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The Presence-Absence Coliform Test for Monitoring Drinking Water Quality

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Synopsis

The concern for improved monitoring of the sanitary quality of drinking water has prompted interest in alternative methods for the detection of total coliform bacteria. A simplified qualitative presence-absence test has been proposed as an alternate procedure for detecting coliform bacteria in potable water.

In this paper data from four comparative studies were analyzed to compare the recovery of total coliform bacteria from drinking water using the presence-absence test, the multiple fermentation tube procedure, and the membrane filter technique. The four studies were of water samples taken from four different geographic areas of the United States: Hawaii, New England (Vermont and New Hampshire), Oregon, and Pennsylvania. Analysis of the results of these studies were compared, based upon the number of positive samples detected by each method.

Combined recoveries showed the presence-absence test detected significantly higher numbers of samples with coliforms than either the fermentation tube or membrane filter methods, $P<0.01$. *The fermentation tube procedure detected significantly more positive samples than the membrane filter technique,* $P<0.01$. *Based upon the analysis of the combined data base, it is clear that the presence-absence test is as sensitive as the current coliform methods for the examination of potable water. The presence-absence test offers a viable alternative to water utility companies that elect to use the frequency-of-occurrence approach for compliance monitoring.*

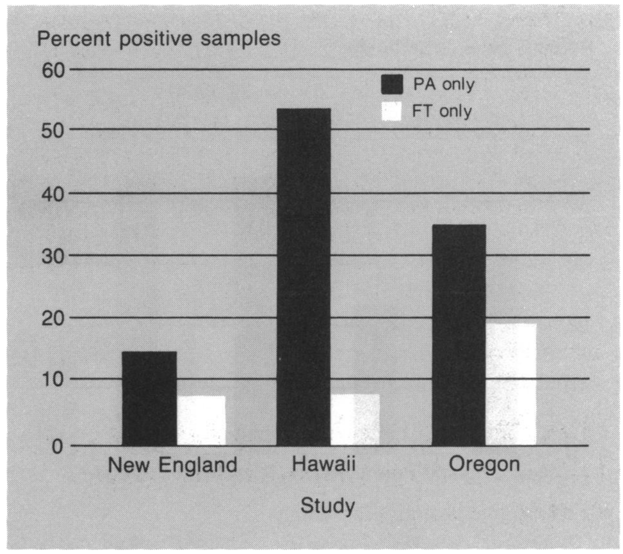
IN THE UNITED STATES, the total coliform group of bacteria serve as the principal microbiological parameter for determining the sanitary quality of drinking water. The National Interim Primary Drinking Water Regulations require that all public water supplies be monitored for coliform bacteria with the use of either the multiple fermentation tube (FT) procedure or the membrane filter (MF) technique (*1*).

Proposed changes to existing regulations call for amending the recommended maximum contaminant level for coliform bacteria to 0 organisms per 100 ml and adopting a frequency-of-occurrence concept for compliance monitoring (*2*). This type of monitoring system would be used to establish a coliform compliance limit based on the fraction of samples containing coliform bacteria during a given period (*3*). This frequency-of-occurrence protocol, the presence-absence concept, would be used in place of the current system wherein compliance is based upon either the arithmetic average of coliform bacteria detected in water samples by the MF technique or the percentage of positive FTs found over a 30-day period. Such data can be obtained from conventional coliform tests (MF or FT) by translating any coliform count or positive tube results into a coliform occurrence. The concern for improved monitoring, especially for small water systems, has prompted interest in the use of a presence-absence (PA) procedure as an alternative method for detecting coliform bacteria in drinking water to determine compliance with a regulation based on the presence-absence concept.

One of the major concerns of monitoring drinking water for coliforms is to ensure that microbial flora or the different substances found in drinking water do not influence the results of detection methods. A search of the scientific literature indicates a paucity of information concerning the effect of geographic differences on coliform monitoring. In this study, we explore the effect of geographic differences on the PA test in terms of coliform detection compared with MF and FT methods now in use.

