

THE INTERPRETATION OF TESTS FOR *B. COLI COMMUNIS*.*

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(From the Water Purification Works, Columbus, Ohio.)

The presence of *B. coli communis* in water is assumed to indicate the presence of sewage pollution and typhoid germs.

Several species of bacteria resemble the colon bacillus, and if we are going to regard the presence of *B. coli communis* as the criterion of pollution, it is important to have an accurate method for its identification, and, as the operation of water purification works is modified as the numbers of *B. coli* increase or decrease it is also important that the method be rapid.

At the present time the fermentation of sugar solutions offers the readiest means for identification. This method has been used for years, but some new suggestions made by Mr. D. D. Jackson¹ in regard to the use of dulcitol, raffinose, and mannitol looked so promising that a series of comparative tests in various media have been carried out at the Columbus Water Purification Works. Tests were made on many duplicate cultures of bacteria isolated from the river and purified water at the plant, and upon duplicate cultures obtained from nine different laboratories. Summaries of these results will be given in the following pages.

In our daily routine analysis of the river and filtered water for the presence of *B. coli* it has been our custom to transplant cultures from an agar slant into lactose bile, and if gas develops the culture is then transplanted into milk, nitrate solution, Dunham's peptone solution for indol, and gelatin (as outlined in "Standard Methods"). We found that, out of a total of 3,000 cultures, about 65 per cent of the lactose-bile fermenting organisms gave positive tests in these media; 28 per cent failed to produce indol; 5 per cent did not reduce nitrate; and there were some that liquefied gelatin.

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¹ *Jour. Infect. Dis.*, 1911, 8, p. 241.

At the end of each month for two or three months, a number of cultures were chosen from those tested during the month. These cultures were purified and revived according to the standard method and were transplanted in duplicate into lactose bile, dextrose broth, saccharose broth, dulcete broth, Dunham's solution for indol, nitrate solution, gelatin, milk, neutral red dextrose broth, Endo medium, dextrose broth to which NaOH was added after incubation for the presence or absence of red color, and esculin bile solution.

The different strains of lactose-bile fermenting organisms that we have isolated, by the use of these media, are shown in Table 1.

TABLE 1.

	Gas in Dulcete	Gas in Saccharose	Indol	Nitrate Reduced	No. of Days to Blacken Esculin Bile Solution	NaOH Red Test in Dextrose
Culture 1.	o	o	o	+	10	o
" 2.	o	o	+	+	10	o
" 3.	+	o	+	o	8	o
" 4.	+	o	o	+	10	o
" 5.	+	o	+	+	2	o
" 6.	o	+	o	+	10	o
" 7.	o	+	+	+	10	+
" 8.	+	+	o	+	4	+
" 9.	+	+	o	+	1	+

These organisms all ferment dextrose and lactose, coagulate milk, give positive reactions on Endo medium and in neutral red broth, and do not liquefy gelatin.

These different lactose-bile fermenting organisms are non-chromogenic, but we have isolated two organisms that give gas in lactose bile that are easily separated from the group by the fact that one produces a brown pigment and the other a yellow. These seem to be rather common in our raw water supply, so that the bile presumptive test for *B. coli* would often be misleading.

TABLE 2.

Culture	Gas in Dulcete	Indol	Nitrate
1.	o	o	+
2.	o	+	+
3.	+	+	o
4.	+	o	+
5.	+	+	+

It will be noticed that these cultures may be divided into two classes on the basis of the positive and negative fermentation of

saccharose. Cultures 1, 2, 3, 4, and 5 do not ferment saccharose, and their culture differences may be shown in Table 2.

Cultures 6, 7, 8, and 9 ferment saccharose, and their cultural differences are shown in Table 3.

TABLE 3.

Culture	Gas in Dulcitate	Indol	NaOH Red Test	More than 24 Hrs. to Blacken Esculin
6.....	o	o	o	+
7.....	o	+	+	+
8.....	+	o	+	+
9.....	+	o	+	o

The lactose-bile fermenting organisms obtained from outside laboratories were also run in duplicate in all the above-named media and also in raffinose and mannite. The results are shown in Table 4.

TABLE 4.

