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A Quick Microtechnique for the Brilliant-Green Lactose Bile Confirmed Test

Rhodes Q. Stuart and R. H. Weaver

Department of Bacteriology, University of Kentucky, Lexington, Kentucky

Shortening of the time required to obtain the results of tests for coliform organisms in water and reduction of the amounts of materials used in the tests is often important in public health work. With this in mind a quick microtechnique for the confirmed test has been developed using the principle of utilizing large inocula in small

amounts of media as proposed by Arnold and Weaver, 1948, and Hannan and Weaver, 1948.

Experimental

In the test, 5 x 50 mm microtubes are filled 1/3 full with portions of broth cultures from positive or doubtful presumptive tests and placed in a micro-serological-tube rack which is then set in a 37 C water bath. The tubes are now filled to the 2/3 level with brilliant-green lactose bile broth containing 10 per cent bile, which has been preheated to 37 C. Finally, the remaining space in the tubes is filled with sterile 1 percent melted agar which is at 50 C. After the agar becomes solid the tubes are inverted and immersed in the water bath. In our work we have examined the tubes at 15 min intervals for the first hours and at the end of the third and fourth hours for bubbles of gas. We also have examined them after 5 and 6 hr periods of incubation but we found this to be undesirable.

The microtubes may be adequately "sterilized" by placing them in a beaker of distilled water and boiling for 15 minutes.

Capillary pipettes may be employed for filling the microtubes. We make these to have a length of approximately 6 inches, including 3 inches of capillary. We plug the bulb end of the pipettes with cotton and pierce the pipettes through a paper cover that has been placed across the open end of a number 2½ can and secured in place with a string. After approximately 10 pipettes have been placed in a can the ends of the pipettes may be protected by a piece of wrapping paper. They may then be sterilized in the hot air oven. Medicine dropper bulbs may be placed on the pipettes when they are to be used.

In the preparation of the medium, a solution containing 2 percent bacto-peptone, 20 percent bacto-oxgall and brilliant green in a concentration of 1:2,500 is made. It is adjusted to pH 7.3 by potentiometric methods, tubed in 25 ml amounts and sterilized in the autoclave. When the medium is to be used, a 25-ml portion of the solution is mixed with a sterile 25-ml portion of 10 percent lactose solution to give a medium containing 1 percent peptone, 10 percent bile, 5 percent lactose and brilliant green in a concentration of 1:5,000. The final pH should be adjusted, if necessary, to 7.1-7.4.

It is essential that the portion of the presumptive culture that is used as the inoculum and the medium be at 37 C when the confirmed test cultures are prepared, otherwise false positive results are pro-

duced by the liberation of gas in the tubes when they warm up to the temperature of the water bath.

If completed tests are to be made from tubes which give positive confirmed tests with the microtechnique, the agar cap may be loosened by heating the end of the tube carefully with a small flame. After removal of the agar, streaks may be made in the usual fashion on eosin methylene-blue or Endo agar.

The approximate optimum concentrations of ingredients in the medium and other conditions for the tests were arrived at in preliminary experiments that will not be described in detail.

The efficiency of the microtechnique that had been developed was tested on 162 water samples. Many of these samples had been stored in the laboratory for one or more days or had been taken from sources known to give false positives, in order to provide as stringent a test as possible for the procedure. Presumptive tests were made with lactose broth according to the procedure of *Standard Methods for the Examination of Water and Sewage*, Ninth Edition, 1946. Confirmed tests were run in duplicate with the microtechnique and with the Standard Methods macrotechnique from all positive and doubtful presumptive tubes. The microtechnique was performed as has been described and also using a medium containing half as much bile (5 percent). Completed tests were run by Standard Methods procedures from all positive confirmed tests. Eosin methylene-blue agar was used for streaking.

The results of confirmed tests from 63 positive presumptive tubes are summarized in Table I. Confirmations were obtained with the Standard Methods procedure in 62 cases and with both the 5 percent and 10 percent brilliant-green lactose bile broths by the microtechnique in all 63 cases. Completed tests showed all confirmed tests to be due to coliforms.

Twenty-four hours was required to obtain the results of the confirmed tests when the Standard Methods procedure was used. In contrast to this, positive confirmed tests were obtained by the microtechnique in 15 minutes from 42 of the 63 samples tested, and in 30 minutes from 51 of the 63 samples. Positive results from all 63 samples were obtained in 4 hours.

The results of the confirmed test with 99 water samples that yielded doubtful presumptive tests are summarized in Table II. Here it can be seen that the Standard Methods procedure yielded 47 posi-

TABLE I. RESULTS OF MICRO CONFIRMATORY AND MACRO CONFIRMATORY TESTS FROM POSITIVE PRESUMPTIVE TESTS

Medium	Number of positive presumptive tests	Number of positive confirmed tests	Number of negative confirmed tests	Confirmed Tests due to coliform organisms	False confirmed tests (not due to coliform organisms)
Standard Methods Brilliant-Green Lactose Bile Broth.....	63	62	1	62	None
5 Percent Bile Medium with Microtechnique..	63	63	None	63	None
10 Percent Bile Medium with Microtechnique..	63	63	None	63	None

tive confirmed tests, of which 23 proved to be due to coliforms according to the completed tests and the microtechnique yielded 36 positive confirmed tests with each medium, including 20 which proved to be due to coliforms when the 5 percent bile medium was used and 23 when the 10 percent bile medium was used. Although the same number of positives due to coliforms was obtained by the Standard Methods procedure and by the microtechnique with the 10 percent bile medium, 3 samples were positive by the Standard Methods procedure that were negative by the microtechnique and 3 others were positive by the microtechnique although they were negative by the Standard Methods procedure. With the microtechnique using the 10 percent bile medium, 9 of the 23 positive confirmed tests that proved to be due to coliforms were positive within 15 minutes, 13 within 30 minutes and 21 within 4 hours; 2 being positive only after 6 hours. Of the 13 positives that proved not to be due to coliforms, 9 were positive only after 6 hours. From these results it was concluded that the confirmed test could be terminated profitably after 4 hours.

The 5 percent bile medium proved to be slightly less reliable than the 10 percent bile medium.

The results of these studies show the microtechnique as described to be as reliable as the Standard Methods procedure if judged by the number of positive results that are obtained which prove to be due to coliforms according to the completed test. It appears to be slightly superior if judged by the number of false positive confirmed tests obtained. The time required for the test has been shown to vary from 15 minutes to 4 hours (in two cases, 6 hours) as compared to 24 to 48 hours for the Standard Methods procedure.

TABLE II. RESULTS OF MICRO CONFIRMATORY AND MACRO CONFIRMATORY TESTS FROM DOUBTFUL PRESUMPTIVE TESTS

Medium	Number of Doubtful pre-sumptive tests	Number of positive confirmed tests	Number of negative confirmed tests	Confirmed Tests due to coliform organisms	False confirmed tests (not due to coliform organisms)
Standard Methods Brilliant-Green Lactose Bile Broth.....	99	47	52	23	24
5 Percent Bile Medium with Microtechnique..	99	36	63	20	16
10 Percent Bile Medium with Microtechnique..	99	36	63	23	13

SUMMARY

A quick microtechnique has been described for the brilliant-green lactose bile confirmatory test for water analysis. Testing of the microtechnique on 162 water samples showed it to yield results that were slightly more accurate than those obtained with the procedure of the Standard Methods for the Examination of Water and Sewage, Ninth Edition, 1946, in that less false positives were produced. The use of the microtechnique resulted in the saving of one to two days in the time required to obtain results and in a considerable saving of materials.

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