The use of a simplified presence-absence approach for examining potable water was first proposed by Weiss and Hunter (*4*). A presence-absence procedure has been used extensively in the Province of Ontario, Canada (*5*), and has been compared with the MF technique (*6*). A PA procedure is currently listed as a tentative method for analyzing drinking water samples in "*Standard Methods for the Examination of Water and Wastewater*" (*7*). The PA test, unlike conventional methods, is a qualitative rather than a quantitative procedure. The procedure is a basic modification of the FT method and uses the same verification procedure. Unlike the FT procedure, 100 ml of sample are routinely analyzed. The procedure consists of inoculating the water sample into a bottle containing the appropriate concentration of PA medium (*7*) and a fermentation tube for gas entrapment. The bottle is incubated for 24 to 48 hours at 35° Celsius (C) and inspected for acid and gas production. If gas is noted in the fermentation tube or an acid reaction (as indicated by a color change of the indicator dye) is observed, a small inoculum of the culture is transferred to a tube of brilliant green

Figure 1. Results of the presence-absence (PA) and multiple fermentation tube (FT) tests in three studies



lactose broth for confirmation. The production of gas in the confirmatory medium within 24 to 48 hours at 35°C is related to coliform occurrence. The test is completed within a maximum time of 96 hours, and the results are reported as coliform present or absent.

The implementation of any new procedure for compliance monitoring requires the accumulation of a sufficient database to ensure that the procedure is comparable to the currently accepted methods. These data are especially important for the monitoring of potable water, where the vast majority of samples contain few or no coliforms (*8*). It is imperative, therefore, that any new procedure be able to detect very low numbers of coliform bacteria at a level of sensitivity equal to that of the procedures currently being employed. Recent research findings have indicated that the PA test is more sensitive than either the FT or MF procedures (*9,10*), while others have found the PA test to be at least as sensitive as the FT and MF procedures (*8,11*). The objective of our investigation was to evaluate the coliform data from four studies conducted in different geographic areas to determine the equivalency of the standard PA test with the conventional MF and FT procedures. Only those studies using currently acceptable protocols for coliform detection (*7*) are included in this analysis.

Methods

Four comparative studies relating to the standard PA test and other coliform detection methods have

Figure 2. Results of the presence absence (PA) and membrane filter (MF) tests in four studies

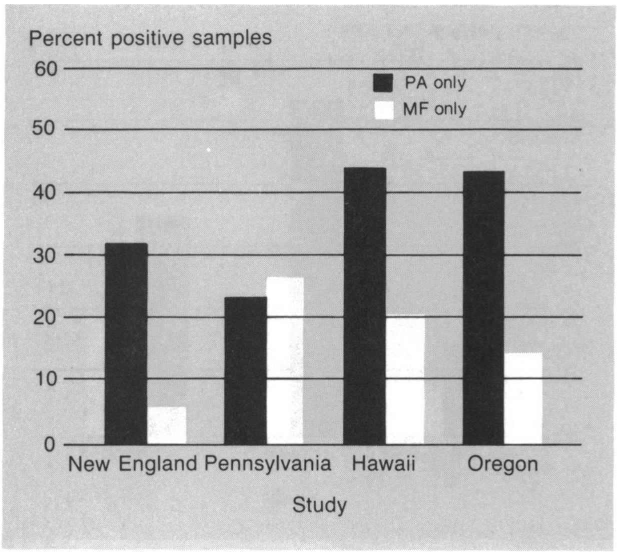
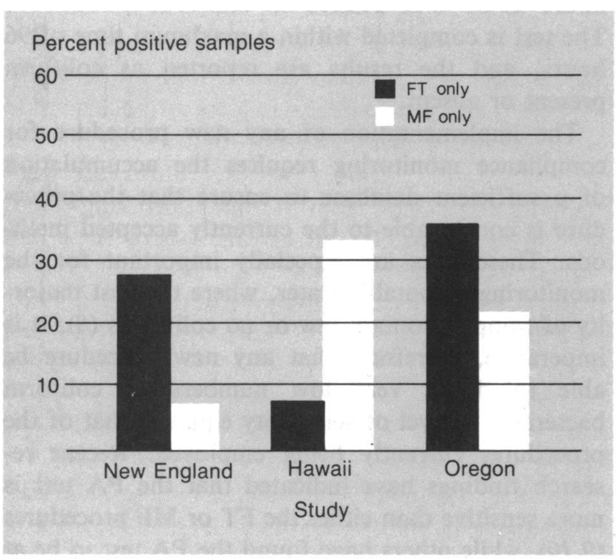


