

# Routine Use of a Modified Eijkman Medium in the Examination of Oysters, Crabmeat, and Other Substances

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THE Eijkman test for *Bacillus coli* has been used and studied in the laboratories of the Bureau of Bacteriology of the Maryland State Department of Health since 1929. One of the authors (Perry, 1929), first applied the test to a number of cultures of *Bacillus coli* recovered from oysters and oyster bearing waters. He found "such cultures invariably produce indol from a suitable medium and were able to ferment the dextrose of Eijkman broth with gas at 46° C." He also found that "only 11.2 per cent of 223 cultures of lactose-fermenters (coli-aerogenes group) from oyster and water samples from four areas were *Bacillus coli*. The four areas differed geographically, topographically, and in respect to their sanitary features. The presence of *Bacillus coli* was found in close agreement with probable fecal pollution."

In Maryland, a systematic study of oyster producing waters and the oysters themselves has been in progress since 1925. It became obvious from these studies that an evaluation of the coli-aerogenes group content of oysters is of little, if any, practical value (Perry, 1928). While considerable difference of opinion exists among bacteriologists and others whether *B. coli* or the coli-aerogenes group should be used as an index of pollution in drinking waters, in the field of fecal pollution in oysters and

oyster waters the coli-aerogenes group has been found unsatisfactory as it gives information of little if any value for oysters and of comparatively less value than *B. coli* for oyster bearing waters. For the past year (1934), determinations of the *B. coli* content (score and probable number) of oysters using the modified Eijkman medium of Perry and Hajna\* (1933), and standard lactose broth (American Public Health Association) have replaced those of the coli-aerogenes group. As yet, no attempt has been made to correlate these data with pollution. Most of the samples have been taken from waters free of any significant fecal pollution and the *B. coli* scores of the oysters usually have been very low. While the coli-aerogenes group has been much more satisfactory as an index of possible fecal pollution of oyster waters than of oysters themselves, it has not been entirely satisfactory even for oyster waters. The coli-aerogenes group content of many waters of unquestionable purity, from the standpoint of growing oysters, has

\* Modified Eijkman Medium †

Dextrose . . . . .	3 grams
Peptone (Difco) . . . . .	15 grams
K <sub>2</sub> HPO <sub>4</sub> . . . . .	4 grams
KH <sub>2</sub> PO <sub>4</sub> . . . . .	1.5 grams
NaCl . . . . .	5 grams
Dist. water . . . . .	1,000 c.c.

† If inoculum is by loop or needle, use formula as given. If by pipette, adjust so that final concentration with inoculum will be given.

been far in excess of the amount permitted by the Drinking Water Standards of the U. S. Public Health Service. This experience indicates that *B. coli* would be a much better index of fecal pollution for both oysters and oyster waters than the coli-aerogenes group. The writers also believe that *B. coli* would be just as superior to the coli-aerogenes group as an index of possible fecal pollution when applied to drinking waters.

The Eijkman test has, we believe, many practical advantages as a presumptive test for *B. coli* in oysters, water, crabmeat, and other substances. These advantages have been given by Leiter (1929) and others. That the original Eijkman medium (1904) was not entirely satisfactory has already been pointed out by the authors (1933). More *B. coli* was recovered from fresh human feces using the modified Eijkman medium referred to than could be recovered with lactose broth. In this paper, data are presented on the practical application of this test to samples of oysters, water, crabmeat, etc.

#### METHOD OF EXAMINING OYSTERS, ETC.

The method of examination was as follows: Five tubes of each medium (modified Eijkman medium and Standard lactose broth) were inoculated with like amounts of water, oyster shell liquor, etc. In the case of oysters, 5 tubes were inoculated with 1 c.c. each of oyster shell liquor and 5 with 0.1 c.c. (1 c.c. of a 1 in 10 dilution). In most water samples, 5 tubes were inoculated with 10 c.c. each, and 5 with 1 c.c. each. The amounts of other substances used varied according to experience but like amounts were used for both media except where noted. Lactose broth tubes were examined according to Standard Methods of Water Analysis of the American Public Health Association while the method used for the Eijkman broth tubes was that described by the authors (1933).

