A Research Note

Extended Incubation of LST and BGB Tubes and the MPN Estimates of Coliforms

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ABSTRACT

Extending the incubation time past the standard 48 h for presumptive lauryl sulfate tryptose (LST) broth and confirmed brilliant green bile (BGB) broth tests for coliforms resulted in increases in most probable number (MPN) estimates. Whereas 40% of the samples showed an increase in the presumptive MPN when the incubation was extended from 48 to 72 h, only 5% showed confirmed MPNs, which exceeded the 95% confidence limits established for the 48-h confirmed MPNs. Extending the incubation of BGB tubes to 72 h resulted in less than 5% of the samples exhibiting increased MPNs, which exceeded the 48-h 95% confidence limits. Some loss in viability of coliforms was observed when LST tubes were incubated beyond 72 h. The study demonstrates the importance of adhering to standard incubation times for interlaboratory comparisons and ensuring regulatory compliance.

Key Words: Coliforms, MPN, LST broth, BGB broth

to evaluate the incidence of increases in the MPN estimates when incubation is extended to 72 h.

MATERIALS AND METHODS

The experimental work was carried out in the authors' four laboratories. Coliform populations were estimated in 106 food and water samples, which were part of routine sample submissions to the laboratories. A variety of products were analyzed and the number and type were as follows: 26 red meat, 8 poultry, 9 dairy, 21 seafood, 36 water and 6 miscellaneous foods. The presumptive and confirmed coliform tests were carried out following conventional methodology as described in the sixth edition of the Bacteriological Analytical Manual (5). Five tubes per dilution were used. Dehydrated LST broth (Difco Laboratories, Detroit, MI) and BGB 2% broth (Difco) were used; all four laboratories used the same lots of media.

The protocol used to evaluate the effects of extended incubation for both the LST and BGB tubes is shown in Fig 1. The LST tubes were incubated for 24 ± 2 h and the number of positive tubes recorded. All tubes were incubated an additional 24 ± 2 h and the new positives recorded. All positive tubes were transferred to BGB for the confirmation of coliforms. All LST tubes were incubated for a further 24 ± 2 h to a total of 72 h incubation and new positives recorded. All positive tubes were transferred to BGB for confirmation of coliforms in new positive tubes and verification that 24 and 48-h positives would continue to demonstrate the presence of confirmed coliforms.

The BGB tubes were incubated for 24 ± 2 h and the number of positives recorded. All tubes were incubated an additional 24 ± 2 h and new positives recorded. All positive tubes were streaked on MacConkey agar and incubated at 35 ± 1°C for 24 ± 2 h. The presence of lactose positive colonies (red or pink), which microscopic examination indicated were gram-negative non-sporeforming rods used as verification of the presence of coliforms. Negative BGB tubes were incubated for an additional 24 ± 2 h and new positives recorded and verified on MacConkey agar for the presence of coliforms.

Most probable number tables from the 16th edition of Standard Methods for the Examination of Water and Wastewater (2) were used to establish MPNs.
EXTENDED INCUBATION OF MPN TESTS

RESULTS

Of the 106 samples analyzed, 92 demonstrated the presence of presumptive coliforms. Four samples contained presumptive coliforms, which were not confirmed. For seven of the samples all five tubes of all dilutions used for both the presumptive and confirmed tests were positive. As a result, these samples were not used in the comparisons. Since no presumptive coliforms were detected in 14 samples, only 85 sample results were available to show changes in numbers of confirmed coliforms.

Extending the incubation time of the LST tests from both 24 h to 48 h and 48 h to 72 h resulted in an increase in the number of positive tubes. While a large number (67%) of the samples tested showed an increase in the MPN as a result of continuing the incubation from 24 to 48 h, further incubation to 72 h resulted in an additional 40.2% of the samples exhibiting higher MPNs (Table 1). To evaluate the extent of the MPN increases, a comparison of the increases relative to the upper 95% confidence limits established by the 48 h MPN was made. While 40.2% of the samples showed an increased presumptive MPN, only 16.3% of the samples showed an increase, which exceeded the upper 95% confidence limit. It is important to note that only 5.4% of those samples demonstrated increased confirmed coliform MPNs (Table 1). The samples exhibiting these confirmed increases were all raw meat samples being either ground beef or ground turkey.

The BGB media used for confirming the presence of coliforms also showed additional tubes with gas production when incubated for 72 h compared to 48 h. Of the 85 samples which were presumptive positive, confirmatory testing of the 48 h LST tubes revealed that MPN values increased in 14 (16.5%) of the samples when incubation was extended from 48 to 72 h (Table 2). However, only 4 samples (4.7%) increased beyond the 95% upper confidence limit set by the 48-h MPN. Similar results were observed when the 72 h incubated LST tubes were confirmed. Extending incubation of the BGB tubes from 48 h to 72 h resulted in only a 3.5% increase in the number of samples showing MPNs, which exceeded the 48-h 95% upper confidence limit.