Figure 3. Results of the multiple fermentation tube (FT) and membrane filter (MF) tests in three studies



been conducted in geographically distinct locations (*8–11*). The results of these studies were compared based upon the number of positive samples detected by the various methods. The PA test was compared with the FT procedure and the MF technique for each study individually and for the combined studies. The same analysis was done for the FT and MF comparison. Statistical analysis of the data was accomplished using McNemar's test (*12*) to compare the overall proportion of positive samples detected by the different methods. Large McNemar's chi-square values indicate a significant difference between the two methods. All statistical tests were done at the 0.05 significance level.

Results

In the first major comparative study, Jacobs and coworkers surveyed 15 small community water systems in New England (*9*). Samples were analyzed using the PA, FT, and MF procedures. A 10-tube FT procedure was used, thus allowing 100 ml of water to be examined by each of the three methods. A total of 1,483 samples were analyzed. The PA test and the FT procedure collectively detected 323 positive samples with either one or both tests being positive. The percent positive by the PA test only was 14.9 percent as opposed to 8.4 percent by the FT procedure only (fig. 1). The number of samples positive by either the PA or MF technique, or by both, was 316, with 31.6 percent being detected only by the PA test and 6.3 percent by the MF (fig. 2). Of the 299 samples that were positive by either the FT or MF method, or both, 27.8 percent were positive by the FT only and 8.0 percent were positive by the MF only (fig. 3). McNemar's statistic for each comparison and the corresponding *P* value are shown in the table. The PA test recovered significantly more coliforms than the FT or MF methods, and the FT procedure recovered significantly more coliforms than the MF technique.

In the Pennsylvania study (*8*), 2,601 water samples were analyzed by the PA and MF procedures. The two procedures collectively recovered 569 positive samples. Of these positive samples, 23.2 percent were positive by the PA test alone, and 26.7 percent were positive only by the MF technique (fig. 2). This data set shows more samples positive by the MF than by the PA, but the difference is not statistically significant (see table).

Two hundred water samples collected from potable water systems in Hawaii were analyzed using the PA, FT, and MF methods (*11*). In that study, a 5-tube (50 ml) FT procedure was used. The PA and FT collectively detected 34 positive samples, of which 52.9 percent were positive by the PA only and 8.8 percent by the FT only (fig. 1). A total of 39 samples were found to be positive by the PA and MF, with 43.6 percent being positive by the PA alone and 20.5 percent by the MF alone (fig. 2). Of the 24 samples positive by the FT and MF, 8.3 percent were positive only by the FT, contrasted with 33.3 percent positive by the MF only (fig. 3). Statistical analyses of these data (table) show the PA test to be significantly better than the

FT for coliform recovery. The PA test had a frequency of occurrence greater than twice that of the MF technique, but the results were not significantly different—probably because of the small number of samples analyzed. The FT procedure was not shown to be better than the MF technique for the recovery of coliform organisms. It was noted that the smaller sample volume (50 ml) used in the FT procedure as opposed to the 100 ml volume used in the MF procedure may be responsible for the lack of a significant difference (*11*).

The Oregon study (*10*), which employed the same microbiological methods as the New England study, analyzed water samples from 10 small public water supplies. A total of 1,560 samples were processed. Of all the samples, 322 were positive by the PA or FT test, or both, with 34.5 percent positive by the PA only and 19.6 percent by the FT only (fig. 1). The PA and MF collectively detected 305 positive samples, with 43.0 percent by PA only and 15.1 percent by MF only (fig. 2). A total of 271 samples were positive by the FT and MF methods, with 35.8 percent being positive by FT only and 22.1 percent positive by MF only (fig. 3). The results of statistical analyses of these data are listed in the table. These results are similar to those found in the New England study, with the PA test being significantly better than the FT and MF procedures and the FT procedure detecting more coliforms than the MF technique.

