The Coliform Group

II. Reactions in EC Medium at 45 C


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Escherichia coli has been suggested by several research workers as a more accurate indicator of fecal contamination than the more inclusive "coliform group." Media and procedures for the enumeration of E. coli have been proposed by Eijkman (1904), Leiter (1929), Perry and Hajna (1933, 1944), Hajna and Perry (1939, 1943), Vaughn et al. (1951), and Levine et al. (1955). Some of these procedures apparently yield satisfactory results with certain samples of water, milk, or food products; however, there is no universal agreement as to their criteria of application and significance of results in water bacteriology. There are also uncertainties regarding the significance of E. coli when assayed by rapid tests as compared to the traditional IMViC classification.

This investigation was undertaken to determine the reactions of 12 IMViC types of coliform bacteria in EC medium after 24 hr incubation at 45 C. It is believed the resultant data will be of value in choosing the procedure and in the interpretation of test results for E. coli and the coliform group.

Materials and Methods

The coliform group of bacteria for this investigation was defined as aerobic and facultative anaerobic gram-negative, nonsporeforming bacteria which fermented lactose with gas formation in 48 hr, or less, when incubated at 35 C (Standard Methods for the Examination of Water, Sewage, and Industrial Wastes. APHA, 1955a). All of the cultures were recently isolated from surface water samples obtained from various locations and with varying degrees of pollution (Clark et al., 1957). Procedures described as tentative methods in Standard Methods for the Examination of Water, Sewage, and Industrial Wastes (APHA, 1955b) were used for indole, methyl red, and Voges-Proskauer tests, and citrate agar of Simmons (1926) was used for the citrate test. The EC medium of Hajna and Perry (1943) was used as a confirmatory test for the various coliform bacteria. It was reconstituted from the dehydrated product, tubed with an inverted vial for the observation of gas production and sterilized in an autoclave at 121 C for 15 min.

Each culture of the coliform bacteria was examined for indole and acetyl methyl carbinol production, methyl red reaction, utilization of citrate, and gas formation in EC medium in 24 hr at 45.0 C in a constant temperature water bath. In reading the EC tubes, the presence of gas in any quantity was considered a positive reaction and absence of gas (in 24 hr incubation) was a negative reaction.

The procedure used in this study differed from the recommendations of Perry and Hajna (1944) in that they suggested air incubation at 45.5 C for 48 hr.

Results

There were 5794 cultures of coliform bacteria from untreated surface water supplies of 14 treatment plants examined. Of the 1358 E. coli included, 83.7 per cent showed positive EC reactions. When the E. coli strains are subdivided into varieties I and II (see table 1), the percentage of positive results were 92.7 and 21.8, respectively. The remaining 10 IMViC types yielded 7.8 per cent positive tests for 4436 cultures. The data summa-

<table>
<thead>
<tr>
<th>IMViC Type</th>
<th>Commonly Designated Source*</th>
<th>Commonly Designated Source*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feal</td>
<td>Non-fecal</td>
</tr>
<tr>
<td></td>
<td>No. cultures examined</td>
<td>Positive EC reactions</td>
</tr>
<tr>
<td>+ + + + + +</td>
<td>1384</td>
<td>1106</td>
</tr>
<tr>
<td>+ + + +</td>
<td>174</td>
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<td>1688</td>
<td>179</td>
</tr>
<tr>
<td>+ + + + + +</td>
<td>237</td>
<td>8</td>
</tr>
<tr>
<td>+ + + + + + +</td>
<td>433</td>
<td>10</td>
</tr>
<tr>
<td>+ + + + + + + +</td>
<td>989</td>
<td>18</td>
</tr>
<tr>
<td>Total ..........</td>
<td>1358</td>
<td>1136</td>
</tr>
</tbody>
</table>

rized in table 1 also show that type ++-- gave 77.2 per cent positive reactions.

**DISCUSSION**

With 92.7 per cent of the *E. coli* variety I (+ + --) showing positive EC reactions, there is relatively good agreement in the classification of this organism. However, only 21.8 per cent of the so-called *E. coli* variety II (− -- --) reacted positively in EC medium, thus reducing the correlation for the two *E. coli* varieties collectively to 83.7 per cent. The IMViC type ++-- comprised only a small part of the cultures studied, but showed 77.2 per cent positive EC tests. The reactions of *E. coli* variety II and of ++-- type to the EC test at 45°C suggest the need for further work to determine the fecal or nonfecal origin of these organisms. The latter appears to be more closely related to *E. coli* variety I on the basis of growth with production of gas at 45°C than does the indole negative variety II. These results are comparable to those reported by Clark et al. (1957), using a boric acid lactose broth reaction at 43°C. Both the boric acid lactose broth and EC procedures seem to have approximately the same capabilities in differentiating the coliform group.

It is not the purpose of this study to establish the value of any single type of coliform bacteria as a sanitary indicator of fecal pollution. However, the results as reported strongly suggest that added efforts should be made to locate sources of fecal pollution of waters containing EC positive coliforms. Because other biochemical characteristics may show better correlations with fecal or nonfecal origins of the coliform group, further consideration should be given to the use of other measurements in addition to the conventional IMViC reactions and growth at elevated temperatures.

**SUMMARY**

Coliform cultures of 12 IMViC types were incubated in EC medium in a water bath for 24 hr at 45.0°C (±0.3). Positive tests were recorded for 92.7 per cent of *Escherichia coli* variety I, 21.8 per cent of *E. coli* variety II, and 83.7 per cent when considered together as coliform bacteria commonly designated as of fecal origin. The nonfecal types of coliform cultures showed 7.8 per cent positive tests. A high percentage of ++-- strains grew at 45.0°C, suggesting a close relationship to the *E. coli* variety I.

Sources of samples containing positive EC coliforms should be carefully inspected for possible fecal contamination.

The EC broth procedure as described appears suitable for the rapid enumeration of *E. coli* in the investigation of field problems and other kinds of control work.

**REFERENCES**