Cultures from Outside Laboratories	Gas in Dulcitate	Gas in Saccharose	Gas in Mannite	Gas in Raffinose	Indol	Gelatin Liquefied	No. of Days Required to Blacken Esculin	NaOH Red Test in Dextrose
<i>B. coli</i> 1.....	+	—	+	+	+	o	4	o
" 2.....	+	—	+	+	+	o	4	o
" 3.....	+	o	+	o	+	o	2	o
" 4.....	+	o	+	o	+	o	3	o
" 5.....	+	o	+	o	+	o	3	o
" 6.....	+	o	+	o	+	o	2	o
" 7.....	+	o	+	o	+	o	2	o
" 8.....	o	o	+	o	+	o	3	o
" 9.....	+	o	+	o	+	o	3	o
" 10.....	+	+	+	+	+	o	8	o
" 11.....	+	+	+	+	+	o	5	o
" 12.....	+	o	+	o	+	o	11	o
" 13.....	—	+	+	+	+	o	2	o
" 14.....	—	+	+	+	+	o	5	o
" 15.....	—	+	+	+	+	o	5	o
<i>B. coli communior</i> A.....	+	+	+	+	o	o	—	+
<i>B. aerogenes</i> 1.....	+	+	—	—	+	o	3	o
<i>B. aerogenes</i> 2.....	+	+	+	+	+	o	8	o
<i>B. lactis aerogenes</i>	+	+	+	+	o	o	1	+
<i>B. cloacae</i>	+	+	+	+	o	+	5	+
<i>B. aerogenes capsulatus</i>	—	+	—	—	o	+	—	+
<i>B. acidi lactici</i>	o	o	+	o	+	o	o	o

These cultures all ferment dextrose and lactose, coagulate milk, reduce nitrate, and give positive reactions on Endo medium and in neutral red broth.

The esculin bile solution used in this work was made according to the following formula.¹

¹ Harrison and Vanderleek, *Centralbl. f. Bakt.*, Abt. 1, Orig. 1910, 51, p. 607.

- 1 per cent Witte's peptone
- 0.5 per cent sodium taurocholate (commercial)
- 0.1 per cent esculin
- 0.05 per cent ferric citrate
- 100 c.c. tap water.

After steaming 15 to 20 minutes the medium is filtered, tubed, and sterilized (fractional sterilization).

It will be noticed in the table that the time necessary to change the color of this solution from a clayish color to a jet black ranges from less than one day to 11 days. *B. lactis aerogenes* is the only lactose-bile fermenting bacterium that we have been able to isolate that produces the black color in less than one day. *B. coli communior* requires two to three days, and some of the other forms require a much longer time.

Another thing to be noticed is the close similarity of *B. coli communior*, *B. acidi lactici*, *B. aerogenes*, and *B. coli communior*. By the standard confirmatory tests these four strains of lactose-bile fermenting organisms would all be called *B. coli*.

The value of saccharose and dulcite broth as a differential test for these strains is shown in Table 5.

TABLE 5.

Species	Gas in Saccharose	Gas in Dulcite
<i>B. coli communior</i>	+	+
<i>B. aerogenes</i>	+	+
<i>B. coli communior</i>	o	+
<i>B. acidi lactici</i>	o	o

The different organisms that we have studied may be grouped into two classes: (1) those that ferment saccharose; (2) those that do not ferment saccharose.

Non-saccharose fermenters: *B. coli communior* and *B. acidi lactici*. Saccharose fermenters: *B. aerogenes*, *B. coli communior*, *B. lactis aerogenes*, *B. aerogenes capsulatus*, and *B. cloacae*.

B. coli is differentiated from *B. acidi lactici* by one (*B. coli*) fermenting dulcite and the other (*B. acidi lactici*) not fermenting dulcite.

The five organisms listed under the heading saccharose fermenters all produce gas from the various sugars; however, not to

the same extent. *B. lactis aerogenes* produces from 80 to 100 per cent gas in both lactose bile and dextrose broth. *B. aerogenes capsulatus* produces much gas in bile (75 to 90 per cent) but only about 25 to 30 per cent in dextrose. *B. cloacae* only gives about 15 per cent gas in lactose bile. The other cultures range from 35 to 60 per cent gas in 48 hours. The most striking cultural characteristics of the organisms in this class are shown in Table 6.

TABLE 6.

