tetrathionates revealed inhomogeneity in the distribution of brilliant green in one bottle of one brand. The importance of the salmonella serotype and even the colonial variant used for the pure culture inoculum was also demonstrated.

INTRODUCTION

Microbiologists are currently interested in testing their relative abilities to carry out certain routine bacteriological procedures. This has led to a series of laboratories simultaneously examining circulated samples containing known organisms. The results of these trials are useful in the initiation of attempts at improving performance and may also give some microbiologists confidence in techniques already used. Quality control trials are also possible on an international basis (Edel & Kampelmacher, 1968, 1969) and even to a limited extent on an inter-continental basis (Price & Morgan, 1970), but considerable information can likewise be obtained when they are performed on a smaller scale. Under these conditions, variables introduced by the use of a series of uncoordinated techniques in the hands of a multiplicity of laboratories are avoided.

In this paper we report a study of several brands of two different media for

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salmonella enrichment when inoculated with different materials. The three inocula were (i) sewage-polluted natural water, (ii) pure cultures of salmonella serotypes, and (iii) naturally infected samples of chicken giblets. The use of the first follows the description of a test by Morgan (1974), who found that 25 ml. quantities of the River Taff in Glamorgan were suitable test materials for differentiating satisfactory and unsatisfactory salmonella enrichment media. The second follows the method described by Stokes (1968) and the third was prompted by the somewhat equivocal experience of Vassiliadis *et al.* (1972) with Muller–Kauffmann tetrathionate broth.

So remarkable was one of the results that it was thought well to repeat a part of the investigation elsewhere (Public Health Laboratory, Newcastle). A few problems arose incidental to the main investigation and these are also reported.

METHODS

Media

Tetrathionate

Muller–Kauffmann tetrathionate broth (Medium F) was used at Cardiff. The method of preparation is described elsewhere (Harvey & Price, 1974). This enrichment broth was incubated at 43° C. after inoculation. Five brands of commercial tetrathionate (A–E) were made and used according to their manufacturers' instructions except that E was incubated after inoculation at both 37° and 43° C. (E_{37} and E_{43}). At Newcastle, tetrathionate broth (G) was made according to the formula of Knox, Gell & Pollock (1942), but chalk was omitted as its buffering power seemed negligible. Medium G was incubated at 37° C. Single-strength media were used for pure cultures and chicken giblets, but double-strength medium was used for the polluted-water samples.

Selenite

Five brands of commercial selenite F medium (H–L) were prepared according to the makers' instructions. Medium M was the laboratory formulation of selenite used at Cardiff (Hobbs & Allison, 1945).

Brilliant-green MacConkey agar (Harvey, 1956) was used for all subcultures from enrichment media in Cardiff. These were made after 24 hr. incubation from selenite broths and after 24 and 48 hr. incubation from tetrathionate broths.

Inocula

Polluted water

Twenty-two samples of river water were divided into portions of 25 ml. so that 4–16 tests could be made with each enrichment broth investigated.

Pure cultures

The technique described by Stokes (1968) was followed. In medical microbiology a most important property of liquid media is that they should be able to support the growth of small inocula of the organisms they are designed to isolate. The

Table 1. *Comparisons between tetrathionate broths used as enrichment media, sub-cultured after* 24 *and* 48 *hr* on brilliant-green MacConkey agar* (*tested with* 25 *ml. of sewage-polluted water*)

Sample No.	No. of tests	No. of positive cultures from each enrichment broth						
		A	B	C	D	E_{37}	E_{43}	F
1	8	0	0	0	0	0	0	2
2	8	0	2	0	0	0	0	3
3	8	0	1	1	1	0	0	0
4	8	0	0	0	0	0	0	0
5	8	0	1	2	0	0	0	0
6	8	0	0	0	0	0	0	4
7	8	0	0	0	0	0	0	7
8	8	0	2	0	0	0	0	5
9	8	0	3	5	1	0	2	8
10	4	0	0	1	0	0	1	4
11	16	3	5	5	0	0	0	10
Total	92	3	14	14	2	0	3	43

Order of media success: $F > B = C > A = E_{43} > D > E_{37}$.

* In the tables the results of the 24 and 48 hr. subcultures have been combined.

colony-counting method (Miles & Misra, 1938) can be used to estimate the number of organisms inoculated into a medium under investigation. A preliminary count is made of a smooth broth culture of the test organism to determine which dilution contains only a few viable organisms per drop. In our hands, we found that a 0·02 ml. drop of a 10^{-6} dilution of a broth culture was the most suitable inoculum.

