EXPERIENCE WITH LACTOSE-BILE MEDIUM FOR THE DETECTION OF *B. COLI* IN WATER.

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THE discovery of *B. coli* in 1885 by Escherich, coupled with the knowledge that it is a normal inhabitant of the intestine of man and many animals, and therefore is found in sewage and polluted water, has induced water-bacteriologists to pay more attention to its isolation than to any other part of their work. Various methods have been proposed and tried for the detection of this organism in water. The one considered most reliable and meeting with general approval has involved the planting of a portion of the sample, either directly or after revivifying in broth, into plates of lactose-litmus agar; and then putting those colonies developing *B. coli* characteristics through the different tests as recommended for the identification of this organism. This procedure although accurate, is at the best a slow, tedious, and laborious process. Moreover, it is impracticable when used by the supervisor of a public water-supply, whose duty it is to report on the daily sanitary condition of the water. For example, if two days are required for the water to reach the inhabitants of the city, and it takes seven days to learn the results by this process, the consumer will have used the water five days before he is informed of the danger.

To obviate these difficulties, various rapid methods have been proposed and used by different water-analysists. Nearly all are based on the fact that *B. coli*, when grown in a fermentable sugar, generates a certain amount of gas of which a definite proportion is carbon dioxid. In many cases certain chemicals whose function it is to inhibit the growth of the water bacteria have been added to the sugar solution. The most common is phenol, either alone or in combination with hydrochloric acid. McConkey has recommended the addition of sodium taurocholate to agar; others have used this compound in Smith solution. It would seem that these different methods, all based on the same general principles, ought to give something like concordant results. But unfortunately this is not the case and they

do not check one another even within reasonable limits. The use of phenol has called forth considerable controversy as to its merits. In this laboratory, where it has been used more or less in an experimental way, it is the experience of the author, that when used to test sewage or badly polluted waters, it gives very satisfactory results, but when used to test a questionable water or one containing few *B. coli* or *B. coli* in an attenuated condition, it generally fails to give the positive test. In a case like this Smith solution alone is preferable. It is very evident that a method requiring a previous knowledge of the character of the water for its use is of very little value.

On the other hand, many bacteriologists, recognizing the uncertain value of phenol in media, prefer to use Smith solution without the addition of any reagent having an inhibitory effect. With the use of Smith solution the conditions are just reversed. And in this laboratory, where it has been used since its establishment in 1903, it has been found very satisfactory with good or questionable waters, but with sewage of badly polluted streams, it often completely fails. Frequently with sewages, the fermentation tubes show no gas production whatever, even when inoculated with very small quantities of the sewage.

As a solution of the problem attending the sanitary examination of water, Mr. Daniel D. Jackson,[1] of Mt. Prospect Laboratory, Brooklyn, N. Y., has proposed the use of lactose-bile medium for the fermentation test. This medium consists of undiluted liquid ox bile sterilized when freshly drawn and containing 10 grams of lactose to the liter of bile. The medium has been used in this laboratory for the last five months in conjunction with the presumptive test. In all 1,076 samples have been examined by both methods, and, as far as possible, waters of a varied character have been used ranging from sewages to waters whose purity is beyond question. Not only have comparisons been made as to the relative value of the two methods, but waters, whose sanitary condition is known by actual inspection of the local conditions have been examined with lactose-bile in view of ascertaining how far the results conform to the character of the water.

