Evaluation of the 7-h Membrane Filter Test for Quantitation of Fecal Coliforms in Water

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The 7-h fecal coliform (FC) test for detection of FC organisms in water was evaluated to establish its validity and usefulness for emergency and disaster situations. The tests consisted of routine samples collected for public health surveillance and enforcement purposes. A total of 984 water samples from throughout California were assayed. These included samples from coastal salt waters, rivers, canals, and reservoirs, in addition to potable and miscellaneous freshwater sources. A portion of each sample was tested concurrently by both the 7-h FC test and the most-probable-number FC five-tube test. The 7-h FC test samples were incubated for 7 to 7.25 h at 41.5°C. Overall, greater than 90% agreement was obtained between the methods in determining whether the water quality was acceptable or unacceptable. Statistical analysis of the 984 samples confirmed that the 7-h FC method was a suitable alternative to the most-probable-number FC method for evaluation of freshwater samples. During emergencies or disasters, the 7-h FC test could provide a means for detection of fecal contamination of water with results available in less than 1 day.

With urbanization and the continuing threat of disaster and subsequent contamination of water supplies, the need for rapid determination of the sanitary quality of water is an increasing public health concern. During natural disasters such as floods and earthquakes, water supplies may become contaminated. A method of analysis that is rapid, accurate, sensitive, and economical and that correlates well with accepted standard procedures would be useful.

Reasoner et al. (4) developed the rapid 7-h fecal coliform (FC) test, in which a membrane filter and buffered lactose-based medium are used. These investigators determined that their method was suitable for examination of surface waters and unchlorinated sewage. The 7-h FC test offers a considerable time-saving advantage over standard and proposed (1, 2, 5, 6) FC tests, all of which require a minimum 24 h of incubation.

This study was undertaken to evaluate the 7-h FC test by using samples collected throughout California. Data are presented from a comparative study performed by 10 public health laboratories in California representing northern and southern coastal counties, major valleys, and mountainous and desert regions. Water samples were tested from a variety of sources: coastal waters, reservoirs, rivers, and treated drinking-water supplies from both surface and deep wells. The samples were simultaneously tested by the standard most-probable-number (MPN) test (1) and the 7-h FC method.

MATERIALS AND METHODS

Sample sources and collection. Ten public health laboratories throughout California participated in the study, testing samples from various water sources available within their jurisdictions. The participating laboratories were the California State Department of Health Services’ Sanitation and Radiation Laboratory and the Public Health Laboratories of the following counties: Los Angeles, Mendocino, Monterey, Orange, Riverside, San Diego, San Francisco, San Mateo, and Tulare.

A total of 984 samples were divided into six categories for tabulation. The samples consisted of 450 potable, 299 salt, 77 reservoir, 111 river, 29 canal, and 18 miscellaneous waters. At least 250 ml of water was collected from each source, transported to the laboratory, and held at 4°C. All samples were tested within 30 h of collection. The five-tube MPN FC and the 7-h FC filter tests were performed concurrently in each participating laboratory.

Medium for the 7-h FC test. Each participating laboratory received 0.25 lb (113 g) of Difco m-7h FC medium (lot no. QC0233). The dehydrated medium was donated to the California State Department of Health Services’ Sanitation and Radiation Laboratory by Difco Laboratories. It was portioned and sealed in sterile 8-oz (225-ml) plastic bottles and shipped to each laboratory. The m-7h FC medium was prepared by using 46.5 g of dehydrated powder suspended in 1.0 liter of distilled or deionized water and heated to boiling. After the ingredients were dissolved, the solution was heated for an additional 5 min. The pH was adjusted to 7.3 ± 0.2 at 25°C. Four to six milliliters of medium was then dispensed into sterile petri dishes (60 by 15 mm). The poured plates were stored in a tight container at 4°C and used within 2 weeks. Quality control for each batch of freshly prepared medium and on each day of testing was performed by using Escherichia coli as a positive control and Enterobacter aerogenes as a negative control.

Membrane filter procedure. Samples (100 ml) of potable water were filtered and placed on m-7h FC medium as described previously (4). Nonpotable water was processed in 10- and 100-ml volumes. The m-7h FC medium was then
maintained at 41.5°C for 7 to 7.25 h. In two participating laboratories, the plates were placed in waterproof plastic bags and incubated in a water bath. In the other laboratories, standard incubators were used; a maximum of two plates were stacked vertically to provide more rapid equilibration and uniform incubation temperature. At the end of the incubation period, yellow colonies were counted and recorded.

**MPN FC procedure.** The five-tube MPN procedure was performed by using lauryl tryptose broth and brilliant green lactose bile broth (BGLBB) for confirmation of total coliforms, as well as EC medium for confirmation of FC (1). For potable water samples, either 10-ml portions or 10-, 1-, and 0.1-ml portions were used. For all other samples, either 10-, 1-, and 0.1-ml portions or 10-, 1-, and 0.01-ml portions were used. Tubes were examined for gas production at 24 ± 2 and 48 ± 3 h. Samples in tubes showing growth and gas production were inoculated to BGLBB and EC medium for confirmation.