Chicken giblets

These were readily available as a naturally infected material at Cardiff. About 25 % of these specimens could be expected to yield salmonellas. The inocula used weighed 25–50 g. Samples were chopped with scissors and put into 200 ml. of enrichment medium.

RESULTS

Tables 1 and 2 record the number of isolations of salmonellas from polluted waters and small inocula of pure cultures sown into various tetrathionate broths. There was partial correlation between the results obtained from the polluted water and the pure cultures. Media A, E_{37} and E_{43} were unsatisfactory by both methods and B, C and F were satisfactory. The methods disagreed about D. The polluted-water inocula discriminated more sharply between satisfactory and unsatisfactory tetrathionate enrichment broths.

Tables 3 and 4 show the results of testing selenite F broth with polluted water and small numbers of pure cultures of salmonellas. The polluted-water inocula distinguished between the various brands of selenite. The pure culture inocula did not. All brands of selenite were always successful with the small and roughly similar sized inocula of pure cultures, whereas several tetrathionates were not.

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Table 2. *Identical samples (as Table 1) tested with small numbers of organisms from a pure culture of* Salmonella typhimurium, *phage-type* 1, *var.* 5

Test no.	Approx. no. of organisms inoculated	Growth or no growth of organisms in each enrichment broth						
		A	B	C	D	E_{37}	E_{43}	F
1	6	−	+	+	+	−	+	+
2	6	−	+	+	+	−	−	+
3	13	−	+	+	+	−	−	+
4	11	+	+	+	+	−	−	+
5	16	−	+	+	+	−	−	+
6	4	−	+	+	+	+	−	+
7	8	−	+	+	+	−	−	+
8	13	−	+	+	+	−	−	+
9	20	−	+	+	+	−	−	+
10	28	+	+	+	+	+	−	+
11	3	−	+	+	+	−	−	+
No. of tests positive (out of 11)		2	11	11	11	2	1	11

Order of media success: B = C = D = F > A = E_{37} > E_{43}.

Table 3. *Comparisons between selenite F broths used as enrichment media, sub-cultured after* 24 *hr on brilliant-green MacConkey agar tested with* 25 *ml. of sewage-polluted water*

Sample no.	No. of tests	No. of positive cultures from each enrichment broth					
		H	I	J	K	L	M
1	8	0	6	6	1	6	7
2	8	1	2	4	3	6	4
3	8	6	5	8	6	6	4
4	8	8	8	8	7	8	8
5	8	2	2	3	1	7	5
6	4	2	4	3	0	2	3
7	4	0	2	2	1	3	2
8	8	3	1	1	0	3	2
9	8	2	3	2	0	2	7
10	8	2	6	4	2	5	7
11	8	3	5	7	1	4	5
Total	80	29	44	48	22	52	54

Order of media success: M > L > J > I > H > K.

With the polluted-water inocula, selenite was not always successful and the failure rate easily distinguished the different brands. It must be emphasized that the water samples used for testing selenite were different from those used to test tetrathionate. Both, however, came from the same river source.

Table 4. *Identical samples (as Table 3) tested with small numbers of organisms from a pure culture of* Salmonella typhimurium, *phage-type 1, var. 5*

Test no.	Approx. no. of organisms inoculated	Growth or no growth of organisms in each enrichment broth					
		H	I	J	K	L	M
1	5	+	+	+	+	+	+
2	8	+	+	+	+	+	+
3	12	+	+	+	+	+	+
4	15	+	+	+	+	+	+
5	8	+	+	+	+	+	+
6	5	+	+	+	+	+	+
7	11	+	+	+	+	+	+
8	10	+	+	+	+	+	+
9	8	+	+	+	+	+	+
10	8	+	+	+	+	+	+
11	11	+	+	+	+	+	+
No. of tests positive (out of 11)		11	11	11	11	11	11

Order of media success: H = I = J = K = L = M.

Table 5. *Tetrathionate A tested against Tetrathionate F*

	Organisms in 0·02 ml. of 10^{-6} dilution	Dilution of test culture							Medium used
		Undiluted	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
1	4	+	+	+	−	−	−	−	A
		·	·	·	·	·	+	+	F
2	5	−	−	−	−	−	−	−	A
		·	·	·	·	·	+	+	F
3	3	−	−	−	−	−	−	−	A
		·	·	·	·	·	+	+	F
4	28	+	+	+	+	+	+	+	A
		·	·	·	·	·	+	+	F
5	3	+	+	+	+	+	−	−	A
		·	·	·	·	·	+	+	F
6	19	+	−	−	−	−	−	−	A
		·	·	·	·	·	+	+	F
7	20	+	+	+	+	+	+	+	A
		·	·	·	·	·	+	+	F
8	3	+	+	+	+	+	−	−	A
		·	·	·	·	·	+	+	F

+, Growth. −, No growth.

