The Coliform Group

II. Reactions in EC Medium at 45 C


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Escherichia coli has been suggested by several re-
search 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 signif-
cance of results in water bacteriology. There are also
uncertainties regarding the significance of E. coli
when assayed by rapid tests as compared to the tradi-
tional 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 in-
cubated at 35 C (Standard Methods for the Examin-

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 posi-
tive 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, respec-
tively. 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>
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<tbody>
<tr>
<td></td>
<td>Feal</td>
</tr>
<tr>
<td></td>
<td>Number of cultures examined</td>
</tr>
<tr>
<td>+ + + +</td>
<td>1184</td>
</tr>
<tr>
<td>+ + +</td>
<td>174</td>
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<td>+ + +</td>
<td>453</td>
</tr>
<tr>
<td>+ + +</td>
<td>989</td>
</tr>
<tr>
<td>Total</td>
<td>1358</td>
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</tbody>
</table>

* According to Standard Methods for the Examination of
Water, Sewage, and Industrial Wastes (10th edition, 1955), page
391, Table 22—Coliform Group—Reaction Classification.
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**


