Further Evaluation of EC Medium for the Isolation of Coliform Bacteria and Escherichia coli*

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AT the St. Louis meeting of the American Public Health Association in October, 1942, the authors had the pleasure of discussing their experience with a new EC medium (buffered tryptose lactose bile salt †) for the isolation of coliform bacteria at 37° C. and of *Escherichia coli* at 45.5° C.[1] In the examination of 147 samples of drinking water of various types, not a single false presumptive was encountered among 1,176 gas tubes while 58.5 per cent of false presumptives were obtained with standard lactose broth. At the same time 14.2 per cent more of the EC tubes were positive for coliforms. The medium was found to have comparable sensitivity to Mallmann and Darby's [2] LST (lauryl sulfate tryptose) medium when tested with 33 stool specimens and 25 samples of raw sewage.

Evaluation of the medium in laboratories other than that of the authors, seemed desirable. However, due to war conditions many laboratories were unable to participate in such an evaluation. Results are presented here from eleven laboratories including that of the authors. Some results from the participating laboratories have had to be excluded because of technical errors.

The completed test for coliform bacteria was used in this study. It is well known that the more steps involved in a routine examination, the greater the number of errors, and it is probable that some of the results presented here are highly inaccurate due to such technical errors. If the short confirmatory procedure of transferring a loopful of culture from the primary gas tube to a secondary tube of brilliant green bile broth had been used, such errors would probably have been largely eliminated and a far better evaluation of the several media might have been obtained. The good results obtained in some laboratories indicate the potential values of these media.

It appears that in actual routine work an unconfirmed presumptive test with either the EC medium or the LST medium is probably much more reliable than the standard completed test. The use of a single primary medium such as EC or LST as a completed test would be valid, however, only if gas resulting from actual fermentation of lactose is recorded. With both these media abundant gas is almost invariably present within 24 hours if positive for any bacteria of the coliform group. Slight traces (less than 1 per cent) of gas in these two media without

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† "EC" refers to *Escherichia coli* though the medium is just as valuable for isolation of coliform bacteria at 37° C. as it is for the isolation of *Esch. coli* at 45.5° C.

effervescence are probably nonspecific gas, for it is well known that such traces of gas may result from various nonspecific causes. It is a routine practice in the authors' laboratory to test for questionable gas by shaking. If definite fermentation of lactose has occurred, effervescent gas will be very obvious. Slight traces of gas have never been found by the authors in either EC medium or LST medium after 24 hours' incubation unless slow lactose fermenters are present. Whenever coliform bacteria have been present, abundant gas has been produced. Slight traces of nonspecific gas without effervescence do occur in some laboratories for the reasons noted. Such traces of nonspecific gas can usually be avoided by suitable technical precautions and their nonspecific nature determined by shaking. Traces of gas, however, should always be confirmed.

In order to show the wide variations in results obtained by the participating laboratories, the results were separately compiled. The results also were compiled according to the type of material under examination. In accordance with our earlier report,[1] very few false presumptive tests were obtained with the EC medium by bacteriologists in five of the participating laboratories (A, B, C, D, and E). Only six, or 0.6 per cent, false presumptives were found among 945 EC gas tubes. It is doubtful, however, if the six so-called false presumptives were really false, as slow-lactose-fermenters were recovered from two similar tubes and possibly were present in these six tubes had they been available for critical reëxamination. The results with the LST medium were also excellent. With the same samples, 654 LST gas tubes were obtained against 650 with the EC medium. There were no false positive EC gas tubes, however, while 15, or 2.3 per cent, were found with the LST medium. False "false presumptives" due to slow-lactose-fermenters, could be expected to be the same with both media. The percentage of false presumptives using standard lactose broth (SLB) varied from 0 to 97.9 per cent and averaged 24.2 per cent.