#### COMPARATIVE NUMBER OF TUBES AND SAMPLES OF OYSTERS, CRABMEAT, AND WATER IN WHICH FERMENTATION AND CONFIRMATION FOR BACILLUS COLI AND COLI-AEROGENES GROUP OCCURRED FOR EIJKMAN AND LACTOSE BROTH

In Table I comparative data are given for the number of tubes of Eijkman and lactose broth, inoculated with equal quantities of shell liquor from oysters, crabmeat, and water, in which fermentation and confirmation for *B. coli* and other coli-aerogenes organisms was made. On the basis of these data, the following deductions may be made.

The modified Eijkman medium is more selective and efficient for the isolation of *B. coli* than lactose broth. Approximately 6.4 per cent of the lactose broth tubes inoculated were confirmed for *B. coli* while 8.9 per cent of the inoculated Eijkman tubes were confirmed for *B. coli* in spite of the fact that nearly twice as many of the lactose broth as of the Eijkman broth tubes showed gas fermentation. Over twice as many of the Eijkman tubes as of the lactose broth tubes inoculated from oysters and crabmeat were confirmed for *B. coli* while a slightly larger per cent of the standard lactose broth tubes inoculated from oyster waters were confirmed for *B. coli*. Of the lactose broth tubes with gas 18.7 per cent were confirmed while 54.3 per cent of the Eijkman tubes were confirmed.

Since the Eijkman test has been designed to eliminate members of the coli-aerogenes group not *B. coli* it should be expected that many less confirmations for this group would be made from the Eijkman broth tubes. Accordingly, 27.6 per cent of the lactose broth tubes were confirmed for members of the coli-aerogenes group against 14.1 per cent of the Eijkman tubes. These figures include *B. coli* of course. The percentage of non-*B. coli* members of the coli-aerogenes group are 21.2 for

TABLE I  
COMPARATIVE RESULTS, BOTH PRESUMPTIVE AND CONFIRMED, FOR *Bacillus coli* AND COLI-AEROGENES ORGANISMS IN EIJKMAN MEDIUM AND STANDARD LACTOSE BROTH FOR OYSTERS, OYSTER WATERS, AND CRABMEAT

	Oysters (shucked) 182 Samples		Oyster Water 344 Samples		Crabmeat * 229 Samples		Crabmeat † 78 Samples	
	Lactose Broth	Eijkman Broth	Lactose Broth	Eijkman Broth	Lactose Broth	Eijkman Broth	Lactose Broth	Eijkman Broth
	Total tubes inoculated.....	2,195	2,195	3,239	3,233	891	2,170	801
Tubes having gas at 24 and 48 hr.....	1,068	422	853	406	288	425	237	126
(a) Tubes having gas at 24 hr.....	451	176	375	191	198	328	151	89
(b) Tubes having gas at 48 hr.....	617	246	478	215	90	97	86	37
Total confirmations, coli-aerogenes group...	890	366	637	352	243	363	196	105
(a) Number of 24 hr. gas tubes confirmed	374	135	338	167	181	301	134	82
(b) Number of 48 hr. gas tubes confirmed	516	231	299	185	62	62	62	23
Total confirmations, <i>Bacillus coli</i> .....	86	204	284	213	50	252	37	80
(a) Number of 24 hr. gas tubes confirmed	42	62	157	141	44	225	31	70
(b) Number of 48 hr. gas tubes confirmed	44	142	127	72	6	27	6	10
Per cent of tubes with fermentation at 24 and 48 hr. . . . .	48.7	19.2	26.3	12.6	32.3	19.6	29.6	15.7
Per cent of inoculated tubes confirmed for <i>Bacillus coli</i> . . . . .	3.9	9.3	8.79	6.59	5.6	11.6	4.6	10.0
Per cent of gas tubes confirmed for <i>Bacillus</i> <i>coli</i> . . . . .	8.1	48.3	33.3	51.9	17.7	59.2	15.6	63.5

\* Determinations made on 88 samples in lactose and 229 in Eijkman broth

† Determinations made on same samples for lactose and Eijkman broth

lactose broth and 5.2 per cent for Eijkman broth.