Aside from the fact that the presumptive test is unreliable in the examination of polluted waters, there is another factor which tends

[1] *Biological Studies by the Pupils of William Thompson Sedgwick*, University of Chicago Press, 1906.

to throw discredit on its results. Reference is now made to the large number of anomalies which this method furnishes. For example: if fermentation tubes of Smith solution are inoculated with 0.1, 1.0, and 10.0 c.c. portions of the sample, frequently a positive test is obtained in the 0.1 but not in the 1.0 and 10.0 c.c. tubes. And, what is still more puzzling, occasionally, the sample gives a test in the 0.1 and 10.0 c.c. inoculations, but not in the 1.0 c.c. With bile medium, while occasional anomalies have been found, there has been a great reduction, as may be seen from the following data: Of 1,063 samples, duplicate analyses were made in lactose-bile and Smith solution. In the 1.0 c.c. portions 2.73 per cent gave anomalies by the former and 8.00 per cent by the latter method. With the 10.0 c.c. portions, the bile medium gave 4.73 per cent against 13.73 per cent anomalies by Smith. In both cases, the reduction in anomalies was approximately two-thirds.

With the use of the presumptive test for *B. coli*, it has been the practice to consider the test positive, when the total gas production was 25–70 per cent of total volume, and the carbon dioxid 25–40 per cent of this amount. Whipple, Irons, and other authorities agree that the percentage of carbon dioxid should be about 33 per cent of total gas evolved. Experiments in this laboratory, with pure cultures of *B. coli* in fermentation tubes of Smith solution and lactose-bile show that with the former there is a wide range in the percentages of carbon dioxid found. Nineteen inoculations in Smith solution gave an average of 27 per cent carbon dioxid. With the lactose-bile, the results obtained were more uniform, and the average percentage of carbon dioxid obtained was appreciably higher, being 37 per cent. In actual experience, with tests on polluted streams, it has been found that the percentage of carbon dioxid as found by lactose-bile medium has been about 39 against 30 with Smith. Table 1 illustrates this point very clearly.

In this table, the 1–1,000 and 1–100 dilutions are samples of sewages and street wash from which *B. coli* have been repeatedly isolated. As other examples to illustrate the differences in the percentages of carbon dioxid as found by the two methods, tests were made on two badly polluted streams. The first receives sink drainage and street wash; the second, barnyard drainage and street wash. Twenty

TABLE 1.

Dilution	Smith Solution		Lactose-Bile Solution	
	No. Sample Examined	Per Cent CO_2	No. Samples Examined	Per Cent CO.
1–1,000	9	30.00	14	39.17
1–100	14	27.40	26	39.62
1–10	352	30.51	214	39.93
1	526	29.29	430	39.65
10	649	29.71	618	38.89

samples from each stream were analyzed, and with the first, the average percentage of carbon dioxid (including 0.1, 1.0, and 10.0 c.c. portions) was 29.24 by Smith against 40.09 by lactose-bile. With the second stream, averages of 28.96 and 39.20 per cent carbon dioxid were found by tests with Smith and lactose-bile solutions, respectively.

In the second portion of this paper it is the object, first, to show the relative value of the two methods when used on different classes of waters; and second, and more important, to show how the results obtained by the lactose-bile method compare with the known facts ascertained by inspection of the local conditions in which the waters under consideration are found.

For this purpose, series representing polluted and unpolluted waters have been selected and arranged according to their sanitary qualities as shown in Table 2, and the average percentage of samples giving positive tests for *B. coli* with Smith and lactose-bile solutions recorded in the subsequent columns. For the wells, these figures are an average of 50 analyses; for the surface waters and sewage, an average of about 20 analyses. Samples numbered 1 are waters from deep, driven wells not open at the top; those numbered 2 are waters from shallow, open, dug wells. Waters numbered 3, 4, 5, 6, 7, and 8 are from lakes situated in sparsely inhabited regions where there is little danger of pollution. The next group of waters (Nos. 9. 10, and 11) are more or less polluted. No. 12 is a badly polluted stream; No. 13 principally a mixture of street wash and household drainage; No. 14 is a septic sewage.

An examination of Table 2 shows that, with the first eight waters, there is very little difference in the figures obtained by either method. With well waters, it is extremely rare that *B. coli* is found in

the 10 c.c., and it has never been found in the 0.1 and 1.0 c.c. portions although large numbers of these waters have been examined with lactose-bile medium. With the waters from the lakes, which are situated in sparsely inhabited regions, the small amounts of coli are due, no doubt, to the effect of small animals. The results in all these samples indicate waters of an excellent quality.

