# WOOD APPLICATORS FOR THE CONFIRMATORY TEST IN THE BACTERIOLOGICAL ANALYSIS OF WATER

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IN THE confirmatory test for the coliform group the manual "Standard Methods for the Examination of Water and Wastewater" (1) permits either use of brilliant green lactose bile (BGLB) broth fermentation tube or the use of Endo or eosin methylene blue agar plates. The fermentation tube procedure is the method of choice in most laboratories because of its simplicity and reliability. This procedure calls for the transfer of one loopful of broth from a positive "primary" fermentation tube to a fermentation tube containing brilliant green lactose bile. For many years the inoculation of BGLB tubes has been done in the division of laboratories, Michigan Department of Health, with sterile 1-ml. pipettes instead of a wire loop, to save time. One or two drops of inoculum are transferred with the pipette. Recent experience indicated, however, that, if reliable, sterile hardwood applicators would provide a more convenient and less expensive means of inoculating confirmatory BGLB tubes than either the wire loop or pipette.

# Methods

The results of three parallel sets of confirmatory tests using three transfer methods (a wire lope to save time. One or two drops of inocucompared for 606 presumptive tubes. The wire loop was 3 mm. in diameter and made of 24gauge wire as specified in Standard Methods. The pipettes were of 1-ml. capacity and were sterilized and stored in metal containers. The applicators were hardwood sticks,  $\frac{3}{32}$ -inch

Mr. McGuire is chief, sanitary bacteriology and chemistry section, division of laboratories, Michigan Department of Health, Lansing. round and 7 inches long, packaged in test tubes 1 inch in diameter and sterilized by dry heat. The applicators were dropped into the positive lactose tubes. To transfer the inoculum, each applicator was removed from its lactose tube, plunged to the bottom of the BGLB tube, agitated slightly, then removed and discarded.

The 606 primary lactose broth fermentation tubes studied represented 121 water supplies (107 well waters and 14 lake and stream samples). Approximately two-thirds (413) of the tubes were positive (fermented lactose) in the presumptive test. The remaining 193 tubes were negative in the 48-hour presumptive test but for the purposes of this study were subjected to the confirmatory test. The 121 water supply samples were examined on 8 different days (table 1). The sequence in which the three transfer methods were used was rotated between the different series of samples to eliminate any influence of time or order of transfer. All doubtful tubes were carefully shaken just before observation for visible gas.

# Results

Comparison by individual tubes. The applicator technique showed the highest number of confirmations. Results exceeded the loop method by 48 tubes for the 24-hour incubation period and 23 tubes for the final or 48-hour incubation period (table 1). Transfer by glass pipette gave 24 more positive tubes after 24 hours of incubation than did the loop procedure and 14 more tubes at the end of 48 hours.

Of 413 positive presumptive lactose broth tubes studied, 377 (91.3 percent) agreed by all 3 transfer procedures in the confirmatory test (table 2). Of 193 negative presumptive lactose

| Number of tubes examined   | Number of positive confirmed<br>tubes, 24-hour incubation at<br>35° C.   |   |  | Number of positive confirmed<br>tubes, 48-hour incubation at<br>35° C. |  |  | Additional tubes con-<br>firmed over loop tech-<br>nique |  |
|--|--|---|--|--|--|--|--|--|
|  | Loop   | Pipette                                     | Applicator   | Loop   | Pipette                                      | Applicator                                   | Pipette  | Applicator                                 |
| 72         68         46         72         102         72         102 | $     \begin{array}{r}       14 \\       24 \\       9 \\       15 \\       55 \\       16 \\       8 \\       63 \\     \end{array} $ | 11<br>32<br>11<br>15<br>48<br>21<br>9<br>81 | $     14 \\     30 \\     15 \\     15 \\     60 \\     22 \\     10 \\     86   $ | $16 \\ 34 \\ 19 \\ 19 \\ 58 \\ 21 \\ 19 \\ 84$                         | 16<br>39<br>19<br>22<br>58<br>22<br>19<br>89 | 16<br>39<br>19<br>26<br>62<br>23<br>17<br>91 | 0<br>5<br>0<br>3<br>0<br>0<br>1<br>0<br>5                | 0<br>5<br>0<br>7<br>4<br>2<br>2<br>-2<br>7 |
| 606  | 204  | 228   | 252  | 270  | 284  | 293  | 14   | 23   |

Table 1. Results of three confirmed test methods

broth tubes examined, 191 (99 percent) were confirmed by all 3 transfer methods. A total of 568 tubes (93.7 percent) were in agreement by all transfer procedures. The results by 1 method disagreed with the results by the other 2 methods in the remaining 38 tubes. It will be observed that 17 lactose fermentation tubes that the loop method failed to confirm were confirmed by the pipette and applicator methods. The pipette method failed to confirm seven tubes that were confirmed by the other two methods. In no instance did the appli-