**Data collection and tabulation.** Results were recorded on standardized data collection forms, with specimen number, collection date, test date, water source, and remarks being noted. On the basis of the test results, potable waters and waters which could be used for bathing or other body contact recreational purposes (rivers, reservoirs, and salt waters) were classified as satisfactory or unsatisfactory (see Table 2). Potable waters containing any detectable FC were classified as unsatisfactory by the testing method used. Samples from rivers, reservoirs, and salt waters were classified as unsatisfactory if they contained >200 FC per 100 ml (7). All other potable and recreational waters were classified as having satisfactory FC levels.

**Statistical methods.** Data analyses were performed by using SPSS/PC+ microcomputer software (3). Correlation was determined by using Student’s two-tailed t test. McNemar’s test for analysis of discordant pairs was used to determine whether there was a significant difference between the two FC methods with regard to ascertaining satisfactory or unsatisfactory status of potable and recreational waters.

**RESULTS**

For purposes of analysis, the samples were grouped into six categories: potable water, river water, reservoir water, saltwater, canal water, and miscellaneous freshwater samples.

Comparison of FC counts obtained for the samples by the two different tests is shown in Table 1. One potable-water sample with a 7-h FC result showing that FC were too numerous to count and nine saltwater samples for which MPN or 7-h FC endpoints were not determined were excluded from the calculations performed in preparing Table 1. Significant correlation of counts was observed for potable samples and samples collected from river, reservoir, and saltwater sources (see Table 1). Average counts obtained by the 7-h FC method were lower than those obtained by the MPN FC method for all categories of specimens.

There was over 90% agreement between the two tests in determining a satisfactory or unsatisfactory status of water for all four groups evaluated. Analysis of discordant pairs by McNemar’s test indicated no significant difference between results of the two types of tests for any category of fresh water tested. However, a significant difference (P < 0.0001) was observed between results obtained with saltwater samples. Twenty-three saltwater samples were classified as unsatisfactory by the MPN method and satisfactory by the 7-h FC method (Table 2).

As a further method of evaluation, predictive values were calculated for the ability of the 7-h FC test to forecast an unsatisfactory MPN FC result (positive predictive value) or satisfactory MPN FC result (negative predictive value). Negative predictive values for potable, river, reservoir, and salt waters were all >90% (Table 3). The negative predictive

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**TABLE 1. Comparison of FC counts per 100 ml obtained by MPN and 7-h FC tests**

<table>
<thead>
<tr>
<th>Sample type</th>
<th>No. of samples</th>
<th>Mean counts</th>
<th>Minimum counts</th>
<th>Maximum counts</th>
<th>Correlation</th>
<th>Two-tailed probability (for correlation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potable water</td>
<td>449</td>
<td>4.4</td>
<td>1.4</td>
<td>&lt;2</td>
<td>1,600</td>
<td>0.785</td>
</tr>
<tr>
<td>River water</td>
<td>111</td>
<td>141</td>
<td>140</td>
<td>&lt;20</td>
<td>2,690</td>
<td>0.849</td>
</tr>
<tr>
<td>Reservoir water</td>
<td>77</td>
<td>452</td>
<td>340</td>
<td>&lt;20</td>
<td>1,850</td>
<td>0.815</td>
</tr>
<tr>
<td>Canal water</td>
<td>29</td>
<td>11</td>
<td>2</td>
<td>&lt;20</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Miscellaneous water</td>
<td>18</td>
<td>59</td>
<td>31</td>
<td>&lt;20</td>
<td>800</td>
<td>—</td>
</tr>
<tr>
<td>Salt water</td>
<td>290</td>
<td>352</td>
<td>108</td>
<td>&lt;2</td>
<td>17,000</td>
<td>0.516</td>
</tr>
</tbody>
</table>

* MPN values of <2.2 for potable waters and <20 for nonpotable waters were set at zero for determining statistics in this table. Also, MPN values of ≥16 for nine potable-water samples and an MPN value of ≥1,600 for one potable-water sample were set at 16 and 1,600, respectively, for calculating statistics in this table.

* Because of small numbers of samples in these categories, correlation and two-tailed probabilities were not calculated.

**TABLE 2. Comparison of the 7-h FC and MPN-FC tests for determining satisfactory or unsatisfactory FC levels in water samples**

<table>
<thead>
<tr>
<th>Sample type</th>
<th>No. of samples</th>
<th>MPN-S, 7-h FC-S</th>
<th>MPN-S, 7-h FC-U</th>
<th>MPN-U, 7-h FC-S</th>
<th>MPN-U, 7-h FC-U</th>
<th>% Agreement</th>
<th>McNemar’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potable water</td>
<td>450</td>
<td>389</td>
<td>19</td>
<td>11</td>
<td>31</td>
<td>93.3</td>
<td>0.201</td>
</tr>
<tr>
<td>River water</td>
<td>111</td>
<td>85</td>
<td>4</td>
<td>7</td>
<td>15</td>
<td>90.1</td>
<td>0.549</td>
</tr>
<tr>
<td>Reservoir water</td>
<td>77</td>
<td>41</td>
<td>2</td>
<td>2</td>
<td>32</td>
<td>94.8</td>
<td>1.000</td>
</tr>
<tr>
<td>Salt water</td>
<td>299</td>
<td>245</td>
<td>1</td>
<td>23</td>
<td>30</td>
<td>92.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* MPN-S, MPN-U. Satisfactory and unsatisfactory, respectively, by the MPN test. 7-h FC-S, 7-h FC-U, Satisfactory and unsatisfactory, respectively, by the 7-h FC test.

