A COMPARISON OF THE EIJKMAN TEST WITH OTHER TESTS FOR DETERMINING ESCHERICHIA COLI IN SEWAGE

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The routine use of a Modified Eijkman method in the examination of oysters, crabmeat, and other substances has been discussed by the authors (1935). Only three sewage samples were reported, a number obviously insufficient from which to draw any conclusion, especially since these observations were made before certain improvements in the Eijkman method had been applied. Therefore, it was thought desirable to study the method further with samples of raw sewage, effluent and sludge in order to evaluate more accurately the authors' modification of the Eijkman test for this purpose.

Brown and Skinner (1930) have criticized the value of the test based on pure cultures, alone. In the present investigation, the dilution method, using triplicate inoculations for each dilution, has been used to compare the Eijkman test with other tests used for the examination of water in different parts of America and with the Standard lactose broth method.

PROCEDURE

High dilutions of sewage were made in order to insure that the last two or three dilutions would be negative, a necessary step in order to calculate most probable numbers with accuracy. All cultures were incubated at 37°C., for approximately twenty-four and forty-eight hours, except the Eijkman tubes which were incubated at 46°C. Eosin-methylene-blue (E.M.B.) agar plates were streaked from tubes and after twenty-four and forty-eight

hours incubation, one or two typical colonies from each plate were inoculated into citrate, Eijkman and lactose broth for confirmation. Cultures which produced typical colonies on E.M.B. plates and formed gas in lactose and Eijkman broths but which were unable to grow in the citrate medium of Koser (1923) were considered as *Escherichia coli*.

MEDIA USED IN THE COMPARISON

Lactose broth and eosin-methylene-blue agar were prepared according to Standard Methods (1933). The buffered lactose broth was of the formula outlined in the non-standard section.

Salle's crystal violet lactose broth (1930), Dominick-Lauter's brom-cresol-purple, methylene blue, lactose broth (Standard Methods, 7th edition), Ritter's basic fuchsin, lactose broth (1932) and Stark and England's formate ricinoleate broth (1935) were prepared according to the directions of the authors. The formula for the modified Eijkman medium of Perry and Hajna (1935), is, in the final concentration, as follows:

	per cent
Bacto-peptone	1.5
Bacto-dextrose	0.3
K_2HPO_4	0.4
KH_2PO_4	0.15
NaCl	0.5

No titration nor filtration is required. The final pH is 6.9.

RESULTS

The results obtained from the examination of ten samples of raw sewage for *Escherichia coli* are given in table 1. Two hundred and seventy tubes of each medium were inoculated. Salle's (crystal-violet lactose broth) had the smallest number (171) of tubes showing gas fermentation. Three more (174) of the modified Eijkman broth tubes had gas. The greatest number (185) of tubes with gas was obtained with Standard lactose broth. Eijkman broth, however, although with next to the smallest number of tubes showing gas, yielded the greatest number (161) of confirmatory tests for *Escherichia coli*. Salle's medium yielded

less than half as many (80) confirmations. Standard lactose broth yielded almost as many (153) confirmations for *Escherichia coli*, but due to gas production by bacteria other than *Escherichia coli* only 82.8 per cent of the tubes with gas confirmed as compared

TABLE 1

Comparative results, both presumptive and confirmed, for Escherichia coli and coli-aerogenes organisms in raw sewage with various media and with Eijkman broth

Number of Samples Examined—10

	STANDARD LACTOSE	STANDARD LACTOSE PLUS K_2HPO_4	EIJKMAN	RITTER'S	SALLE'S	DOMINICK-LAUTER'S	STARK AND ENGLAND'S
Total tubes inoculated	270	270	270	270	270	270	270
Tubes having gas at 24 and 48 hours	185	181	174	177	171	178	183
(a) Tubes having gas at 24 hours	182	180	170	176	155	176	183
(b) Tubes having gas at 48 hours	3	1	4	1	16	2	0
Total confirmations, coli-aerogenes group	182	180	171	176	171	178	183
(a) Number of 24-hours gas tubes confirmed	181	179	167	176	155	176	183
(b) Number of 48-hour gas tubes confirmed	1	1	4	0	16	2	0
Total confirmations, *Esch. coli*	153	105	161	141	80	155	148
(a) Number of 24-hour gas tubes confirmed	152	105	157	141	76	155	148
(b) Number of 48-hour gas tubes confirmed	1	0	4	0	4	0	0
Per cent of tubes with fermentation at 24 and 48 hours	68.5	67.0	63.3	65.5	63.3	65.9	67.8
Per cent of inoculated tubes confirmed for *Esch. coli*	56.7	39.0	59.6	52.2	29.6	57.4	54.9
Per cent of gas tubes confirmed for *Esch. coli*	82.8	58.0	92.5	79.8	46.8	87.0	81.0

with 92.5 per cent of the positive Eijkman tubes. These results indicate that the modified Eijkman method is superior to the other methods for the isolation of *Escherichia coli*.