Tetrathionate A

This medium, one of the commercial brands, had performed badly with both polluted water and pure cultures (Tables 1, 2). It was compared, therefore, with F as control. The two were re-examined by the pure culture technique and the results are given in Table 5. The results with A varied widely and unpredictably. Medium F, in contrast, was consistently successful. Could batches of A vary even

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Table 6. *Concentration of brilliant green in parts per million in samples of medium A*

Concentration	Number of samples in which a particular concentration was found
4	2
5	3
6	4
7	8
8	16
9	10
10	4
12	1

Table 7. *Medium A tested against medium F using subculture of smooth colony from sample* 8 *as test strain*

Sample no.	Organisms in 0·02 ml of 10^{-6} dilution	Organisms necessary to initiate growth	Dilution of test culture: Undiluted	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Medium used
9	7	.	−	−	−	−	−	−	−	A
		.	.	.	.	.	.	−	−	F
10	6	.	−	−	−	−	−	−	−	A
		600	.	.	.	.	+	−	−	F
11	8	.	−	−	−	−	−	−	−	A
		800	.	.	+	+	+	−	−	F

+, Growth on subculture from inoculated tetrathionate broth
−, No growth on subculture.

when made from the same bottle? A clue was obtained when it was noticed that some batches of A were colourless and others deep green. Brilliant green is incorporated as a dry ingredient instead of being added separately. We considered that there might be unequal concentrations of the dye throughout the bottle and arranged for the brilliant-green concentration to be assayed from different parts. The results are given in Table 6. The concentration of brilliant green that should have been present in A was 11 parts per million.

Another reason for the variable results obtained in this investigation of tetrathionate A was potential variation in the sensitivity of the test strain to tetrathionate. This was discovered accidentally. In the viable count plate of sample 8 (Table 5), both smooth-surfaced and rugose colonies were seen after leaving the plate at room temperature for several days. The rugose character of a salmonella colony undersuch circumstances is well known to us (Harvey & Price, 1974) and has been described in detail by Jamieson (1966). Because a smooth suspension or fluid culture is recommended for media testing (Stokes, 1968), the smooth-surfaced colony on the Miles and Misra plate was selected to prepare a fresh test culture. Three further samples of A were tested against F using the new culture. The results were at variance with Table 5 and are recorded in Table 7. It is evident that in samples 10 and 11, between 600 and 800 organisms were required to initiate growth in F, though this had been accomplished with 3–28 organisms of the old culture.

Table 8. *Tests on medium E in Cardiff and Newcastle laboratories*

Serotype	No. of organisms*	Dilution of culture: 10	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Medium†
		Cardiff laboratory								
S. typhimurium	7	+	+	+	−	−	−	−	.	E
		+	+	+	+	+	+	+	.	F
	6	+	+	+	+	−	−	−	.	E
		+	+	+	+	+	+	−	.	F
S. panama	19	+	+	+	+	−	−	−	.	E
		+	+	+	+	+	+	+	.	F
	20	+	+	+	+	−	−	−	.	E-BG
		+	+	+	+	+	+	+	.	F
	10	+	+	+	−	−	−	−	.	E
		+	+	+	+	+	+	+	.	F
S. montevideo	5	+	+	−	−	−	−	−	.	E
		+	+	+	+	+	+	−	.	F
	17	+	+	−	−	−	−	−	.	E-BG
		+	+	+	+	+	+	+	.	F
		Newcastle laboratory								
S. agona	74	.	.	.	+	−	−	−	−	E
		.	.	.	+	−	−	−	−	E-BG
		.	.	.	+	+	+	+	+	G
S. typhimurium	28	.	.	.	+	+	+	+	+	E
		.	.	.	+	+	−	−	−	E-BG
		.	.	.	+	+	+	+	+	G
S. heidelberg	11	.	.	.	−	−	−	−	−	E
		.	.	.	−	−	−	−	−	E-BG
		.	.	.	+	+	+	+	+	G
S. paratyphi B	2	.	.	.	+	+	+	−	−	E
		.	.	.	+	+	−	−	−	E-BG
		.	.	.	+	+	+	+	+	G
S. muenchen	16	.	.	.	−	+	−	−	−	E
		.	.	.	+	−	−	−	−	E-BG
		.	.	.	+	+	+	+	+	G

* The number of organisms in 0·02 ml. of the 10^{-6} dilution.