* A value of <0.05 in McNemar’s test indicates a significant difference.
TABLE 3. Ability of the 7-h FC test to predict satisfactory or unsatisfactory FC levels as measured by the MPN-FC test

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Positive predictive value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Negative predictive value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potable water</td>
<td>62.0% (31/50)</td>
<td>97.2% (389/400)</td>
</tr>
<tr>
<td>River water</td>
<td>78.9% (15/19)</td>
<td>92.4% (8592)</td>
</tr>
<tr>
<td>Reservoir water</td>
<td>94.1% (32/34)</td>
<td>95.3% (41/43)</td>
</tr>
<tr>
<td>Salt water</td>
<td>96.8% (30/31)</td>
<td>91.4% (245258)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Potable waters containing detectable FC were classified as unsatisfactory by the method used. Waters from rivers, reservoirs, or saltwater sources were classified as unsatisfactory if they contained >200 FC per 100 ml. All other water samples were classified as having satisfactory FC levels.

<sup>b</sup> Positive predictive values are given as the number of samples unsatisfactory by both methods/number of samples unsatisfactory by the 7-h FC test.

<sup>c</sup> Negative predictive values are given as the number of samples satisfactory by both methods/number of samples satisfactory by the 7-h FC test.

value for potable samples was 97.2%. The positive predictive value for potable samples was only 62%, which indicates that over one-third of the samples with unsatisfactory results by the 7-h FC test were false-positives as judged against the reference MPN method.

**DISCUSSION**

The 7-h FC test compared favorably with the MPN FC test when used to quantitate FC in potable and other freshwater samples. The procedure was not suitable for measuring FC levels in salt water because of the high proportion of false-negatives (23 of 53). A lower portion of false-negatives (11 of 42) was found when testing potable waters; 10 of these 11 specimens with false-negative 7-h FC results had MPN FC levels of <10/100 ml.

There are at least two possible causes of the low (62%) positive predictive value of the 7-h FC test when used for testing potable waters. First, some of the yellow colonies that appear within 7 h may not be FC. Reasoner et al. (4) reported that over 96% of yellow colonies on m-7h FC medium from surface waters were verified as FC by the MPN technique. However, additional studies, in which yellow colonies from potable-water samples are verified by the MPN technique, are needed. Second, the m-7h FC medium may detect more injured organisms than the MPN technique, although this is less likely, since average counts obtained by the 7-h FC method were lower than those obtained by the MPN FC method.

Current federal recommendations specify that the Enterococcus test be used for monitoring recreational salt waters and either the Enterococcus or E. coli test be used for monitoring fresh recreational waters (8). Both the Enterococcus and E. coli tests require autoclaved media and 48 h of incubation. The 7-h FC test could provide a simpler and more rapid preliminary screening to detect fecal pollution in fresh recreational waters which might be needed for drinking or other purposes in an emergency situation, such as a major earthquake, in which drinking water is unavailable.

The criteria used for evaluating the 7-h FC test as an emergency procedure included ease of use, availability of reagents and materials, accuracy, and correlation with standard methodology. Comments from users, including those in laboratories with no previous experience of membrane filtration methods, were generally favorable. Tests were performed within 8 to 9 h, including incubation time. Yellow FC colonies were easy to distinguish from other colony types.

Although a special medium is needed for the 7-h FC test, it is available from commercial sources upon special request. This alleviates the need for making up medium from individual components in an emergency setting. The medium requires no autoclaving. All materials necessary could be either of the sterile, disposable type or of the reusable type, with boiling water used for sterilization. Thus, the method is ideally suited for emergency use, requiring only an incubator, a stored supply of distilled water, a vacuum, a hot plate, and an alcohol burner. Electric power could be supplied by an emergency generator.

As an alternative to preparation of m-7h FC plates from complete dehydrated medium, they can be prepared from the individual ingredients, which are readily available from commercial suppliers. Future studies are needed to evaluate the effects of batch-to-batch variability of m-7h FC medium prepared from individual ingredients obtained from various manufacturers.

The 7-h FC test yielded higher negative predictive values than positive predictive values for freshwater samples. Thus, the user would have greater confidence in a negative result than in a positive result. Given that the negative result is the desired condition, this further supports the use of the 7-h FC test as an acceptable substitute procedure for determining the quality of potable water in emergency situations. Investigators in the laboratories participating in this study found the 7-h FC test to be a reliable alternative to the standard MPN FC procedure for evaluating freshwater samples.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


