CALIFORNIA'S TEST FOR B. COLI*

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SINCE the establishment of this laboratory, September, 1915, the method for the isolation of B. coli in water samples has been that described in Standard Methods of Water Analysis as the "partially confirmed test," and consists of fermentation and enrichment in lactose broth and streaking on litmus lactose agar plates from broth tubes showing gas. The B. coli group is considered present when typical colon-like colonies develop on the litmus lactose agar plates. This method is similar to but in one particular differs from the procedure adopted by the Treasury Department on October 21st, 1914, for drinking water supplied to the public by common carriers in interstate commerce. The latter procedure involves an additional step over that used in this laboratory, namely: fishing and transplanting of a colon-like colony from the lactose litmus agar plates into a lactose broth tube, to confirm the gas-forming property.

Comparison of Methods

A comparison of the two methods on a large number of water samples from various sources shows the following results:—

	24 hours.	48 hours
Presumptive test—		
Total number	382	782
Partially confirmed test—		
Typical colon-like colonies	97.1%	76.1%
Treasury Department method—		
Fermentation of typical colon-like colonies	97 %	73.4%

Observations were made on the presumptive test after 24 hours and 48 hours. One of the striking features of this comparison is the small number of samples showing B. coli after but 24-hour incubation and the large number of 24-hour presumptive tests which on subsequent confirmation gave positive B. coli by the Treasury Department methods, and by the method used in the laboratory of the Bureau of Sanitary Engineering, 97 and 97.1 per cent., respectively. Of the 48-hour presumptive tests, however, only 73.4 per cent. were confirmed by the Treasury Department method and 76.1 per cent. by the method of this bureau. A difference of 2.7 per cent. is noted in the two methods. It is doubtful whether this difference is of sufficient importance to justify the extra work involved in obtaining the lower and doubtless more correct percentage. The "partially confirmed test" is on the safe side at any rate.

The latest Standard Methods of Water Analysis published by the American Public Health Association, 1917, defines the B. coli group as including all non-spore-forming bacilli, which grow aerobically on solid media and which produce gas in lactose broth. The B. coli tests are grouped under three headings: (1) the presumptive test, consisting only of gas production in lactose broth; (2) the partially confirmed test, consisting of plating a portion of the broth tubes showing gas on litmus lactose agar or endoes media and noting the formation of "typical colon-like colonies"; (3) the completed test, which consists of fishing typical colonies from the litmus lactose agar plates for demonstration of non-spore-forming bacilli and formation of gas in lactose broth.

*From "Bulletin" of State Board of Health.

It was deemed advisable to make a comparison of the three methods and determine the certainty with which the "typical colon-like colony" can be recognized and which of the three methods is most desirable for California conditions, with a view to reducing the work in the laboratory to a minimum without sacrificing the accuracy of the demonstration of B. coli. The following comparison was obtained from an entirely different series of water samples than those given in the preceding table:—

	24 hours.	48 hours.
Presumptive test—total	321	776
Partially completed tests	100%	82.1%
Completed tests	100%	81.2%

Fifty-one per cent. of the tests giving confirmed presence of B. coli after 48 hours failed to indicate the presence of B. coli on 24 hours' incubation. Practically 100 per cent. of the 24-hour presumptive tests were positive when confirmed by the completed test; in fact, only one sample out of a total of 321 could not be confirmed. The difference of 0.9 per cent. between the "partially confirmed" and "completed test" is considered too small to warrant the large amount of time consumed in completing the confirmation.

It must be recognized that the success of the partially confirmed test depends upon one's ability to distinguish by colony appearance between B. coli and similar acid-forming colonies. On litmus lactose agar, the typical colon-like colony appears as a raised red or pink spot in a blue field, or if the field becomes acid, the red or pink color of the colony will be more prominent than that of the field. The colonies on the surface are fat, glistening and smooth with a regular outline. Small deep red colonies with a deep red or brick-colored centre or irregular outline are usually not B. coli. On endo media, the colonies are raised with a regular outline, deep red and with a highly metallic sheen.

On the large number of 48-hour presumptive tests which the Bureau found negative on further confirmation, a large percentage were waters treated with chlorine gas, though the same observation applies to well waters, large reservoirs and streams. The positive presumptive tests in these cases may be attributed to either anaerobic or aerobic lactose-splitting spore-bearing bacilli, which are highly resistant to chlorine. B. welchii was isolated from the river supply at Sacramento, Cal., in 1916, as the anaerobic and aerobic spore-bearing bacteria and lactose-bile. At that time the city laboratory was using only the presumptive test for B. coli. In endeavoring to eliminate gas-forming bacteria from the supply, an overdose of chlorine was consequently added which caused tastes and subsequent complaints by the users of the water. More recently a larger number of aerobic spore-bearing lactose-splitting bacilli have been isolated from both chlorinated and unchlorinated supplies. It is thought that both anaerobic and aerobic spore-bearing bacteria and lactose-splitting bacilli are widely distributed in California waters.

Summary of Experiences

The experience of the Bureau on a wide range of waters has been that only about 50 per cent. of the tests in which B. coli are confirmed, develop gas within 24 hours' incubation. Forty-eight hours' incubation is necessary on tubes in which gas does not develop at 24 hours. However, practically all tubes showing a gas at the end of 24 hours were later confirmed for B. coli. Of those showing gas on 48 hours' incubation less than 85 per cent. could be confirmed.

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