In the broths containing bacteriostatic dyes, there seems to be an inhibiting effect upon *Escherichia coli*. Brom-cresol-purple

methylene-blue broth seems to be the best of the group. The failure of crystal-violet lactose and buffered lactose broths to detect greater numbers of *Escherichia coli* seems to lie in the buffering action of the two media, which favors the *Aerobacter aerogenes* and other members of the colon group over *Escherichia coli* at 37°C.

TABLE 2

Comparative results, both presumptive and confirmed, for Escherichia coli and coli-aerogenes organisms in Eijkman medium and in standard lactose broth

	20 SAMPLES OF RAW SEWAGE		4 SAMPLES OF EFFLUENT		10 SAMPLES OF SLUDGE	
	L	E	L	E	L	E
Total tubes inoculated	624	624	102	102	270	270
Tubes having gas at 24 and 48 hours	370	395	78	45	165	142
(a) Tubes having gas at 24 hours	362	373	66	38	144	121
(b) Tubes having gas at 48 hours	8	22	12	7	21	21
Total confirmations, coli-aerogenes group	365	389	78	39	145	119
(a) Number of 24-hour gas tubes confirmed	360	369	66	37	141	118
(b) Number of 48-hour gas tubes confirmed	5	20	12	2	4	1
Total confirmations, *Escherichia coli*	320	373	16	28	129	117
(a) Number of 24-hour gas tubes confirmed	316	356	13	28	128	117
(b) Number of 48-hour gas tubes confirmed	4	17	3	0	1	0
Per cent of tubes with fermentation at 24 and 48 hours	59.3	63.3	76.5	44.0	61.2	52.6
Per cent of inoculated tubes confirmed for *Escherichia coli*	51.3	59.8	15.7	27.5	47.8	43.3
Per cent of gas tubes confirmed for *Escherichia coli*	87.8	95.8	20.5	62.3	78.3	82.4

Dominick-Lauter's, Stark and England's and Salle's media gave no false presumptives. Ritter's medium and buffered lactose broth each had one false presumptive.

A further comparison of results obtained with twenty samples of raw sewage, four of sewage effluent and ten of sludge, by Standard lactose broth and Eijkman medium is given in table 2.

Of 624 tubes of lactose broth and a like number of Eijkman broth tubes inoculated with raw sewage, 395 or 63.3 per cent of the Eijkman tubes and 370 or 59.3 per cent of the lactose tubes showed gas. It is interesting to note that 95.8 per cent of the positive Eijkman tubes were confirmed for *Escherichia coli* as compared with 87.8 per cent of the lactose broth tubes, thus demonstrating not only the greater efficiency of the Eijkman test for isolation of *Escherichia coli* but also demonstrating that, in these samples of raw sewage, more tubes (373) of the Eijkman broth were confirmed for *Escherichia coli* than could be confirmed (365) for the entire colon group by Standard lactose broth. It is important that this predominance of *Escherichia coli* in raw sewage be carefully considered for it is of the utmost significance in considering the relative merits of *Escherichia coli* as opposed to the use of the entire group as an index of fecal pollution.

Far less of the tubes of both lactose and Eijkman broth inoculated with sewage effluent in which gas fermentation occurred could be confirmed for *Escherichia coli* than in the case of those inoculated with raw sewage. Only 20.5 per cent of the positive lactose broth tubes could be confirmed against 62.3 per cent of the Eijkman tubes. It is interesting to note the ratio of *Escherichia coli* (Eijkman method) to the colon group (Standard lactose broth) for raw sewage and for sewage effluent, which in raw sewage was 1.03 to 1.0 while for sewage effluent it was 1 to 2.8. *Escherichia coli* was found to predominate therefore, in raw sewage but other members of the colon group either outgrew or outlived *Escherichia coli* in the effluent. In sewage sludge *Escherichia coli* tends, apparently, to regain its original relative position for the ratio was found to be approximately 1 to 1.2. More tubes (129 vs. 117) of the lactose broth than of the Eijkman broth inoculated with sewage sludge were confirmed for *Escherichia coli*. The percent of positive gas tubes, however, which confirmed was greater for the Eijkman method (78.3 vs. 82.4). The numbers of samples of both sewage effluent and sludge were small (4 and 10 respectively).

SUMMARY

Certain media which have been proposed as possible substitutes for Standard lactose broth have been compared with the authors' modified Eijkman medium for relative efficiency in the isolation of *Escherichia coli* from raw sewage. The Eijkman test was found superior to Standard lactose broth, buffered lactose broth, Ritter's, Salle's, Dominick-Lauter's, and Stark's and England's broths both in the number of isolations of *Escherichia coli* made and in the number of gas tubes confirmed for *Escherichia coli.*

The relative efficiency of Standard lactose broth and the modified Eijkman broth of the authors for the isolation of *Escherichia coli* from raw sewage, sewage effluent and sludge was tested. *Escherichia coli* was recovered from more of the tubes of Eijkman broth than of lactose except in the case of sewage sludge. Four and a half per cent more of the lactose broth tubes inoculated with sludge confirmed for *Escherichia coli.* A much larger percentage of the Eijkman tubes with gas confirmed in all instances.

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