Gas was present in about twice as many (34.4 per cent) lactose broth as Eijkman broth tubes (16.4 per cent). Of standard lactose broth tubes inoculated with liquor from shucked oysters, gas was present in 48.7 per cent against 19.2 per cent of the Eijkman tubes. Of the oyster water samples, 26.3 per cent were found to produce gas in standard lactose broth against 12.6 per cent in Eijkman broth, while gas was present in 31.0 per cent of the standard lactose broth tubes inoculated from crabmeat against 18.6 per cent of the Eijkman broth tubes. In routine laboratory work, it is not always possible to read gas production at exactly 24 and 48 hours. In many instances the 24 hours readings represent only 18 hours of incubation. If a full 24 hours incubation had been made, a much larger per cent of the Eijkman tubes doubtless would have been confirmed for *B. coli* and a much smaller residual number, consequently, would have been confirmed after 48 hours of incubation.

From these data, it is obvious that, using Eijkman rather than lactose

broth, a bacteriologist has only one-third the number of presumptive tests for *B. coli* to confirm. There is less work involved in confirming presumptive Eijkman tubes than presumptive lactose broth tubes, for on plates made from Eijkman tubes, *B. coli* is often the only organism present while on plates made from lactose broth tubes several organisms of the coli-aerogenes group are frequently present. This necessitates increased effort to select colonies of *B. coli* and to secure pure cultures. It is much more difficult to secure well isolated colonies from lactose broth tubes than from Eijkman broth. Only occasionally are plates, made from Eijkman broth tubes, overgrown. There is, therefore, less than half the work involved in using lactose broth for the routine isolation of *B. coli*.

PROBABLE NUMBERS OF *BACILLUS COLI* AND COLI-AEROGENES ORGANISMS FOUND IN OYSTERS, WATER, CRABMEAT, ETC., USING EIJKMAN BROTH AND STANDARD LACTOSE BROTH

The probable numbers\* of *B. coli*

\* Tables for Rapid Interpretation of Fermentative Tube Results, M. H. McCrady, *Pub. Health J.*, IX, 5 (May), 1918.

and coli-aerogenes group organisms in oysters, crabmeat, oyster waters, milk, ice, sewage, and eggs were determined. These data are presented in Table II. *B. coli* was identified in the same manner as already described. In the last column of Table II are given the ratios of the probable numbers of *B. coli* as determined using Eijkman broth to the numbers as determined using standard lactose broth and in proportion to coli-aerogenes organisms as determined using the standard procedure of the American Public Health Association. While these ratios indicate that the Eijkman method, as used, was only half as efficient in detecting the probable numbers of *B. coli* from oysters, salt water from oyster areas, and milk as standard lactose broth, this is not really true. Much of the data prepared in Table II was collected before certain refinements in the Eijkman test were used. It will be observed, for instance, from Table I, that over twice as much *B. coli* was isolated from oysters using Eijkman broth as in using lactose broth. In our earlier work on fresh human feces (1933 results confirmed by recent work) more *B. coli* was isolated using Eijkman broth than by using standard lactose broth. The number of samples of ice, eggs, and sewage is too small to be of much significance, especially since these observations like-

wise were made before improvements in the Eijkman method had been applied.

A most significant point is the tremendous difference between the average number of *B. coli* in 83 samples of oysters and the average number of coli-aerogenes group organisms. With coli-aerogenes organisms outnumbering *B. coli* 50 to 100 times (depending on the method), comparatively small variations in the probable numbers of *B. coli* as determined by the two methods becomes of comparatively less significance. In oyster waters, the ratio of coli-aerogenes organisms to *B. coli* was 3 to 5 times as great. This is what those with experience in this type of work would expect. In crabmeat 4 to 7 times as many organisms of the coli-aerogenes group as *B. coli* were found.

To the writers, these data signify the tremendous error in trying to evaluate fecal contamination of oysters, in particular, on the basis of the coli-aerogenes group and indicate quite clearly the desirability of using *B. coli* generally as an index of fecal pollution.

#### SUMMARY AND DISCUSSION

A modified Eijkman medium with incubation at approximately 46° C. has been used for the routine examination of samples of oysters, crabmeat, salt water in which oysters grow, sewage, and other substances.