No evaluation of the lauryl sulfate medium at 45.5° C. was attempted but the EC medium was found highly specific for *Escherichia coli* at this temperature. While a small percentage of false presumptives was encountered with the authors' original modified Eijkman medium,[3] only one, or 0.17 per cent, out of 603 EC gas tubes obtained at 45.5° C. failed to confirm for *Escherichia coli.*

Unfortunately, the results from six other collaborating laboratories (F, G, H, I, J, and K) were not so satisfactory. The percentages of false presumptives with Laboratory "F" were small for both the EC and LST medium and relatively small for SLB. Laboratory "G" found relatively low percentages of false presumptives with all three media and the sensitivity of the EC medium was considerably lower than that of the LST or SLB. Laboratory "H" tested all three media on samples of shellfish and of raw and chlorinated water. It is stated in the report from that laboratory that "the adoption of the LST medium as the standard medium would result in coliform indices somewhat larger than at present with SLB and that the use of the EC medium would effect a sharp reduction in the number of false presumptives." The following data have been presented by that laboratory after they had deleted certain questionable samples.

Medium	*Primary Gas Tubes* *	*Completed Test* *
SLB	9.7	4.6
LST	6.7	4.7
EC	4.7	4.4

* as M.P.N. per sample

These computations obviously were not made from the data as presented in this

report, which had from 11.3 to 24.4 per cent false presumptives for the EC medium and from 6.8 to 24.1 per cent for the LST medium. Those for standard lactose broth varied from 13.8 to 56.0 per cent. Significant data were selected.

Detailed results for each of the participating laboratories have been deleted on account of space. A summary of the results from all of the participating laboratories is given in Table 1.

TABLE 1

Comparative Sensitivity and Specificity of EC, LST and SLB Media for the Isolation of Coliform Bacteria

(Condensed Table)

Material, Samples and Tubes	Medium	37° C. Incubation: Coliforms: Gas Tubes	Coliforms: No. Pos.	Coliforms: Per cent False Presump.	Esch. coli: No. Pos.	45.5° C. Incubation: Medium	Tubes Inoc.	Gas Tubes	Esch. coli	Per cent False Presump.
				Laboratories A, B, C, D, E						
* S 711 T 4880	SLB	1,117	847	24.2	620	ME	1,265	600	588	0.3
S 379 T 2790	EC	945	936	0.6	728	EC	1,265	603	602	0.17
	LST	654	639	2.3	506					
				Laboratories F, G, H, I, J, K						
† S 284 T 2436	SLB	1,509	1,253	15.0	127	ME	198	130	109	16.2
S 259 T 2236	EC	1,390	1,210	13.0	123	EC	198	122	101	17.2
	LST	1,383	1,178	14.8	116					

SLB = Standard Lactose Broth
LST = Lauryl Sulfate Tryptose
ME = Modified Eijkman
* Samples of human feces, shucked oysters, milk, sewage, and various types of water
† Samples of shellfish and various types of water

The reason for the high percentages of false presumptives obtained by some of the collaborating laboratories may be due to reading tiny bubbles of air or nonspecific gas as "positive" when there is no fermentation of lactose. Such traces of gas are apt to be entrapped air or that resulting from dextrose formed by hydrolysis of lactose. Air may be forced into some tubes when running water from pipettes. Dr. Black's report [4] on technical errors in the bacteriological examination of milk indicates how widespread technical errors may be. Technical errors are doubtlessly as widespread in water examinations. The impossibility of evaluating any method unless technical errors can be controlled, is obvious. It is hoped that further work may help to determine the validity of these results and the exact cause of probable technical errors.

CONCLUSIONS

The results indicate to the authors that both the LST medium and the EC medium are highly sensitive and specific media for the isolation of coliform bacteria from water, shellfish, and sewage. The EC medium has also given excellent results for isolating both coliform bacteria at 37° C. and *Escherichia coli* at 45.5° C. from milk. If errors probably due to reading gas resulting from entrapped air, hydrolysis of lactose, and often other factors can be eliminated, positive presumptive tests with both the EC medium and the LST medium have been found to be indicative of the presence of coliform

bacteria in almost 100 per cent of cases. While both the EC and LST medium are highly efficient in the isolation of coliform bacteria, the EC medium is somewhat more specific. A presumptive test reading with either the EC or the LST is obtainable which seems to be far more dependable than the usual "confirmed" or "completed" test result. Both of these media offer rapid tests of great dependability which should be of the utmost importance under war conditions and in peacetime for, when properly performed, they give highly dependable results within 24 or, at most, 48 hours.

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