Species	Gelatin Liquefied	Indol	More than 24 Hrs. to Blacken Esculin	NaOH Red Reaction
<i>B. aerogenes</i>	o	+	+	o
<i>B. communior</i>	o	+	+	+
<i>B. lactis aerogenes</i>	o	o	o	+
<i>B. aerogenes capsulatus</i>	+	o	+	+
<i>B. cloacae</i>	+	o	+	+

It will be noticed that *B. aerogenes capsulatus* and *B. cloacae* separate themselves from the group by being gelatin liquefiers. *B. aerogenes* produces indol in Dunham's solution and *B. lactis aerogenes* and *B. communior* do not. *B. lactis aerogenes* can be identified by the very quick aesculin reaction.

It will be seen from the foregoing tables that other bacteria besides *B. coli communis* will produce gas in lactose bile, and we believe that in the differentiation of this organism the purified cultures that give gas in lactose bile should be next transplanted into saccharose. If the culture fails to produce gas in saccharose then subcultures should be made into indol and nitrate broths, and if both show positive reactions then transplant into dulcitate. If this reaction is positive call the strain *B. coli communis*. Those organisms that depart from the above tests may be called lactose-bile fermenting organisms, not *B. coli communis*, or may be classified as belonging to the colon group, or they may be run down to positive identification.

The principal differences observed in the organisms that we have studied in this laboratory are shown in the diagnostic table (Table 7).

The table is a résumé of the most illuminating cultural characteristics shown by the different lactose-bile fermenting organisms and

TABLE 7.

Species	Saccharose	Dulcitol	Gelatin Liquefied	Indol	Nitrate	Esculin Days to Blacken More than One
(A) Unknown.....	o	o	o	o	+	+
(B) Unknown.....	o	o	o	+	+	+
<i>B. acidilactici</i>	o	o	o	+	+	+
(C) Unknown.....	o	+	o	+	+	+
<i>B. coli communis</i>	o	+	o	+	+	+
(D) Unknown.....	+	o	o	+	+	+
(E) Unknown.....	+	+	o	+	+	+
<i>B. communior</i>	+	+	o	+	+	+
<i>B. lactis aerogenes</i>	+	+	+	+	+	+
<i>B. cloacae</i>	+	+	+	+	+	+
<i>B. aerogenes cap.</i>	+	+	+	+	+	+
<i>B. aerogenes</i>	+	o	+	+	+	+

it will be noticed that according to the differential tests that have been adopted as standards, we do not differentiate between *B. coli communis* and at least three other distinct species. In the diagnostic scheme given for the identification of *B. coli communis* it is well to carry out the tests in the order mentioned because it saves work and media, and as the dulcitol is expensive it is best to select very small fermentation tubes, and only use this medium to differentiate between *B. acidilactici* and *B. coli communis*.

In the daily routine analysis of the filtered water for the presence of *B. coli communis* it is our practice to proceed according to the following method.

1. Pour azolitmin lactose Parietti agar plates using 1 c.c. of sample.
2. Inoculate 1 c.c. portion of sample into dextrose broth.
3. Inoculate 1 c.c. portion of sample into lactose bile.
4. Inoculate 50 c.c. portion of sample into 10 c.c. of 10 times normal strength broth.
5. Incubate at 37° to 40° C.
6. At the end of 24 hours transplant 1 c.c. from the 50 c.c. enriched sample into lactose bile.
7. Pour plates from all fermentation tubes that show gas.
8. Fish three characteristic colonies (if present) on to agar slopes.
9. Transplant from agar slope in lactose bile.
10. If no gas develops make a negative report.
11. If gas develops then transplant from agar slope into saccharose broth, and if gas develops classify as belonging to *B. coli* group or lactose-bile fermenting organisms not *B. coli communis*.
12. If no gas develops continue the subculturing into nitrate and indol broths; if these are both positive we regard the bacterium as either *B. coli communis* or *B. acidilactici*.
13. Subculture into dulcitol; if positive gas production, record as *B. coli communis*.

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B. coli is found in sewage and therefore its presence is significant; but as newspaper reporters and other persons are inclined to magnify conditions, the officers in charge of purification works should be careful to identify suspicious organisms as true *B. coli* before throwing suspicion upon their plants and prejudicing the minds of the people. With this end in view the procedure outlined in the diagnostic table places a ready method of proving whether waters do or do not contain *B. coli communis*, and the necessity of such procedure is illustrated by the variety of organisms which less thorough tests would include erroneously in the group.