Extending the incubation time of LST tubes to 72 h resulted in a number of samples (9 out of 85) with reduced confirmed MPNs (Table 3). Only 3.5% of the samples showed a reduction in the confirmed MPN, which fell below the lower 95% confidence limits established by the 48-h confirmed MPN values. The frequency and extent of the decreases was greatest when analyzing raw oyster samples.

DISCUSSION

The importance of incubating LST tubes for at least 48 h was clearly shown in this study since 67% of the samples showed an increased MPN after 48-h incubation. Extending incubation time to 72 h resulted in an additional 40.2% of the samples showing an increased confirmed MPN, which exceeded the upper 95% confidence limit. Similar results were observed when the 72 h incubated LST tubes were confirmed. Extending incubation of the BGB tubes from 48 h to 72 h resulted in only a 3.5% increase in the number of samples showing MPNs, which exceeded the 48-h 95% upper confidence limit.

Extending the incubation of LST tubes to 72 h resulted in a number of samples (9 out of 85) with reduced confirmed MPNs (Table 3). Only 3.5% of the samples showed a reduction in the confirmed MPN, which fell below the lower 95% confidence limits established by the 48-h confirmed MPN values. The frequency and extent of the decreases was greatest when analyzing raw oyster samples.

TABLE 1. The effect of extending the incubation time from 48 to 72 h on the presumptive MPN of coliforms using LST broth.

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>No. of samples exhibiting an increased presumptive MPN after 48-h incubation</th>
<th>No. of samples for which the 72-h presumptive MPN exceeded the 95% confidence limits established for the 48-h MPN</th>
<th>No. of samples for which the 72-h confirmed MPN exceeded the 95% confidence limits established for the 48-h MPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>92</td>
<td>37 (40.2%)</td>
<td>15 (16.3%)</td>
<td>5 (5.4%)</td>
</tr>
</tbody>
</table>

Numbers in brackets are percentages of total samples tested.

TABLE 2. The effect of extending the incubation time from 48 to 72 h on the confirmed MPN of coliforms using BGB broth.

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>No. of samples exhibiting an increased MPN after 48-h incubation</th>
<th>No. of samples for which the 72-h MPN exceeded the 95% confidence limits established for the 48-h MPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>85</td>
<td>14 (16.5%)</td>
<td>4 (4.7%)</td>
</tr>
<tr>
<td>85</td>
<td>17 (20.0%)</td>
<td>3 (3.5%)</td>
</tr>
</tbody>
</table>

Numbers in brackets are percentages of total samples tested.

TABLE 3. The reduction in confirmed coliform MPNs when extending the incubation of LST tubes to 72 h.

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>No. of samples exhibiting a reduced MPN after 48 h</th>
<th>No. of samples for which the 72-h MPN was reduced below the 95% confidence limits established for the 48-h MPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>85</td>
<td>9 (10.6%)</td>
<td>3 (3.5%)</td>
</tr>
</tbody>
</table>

Numbers in brackets are percentages of total samples tested.
tested showed an increase in the MPN as result of continuing the incubation from 24 to 48 h. The increases in the MPNs resulting from the extension of incubation time for both the presumptive and confirmed tests was not unexpected since slow lactose fermenters have been reported as early as 1939 (7). While increases were observed, the number of samples, which showed increases in confirmed coliforms was considered to be low. In the case of presumptive coliform estimates (LST) only 5% of the samples incubated for 72 h showed increases which exceeded the 95% confidence limits established at 48 h. When the incubation period was extended to 72 h for confirmation tests (BGB), less than 5% of the samples showed an increase in MPNs after 48 h which exceeded the 95% confidence limit established at 48 h.

Loss of the viability of coliforms in presumptive LST broth can result in lowered MPNs after extended incubation and are of as much concern as increases in population estimates. Our results showed that extended incubation of LST resulted in 10.5% of the samples showing reductions in the confirmed MPN. Only 3.5% showed reductions, which fell below the 95% confidence level established after 48 h incubation of the LST tubes.

In our opinion, the extent of changes observed were small enough that when extended incubation is necessary the results would continue to be acceptable for routine quality control purposes. In performing quality control tests on many products, the presence of coliforms is often of more concern than establishing their exact numbers. However, this study indicates that when performing coliform estimates for regulatory compliance or when comparing results between laboratories the potential increases or decreases related to extended incubation can be avoided by adhering to standard incubation times.

ACKNOWLEDGMENTS

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REFERENCES