† E, the commercial medium; E-BG, the same medium without brilliant green; F, Cardiff Müller–Kauffmann tetrathionate broth; G, Newcastle tetrathionate broth.

Both control and trial media, therefore, inhibited organisms derived from the smooth colonies though to greatly differing degrees. Reversion to the mixture of smooth and rugose colonies once again allowed small numbers (those contained in 0·02 ml. of a 10^{-6} dilution of a broth culture) to multiply in F, but not in A. The descriptions 'smooth' and 'rugose' refer to the appearance of the colony surface only (Plate 1). Both variants produced uniform turbidity in broth.

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Table 9. *Tests on medium E and medium F using naturally infected poultry offal*

Medium positive or negative	No. in category
E+ F+	33
E+ F−	19
E− F+	33
E− F−	283
Total samples	368

$\chi^2 = 3{\cdot}3$. $0{\cdot}10 > P > 0{\cdot}05$. Not significant.

Tetrathionate E

This also performed badly with pure cultures and polluted water. It was re-investigated by the pure culture technique, but in view of our previous results with smooth and rugose variants of our original test strain, recently isolated salmonellas were used for this study. All these had been cultured from tetrathionate and so were unlikely to be oversensitive to it. Use of salmonella strains with previous experience of an enrichment medium is a valid precaution because certain serotypes are more easily recovered from selenite and others from tetrathionate (Harvey & Price, 1974). E is a medium which is widely used and in formula is comparable with F. Yet, in all tests it required a much larger inoculum to initiate growth. It was because of these remarkable differences between E and F that confirmation of the Cardiff findings was sought and found at the Public Health Laboratory, Newcastle. The results from Newcastle are included in Table 8. In both laboratories the omission of brilliant green from E did not improve its performance.

Differences between E and F were finally investigated using naturally infected chicken giblets as an inoculum. Vassiliadis *et al.* (1972) had produced evidence that Muller–Kauffmann tetrathionate broth was not the optimum enrichment medium for salmonella isolation from poultry samples. We wished for further information on this point. After inoculation with the giblets, both tetrathionates were incubated at 43° C only and were subcultured on brilliant-green MacConkey at 24 and 48 hr. The results are given in Table 9, which shows that with naturally infected poultry samples as inocula, the differences between E and F were narrowed and were indeed not statistically significant.

DISCUSSION

The test inoculum, in the results reported here, is shown to be of primary importance among factors influencing quality control tests of media. Small inocula of pure cultures of salmonellas failed completely to differentiate any of the selenite F broths, though inocula of polluted water easily did so (Tables 3, 4). On the other hand, the pure culture technique, which is simple and universally available, was effective in distinguishing, with one discrepancy, the tetrathionate broths though not so incisively as the polluted water technique (Tables 1, 2). The poultry inocula did suggest that tetrathionate F was superior to tetrathionate E for

salmonella isolation, but the superiority was not statistically significant (Table 9). What was only a suggestion with results obtained with naturally infected poultry samples was unequivocally demonstrated by the use of pure cultures. It has been suggested that most strains of salmonellas grow as well in tetrathionate broth as in ordinary broth because the selected bacteria can metabolize the tetrathionate. A few tests with small numbers of salmonellas suspended in urine as diluent convinced us that this was not so with Medium E. Urine was selected for study as tetrathionate broth is recommended as suitable for the recovery of salmonellas from urine (Stokes, 1968).

Two brands of commercial tetrathionate were further examined for possible causes of their inferiority. Chemical analysis of Medium A showed that the concentration of brilliant green varied considerably in different samples taken from the same bottle. The concentration of this ingredient is critical in selenite brilliant green broth when it is incubated at 43° C. and from past experience we have found a concentration of 1 in 10^6 to be suitable (Harvey & Phillips, 1961). There is no reason to suspect that the concentration of dye is less important in tetrathionate broth. Homogeneous distribution of brilliant green in a dry medium is important as was noted by Hobbs, King & Allison (1945) when preparing bismuth sulphite powder 'K'. It might be argued that the variation of the dye would have been less in large batches of medium. This excuse would hardly be acceptable to laboratories only requiring small batches of medium. The variation in the test strain is interesting. Broth cultures from the rugose variant yielded much smaller effective inocula on eight occasions than those from the smooth variant, yet phage-typing established that both variants were *S. typhimurium*, 1 var. 5. The minimum effective inoculum (that required to initiate growth) of the two variants was the same for laboratory prepared selenite F broth (Medium M).