TABLE II

AVERAGE PROBABLE NUMBER OF *Bacillus coli* RECOVERED FROM OYSTERS, ETC., BY EIJKMAN BROTH AND LACTOSE BROTH AS COMPARED WITH THE AVERAGE NUMBER OF COLI-AEROGENES ORGANISMS FROM LACTOSE BROTH

Substances	Number of Samples	<i>Bacillus coli</i> *		Coli-aerogenes * Lactose Broth (c)	Ratio a-b-c
		Eijkman Broth (a)	Lactose Broth (b)		
Oysters . . . . .	83	3.5	9.1	362	1-2.6-103.2
Oyster waters (salt) . . . . .	229	5.2	9.4	28.8	1-1.8-5.5
Water, fresh (heavily polluted) . . . . .	11	431,634	107,582	1,109,514	1-0.25-2.6
Ice . . . . .	6	270.8	1,483.0	1,512.5	1-5.5-5.6
Sewage . . . . .	3	68,333.3	93,333.3	93,333.3	1-1.4-1.4
Eggs . . . . .	2	14,000+	7,700+	14,000+	1-0.55-1
Milk . . . . .	15	49.3	144.7	369.3	1-2.4-7.5
Crabmeat . . . . .	135	628+	418+	2,741+	1-0.67-4.3

\* Probable Numbers per 100 gm.

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The presumptive and confirmed tests for *B. coli* (so-called fecal type) and organisms of the coli-aerogenes group were made on 182 samples of shucked oysters, 344 samples of salt water from oyster areas, and 307 samples of crabmeat. In this work the term "presumptive" refers to any amount of gas produced in either lactose broth or dextrose (Eijkman) broth. In regard to the shucked oyster samples, approximately 3.9 per cent of 2,195 inoculated lactose broth tubes confirmed for *B. coli* against 9.3 per cent of the same number of tubes of Eijkman medium. Of 3,239 and 3,233 tubes of lactose and Eijkman broth respectively inoculated with salt water from oyster areas a little over 2 per cent more of the lactose broth tubes than the Eijkman broth tubes (8.79 and 6.59 per cent respectively) confirmed for *B. coli*. Of 166 samples of crabmeat inoculated into lactose broth 5.1 per cent of the tubes were confirmed for *B. coli* against 11.2 per cent of 307 samples inoculated into Eijkman broth. On the whole, gas was present in less than half as many Eijkman tubes as lactose broth yet more (2.5 per cent) *B. coli* was recovered from the Eijkman broth tubes than from lactose broth tubes, thus establishing the superiority of the Eijkman method under the circumstances noted.

In another series of samples the probable numbers of *B. coli* isolated from Eijkman broth and lactose broth were compared with the numbers of organisms of the coli-aerogenes group. The data given in Table II were collected in the earlier part of this study when an incubator temperature of approximately 46° C. was used. The variations are believed to be due to a different bacterial flora in these samples which tend to suppress *B. coli* in the Eijkman medium. On latter samples a tube temperature of 46° C. rather

than an incubator temperature of 46° C. was used and more *B. coli* was then recovered apparently, by the Eijkman test than by lactose broth. Such a tube temperature was used in some of the 182 samples of shucked oysters in Table I. In 135 samples of crabmeat and 2 samples of eggs the estimated probable numbers of *B. coli* isolated by the Eijkman method were nearly twice those isolated from lactose broth, while in 11 samples of heavily polluted water 4 times more *B. coli* was isolated from Eijkman broth than lactose broth. In 83 samples of oysters, 6 of ice, 3 of sewage, and 15 of milk greater numbers of *B. coli* were isolated from lactose broth tubes; in oysters (shell) 2.6 times as much, in salt waters from oyster areas 1.8 times as much, in ice 5.5 times as much, in sewage 1.4 times as much and in eggs 2.4 times as much.

The opinion of the authors from their rather extensive experience, is that the Eijkman test, properly adjusted, is a valuable presumptive test for *B. coli* which organism they believe should be the one generally used as an index of fecal contamination of oysters, crabmeat, water, and other substances.

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