The PA test recovered significantly more coliforms than the FT procedure in the New England, Oregon, and Hawaii studies (*8,10,11*). The combined results yielded a highly significant difference in recovery, $P<0.01$ (see table). The PA test also proved to have better recovery than the MF technique in the New England and Oregon studies, but not in the Pennsylvania and Hawaii studies. The combined result, however, was still highly significant with a McNemar's chi-square statistic of 38.6, $P<0.01$. The FT method was superior to the MF method in the New England and Oregon studies, but not in the Hawaii study. Again, the combined result was highly significant, $P<0.01$.

McNemar's test results for individual and combined studies

Methods compared and study	*McNemar's X^2*	*P value*
PA versus FT		
New England	5.3	0.02
Hawaii	9.3	<0.01
Oregon	12.7	<0.01
Combined	25.5	<0.01
PA versus MF		
New England	52.0	<0.01
Pennsylvania	1.3	0.26
Hawaii	2.6	0.11
Oregon	39.9	<0.01
Combined	38.6	<0.01
FT versus MF		
New England	31.4	<0.01
Hawaii	2.5	0.11
Oregon	8.3	<0.01
Combined	28.9	<0.01

NOTE: PA = presence-absence; FT = multiple fermentation tube; MF, membrane siltration.

Discussion

The analysis of the combined database shows the PA test to be very efficient for coliform detection in drinking water, with a level of recovery significantly higher than the other methods. Combined data show the PA test detected significantly more positive samples than either of the two standard coliform methods (FT and MF). These findings also show the FT procedure to be superior to the MF technique for coliform detection and are in keeping with those reported by Clark comparing the PA with the MF in Canada (*6*). Data from the Canada study were not included in this report because of a medium formulation change used in the Canada study. Rice and coworkers, with the use of the PA medium (*7*) in an FT procedure, rather than as a qualitative test, reported no apparent differences in recovery between the PA and standard FT and MF procedures on water samples obtained from a pilot drinking water treatment plant (*13*). Further analysis of these data using a two-factor blocked analysis of variance (ANOVA), rather than the previously used one-way ANOVA, did detect significantly greater coliform recovery for the PA versus the MF, but not for the FT procedure.

We conclude that the PA test possesses the sensitivity required for the detection of total coliform bacteria in potable water samples. This study indicates that the PA test is at least as sensitive as the current standard methods for detecting coliforms and that this evaluation holds true in various geographic regions in the United States. This test offers a viable alternative to water utility companies that elect to use the frequency-of-occurrence approach for compliance monitoring.

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A Comparison of the Childhood Health Status of Normal Birth Weight and Low Birth Weight Infants

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Synopsis

We analyzed previously unavailable data to describe the national health status in 1981 of noninstitutionalized children who were low birth weight infants. They were compared with normal birth weight children. All data contained in the analysis were based on weighted national estimates. Low birth weight children in general were found to have more chronic conditions, more hospitalizations, more days in bed because of illness, more limitations of activity, poorer health status as perceived by parents, and more school days lost because of illness. However, numbers of physician visits were not different even for low birth weight children younger than 2 years, which is inconsistent with the higher proportions of multiple hospitalizations, chronic conditions, and other illness measures.

The proportions of children in the younger age groups at risk for health problems associated with low birth weight should be increasing. The proportion of very low birth weight children in the younger age groups with higher excessive morbidity measures tends to support the possibility. The increased survival of high-risk infants raises concern about their future requirements for special medical and educational services, and about the resulting stress on their families.

Normal birth weight children were found to make a major contribution to the prevalence of morbidity. It is not the children identified as at risk as a result of low birth weight that comprise most of those with illnesses. The physical, social, and psychological environment after birth probably has the largest impact on the health status of our