With the next three waters, which are more or less polluted, the percentages of positive tests with the bile medium are appreciably higher than those found with the Smith solution. The results with the lactose-bile would seem to be more in accordance with the facts. With this group of waters, at times *B. coli* can be found and at others it cannot be detected with Wurtz agar.

If, from the preceeding results, there is any question as to the relative value of the two methods, or as to the value of the lactose-bile method when used for the detection of *B. coli* in polluted waters, it is removed after examining the results which samples Nos. 12, 13, and 14 furnish. In every case analyses by the lactose-bile method gave decidedly higher results than by the Smith; and in the 1.0 and 10.0 c.c. portions the percentages of positive tests were close to 100. With the sewage, while there was an occasional negative result, about 90 per cent of the tests were positive with the bile against only 25 per cent with the Smith. With the three samples, now under

TABLE 2.

No.	Source	Percentage of Samples Giving Positive Smith Solution			Tests for *B. coli* by Lactose-Bile Medium		
		0.1 c.c.	1.0 c.c.	10.0 c.c.	0.1 c.c.	1.0 c.c.	10.0 c.c.
1	Deep wells	0.	0.	0.	0.	0.	0.
2	Shallow wells	0.	1.0	10.0	0.	0.	6.0
3	Lake	15.0	10.0	15.0	5.0	0.	15.0
4	Lake	5.2	15.7	21.0	5.2	5.2	31.0
5	Lake	10.0	5.0	40.0	10.0	5.0	15.0
6	Lake	10.0	10.5	26.0	10.0	10.5	50.0
7	Lake	0.	10.0	5.0	00.	0.0	15.0
8	Lake	10.0	15.0	35.0	0.	5.0	30.0
	Average, Nos. 3, 4, 5, 6, 7, and 8	8.3	11.0	23.6	5.0	4.3	26.0
9	River	47.0	72.2	55.5	50.0	75.0	84.2
10	River	26.3	37.6	73.7	30.0	90.0	85.0
11	River	36.8	55.1	68.9	40.0	73.5	78.1
	Average, Nos. 9, 10, 11	36.7	55.1	66.0	40.0	79.4	82.4
12	Brook	47.7	63.2	72.2	60.0	90.0	84.2
13	Drainage	50.0	73.7	78.9	84.2	90.0	90.0
14	Sewage	25.0	25.0	8.2	87.5	93.7	81.2
	Average, Nos. 12, 13, 14	40.9	53.9	53.1	77.2	91.2	85.1

consideration, the averages of the positive tests obtained by the two methods show that the bile gave nearly twice as many positive tests as the Smith. These three samples give results corresponding very closely to what one would expect and what one should find in the examination of polluted waters.

SUMMARY.

The presumptive test, using lactose-bile medium, is a rapid and practical method for the detection of *B. coli.* If the absorptions are omitted, as the data obtained from experiments seem to show that they may be without detracting from the value of the test, the routine work is generally lessened.

The anomalies obtained by this method are few and much less than when Smith solution is employed.

The percentage of carbon dioxid is decidedly higher when lactose-bile is used for testing waters than when Smith solution is employed. This fact corresponds with the results obtained by experiments with pure cultures of *B. coli* in lactose-bile and Smith solutions.

Both methods give substantially the same results when used for testing "safe" waters, and the results are consistent with the facts learned by observation. With questionable or contaminated waters, the percentage of positive tests obtained with the use of lactose-bile media are appreciably higher than those obtained with the use of Smith solution. With sewages or badly polluted waters, lactose-bile medium gives a decidedly greater number of positive tests than the Smith solution.

For general convenience in the routine work of a laboratory, for practicability in the examination of public water-supplies, and for reliability in testing waters of a varied character, the fermentation test, using lactose-bile medium, is the most promising method that has yet been advanced.