Tetrathionate E is widely used in Europe and in some ways is of greater interest than Medium A. For this reason it was investigated in greater detail (Table 8). Brilliant green is added to E separately from the basic ingredients. Nevertheless, because of the results obtained with A, E was tested with and without brilliant green. Table 8 demonstrates that the presence or absence of dye did not affect the performance of the medium. Both Cardiff and Newcastle laboratories agreed that E would not permit growth of small inocula of pure cultures of some salmonella serotypes. The choice of serotype for the pure culture technique was, however, important. Table 9 records the results obtained with naturally infected poultry samples. The figures provide a contrast to those obtained with pure cultures of salmonellas. The difference between the two media is narrowed and this is thought to be due to the infusion of nutritive substances from the meat inoculum counteracting the inhibitory nature of Medium E. Medium F is thus seen to be a more versatile enrichment broth than E, as it is successful with all three test inocula. As was the case with the dye content of E which was demonstrated in Table 8, the temperature of incubation used for this medium did not affect its performance (Tables 1, 2).

We should like to express our thanks to Dr L. Coles, Glamorgan County Analyst, for the assay of the brilliant-green concentration in Medium A.

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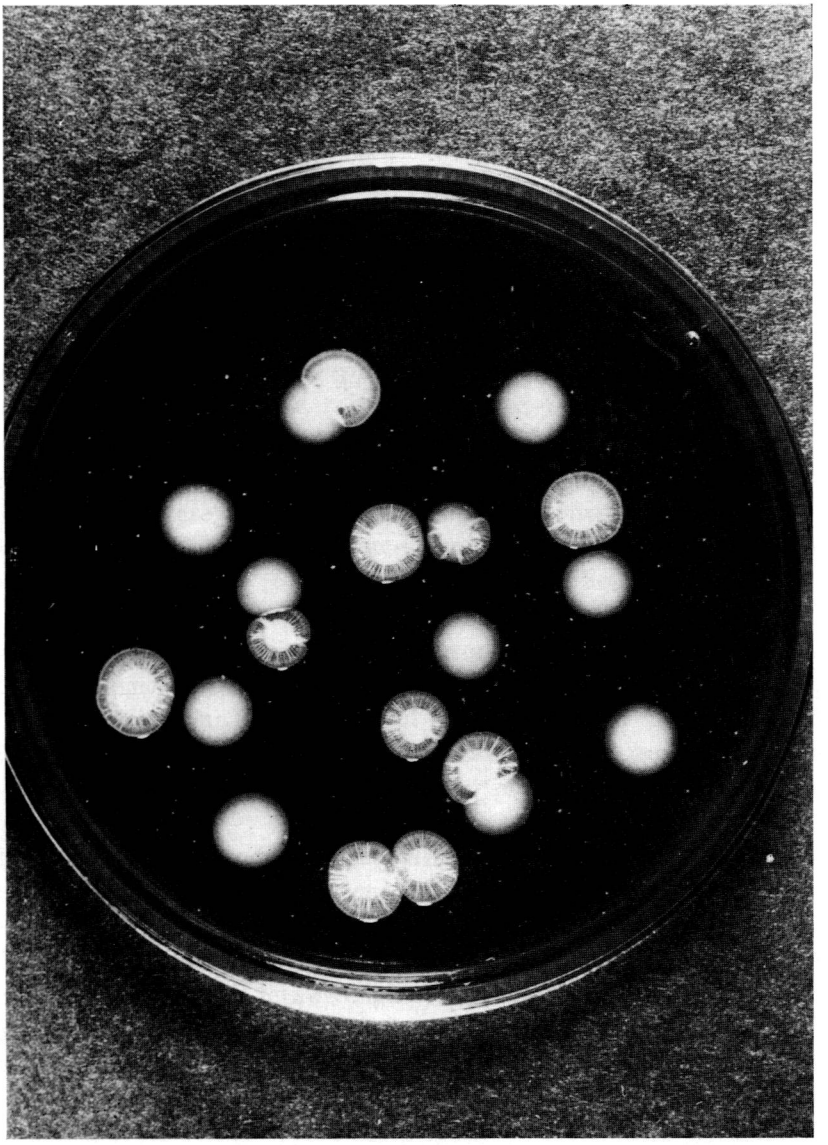
EXPLANATION OF PLATE

Smooth and rugose variants of *S. typhimurium*, phage type 1, var. 5.



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