Table 2. Results for presumptive positive and<br/>presumptive negative lactose tubes by three<br/>transfer methods

| Confirmatory test<br>methods  | Tubes<br>lactose +<br>in pre-<br>sumptive<br>test | Tubes<br>lactose —<br>in pre-<br>sumptive<br>test | Total<br>tubes                     |
|---|---|---|------------------------------------|
| Results in agreement  |   |   |                                    |
|   | 377<br>255<br>122                                 | 191<br>8<br>183                                   | 568<br>263<br>305                  |
| Results not in agreement  |   |   |                                    |
| $\begin{array}{c} \text{Total} \\ \textbf{L}- \textbf{P}+ \textbf{A}+ \\ \textbf{L}+ \textbf{P}- \textbf{A}+ \\ \textbf{L}+ \textbf{P}+ \textbf{A}- \\ \textbf{L}+ \textbf{P}- \textbf{A}- \\ \textbf{L}- \textbf{P}+ \textbf{A}- \\ \textbf{L}- \textbf{P}+ \textbf{A}- \\ \textbf{L}- \textbf{P}- \textbf{A}+ \\ \textbf{L}- \textbf{P}- \textbf{A}+ \end{array}$ | $36 \\ 16 \\ 3 \\ 0 \\ 4 \\ 3 \\ 10$              | $2 \\ 1 \\ 0 \\ 0 \\ 1 \\ 0$                      | 38<br>17<br>3<br>0<br>4<br>4<br>10 |
| Total   | 413   | 193   | 606                                |

Note: L=loop; P=pipette; A=applicator.

cator fail to confirm lactose tubes that were confirmed by the loop and the pipette. In four tubes, only inoculums transferred by the loop were positive, and in three tubes, only those transferred by pipettes were positive. In 10 tubes, only those transferred by the applicators were positive in the confirmatory test.

Among the 193 tubes that were negative in the presumptive test, 10 (5.2 percent) were positive in the confirmatory test. Eight of these were confirmed by all three methods. These 10 tubes represented 7 different water supply samples, each of which produced 1 or more positive presumptive tubes; coliforms were demonstrated in other tubes from the same sample in all cases. These positives were probably due to the variability of the presumptive test, not to faulty transfer technique.

*Comparison by samples.* The 107 well water samples were examined by inoculating five 10-ml. portions and one 1-ml. portion for the presumptive test. Results were classified by the number of lactose positive tubes per sample (table 3). In order to compare these data with standard practice, only positive presumptive tubes were considered. Confirmed test results for 83 of the 107 samples (78 percent) were in agreement by all 3 transfer methods.

With the wood applicator, 15 samples had a higher confirmed coliform index than was indicated by the loop transfer method. With the pipette, five samples had a higher confirmed coliform index than was indicated by the loop method.

Six of the tubes that the loop transfer method

|   | Number of well water samples |  |   |  |  |
|---|------------------------------|--|---|--|--|
| Number of positive<br>presumptive tubes<br>per sample | Total<br>samples             | Samples<br>in which<br>three<br>methods<br>agree | Samples<br>in which<br>three<br>methods<br>disagree |  |  |
|   | 01                           |  |   |  |  |
| 1   | 21                           | 18   | 3   |  |  |
| 2   | 15                           | 8  | 7   |  |  |
| 3   | 16                           | 12   | 4   |  |  |
| 4   | . 9                          | 6  | 3   |  |  |
| 5   | 23                           | 20   | 3   |  |  |
| 6   | 23                           | 19   | 4   |  |  |
| Total   | 107                          | 83   | 24  |  |  |

Table 3. Classification of well water samples by number of positive presumptive tubes and confirmed results

failed to confirm but that were positive by the applicator method were subjected to the completed test for the coliform group. Four of these were also positive by the pipette method. Coliform organisms were demonstrated in each of the six tubes by the completed test.

The time required to transfer 68 tubes by each of the methods was determined. The same amount of time, 16 minutes, was required to make the 68 transfers by both the applicators and the pipettes. The wire loop method required 34 minutes using only one loop. If the time required for cleaning and preparing the glass pipettes is considered, it is apparent that the use of wood applicators offers a distinct saving in personnel time. In addition, the cost of the wood applicators is insignificant (approximately \$1 per 1,000).

These results indicate that the use of wood applicators is a satisfactory method for the inoculation of BGLB fermentation tubes.

#### Discussion

There are three possible explanations for the slight increase in sensitivity with wood applicators. First, in transferring the inoculum by this method the applicator reaches the bottom of both the positive presumptive tube and the confirmatory tube. Thus, since the inoculum is distributed throughout the BGLB, prompt inoculation of the media inside the insert or inverted glass vial is assured. This could be significant in the case of slow fermenters that require a long time to produce visible gas. Second, the applicator may transfer more inoculum than the loop. Third, the possibility of inactivating bacteria with heat from a loop that has not been allowed to cool sufficiently is avoided.

### Summary

Three methods of inoculating brilliant green lactose bile for the confirmatory test in the bacteriological analysis of water have been compared. Of the 606 lactose fermentation tubes studied, 23 more tubes were confirmed by the wood applicator method than by the wire loop method. Fourteen more tubes were confirmed by the pipette method than by the wire loop procedure. The wood applicator method always confirmed tubes that were confirmed by the pipette and the loop methods. Ten tubes were confirmed by only the wood applicator. Seventeen tubes that were confirmed by the applicator and the pipette were not confirmed with the loop method. The inoculation of BGLB tubes with the use of one loop requires approximately twice as much time as either the pipette or wood applicator method of transfer.

This study suggests that Standard Methods should be modified to permit the use of wood applicators or pipettes for the inoculation of BGLB fermentation tubes.

#### REFERENCE

 American Public Health Association: Standard methods for the examination of water and wastewater. Ed. 11, New York, 1960.