Rapid Determination of the Presence of Enteric Bacteria in Water

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A rapid and sensitive method is described for the detection of bacteria in water and various other natural substrates by the isolation of specific bacteriophage. By the addition of large numbers of the organism in question to the sample, the presence of virulent bacteriophage can be demonstrated in as little as 6 to 8 h. Fecal coliform, total coliform, and total coliphage counts were determined for over 150 water samples from several geographical areas over a period of 2 years. Computer analysis of the data shows a high degree of correlation between fecal coliforms and the coliphage present in the samples. With a high correlation coefficient between fecal coliform and coliphage counts, predictions of the fecal coliforms may be made by enumeration of the phage.

With the increasing interest in our water resources, there have been continuing efforts to detect ever lower levels of pathogens. Time-honored methods for determining the bacterial levels of "indicator organisms" are giving way to more precise and simpler direct-count methods of detecting specific organisms. The most-probable-number method has been largely replaced by the membrane filter techniques for total coliforms (TC) or fecal coliforms (FC), but the total, direct enumeration of such pathogens as the salmonellae and shigellae are as yet still impracticable. Total coliform and fecal coliform methods, although specific, take 18 to 24 h. With the detection of bacteriophage, however, the level of fecal contamination can be inferred within 6 to 8 h. This may be accomplished by enumerating the plaques formed when the sample is challenged by susceptible host organisms, thus insuring that each phage particle present will form a plaque. The observed correlation between coliphage and the Escherichia coli host enables one to accurately predict the level of fecal coliforms in the original sample.

To establish the validity of using phage as an indicator of bacteria, it is necessary to know the relationship between them in naturally occurring environments. Quantitation studies were carried out with E. coli and coliphage because most natural water samples do not contain sufficient quantities of Salmonella or Shigella for the purpose of this investigation.

Very little work has been done in the United States in the area of the detection of fecal contamination of water by the use of bacterio-
water. Feigin concluded that the detection of bacteriophage that lyse dysentery bacteria is of sanitary significance when present in a water reservoir.

Instead of adding a known titer of indicator phage to detect the presence of the susceptible host, the procedure may be reversed by adding known indicator bacteria to detect the presence of phage. Takeya (10) detected cholera carriers by utilizing the reverse phage titer rise reaction to detect the kappa-type phage in the bile fluid of the patients. He found the method more rapid and sensitive than culturing for the vibrios.

**MATERIALS AND METHODS**

**Sample sources.** Water samples from six sources were examined independently for TC, FC, and bacteriophage capable of lysing *E. coli* host cells. The samples were collected from the following metropolitan Washington, D.C., and Northern Virginia areas: (i) sewage treatment facility, Manassas, Va.; (ii) Rock Creek, Washington, D.C.; (iii) Holmes Run at Route 95, Alexandria, Va.; (iv) Holmes Run above Lake Barcroft, Bailey’s Crossroads, Va.; (v) Tidal Basin near the Jefferson Monument, Washington, D.C.; and (vi) Potomac River at Haines Point, Washington, D.C. Samples were collected weekly. A sewage treatment facility was included to furnish data for high levels of organisms. Rock Creek may be considered a recreational water since it flows through Rock Creek Park, a large park in Washington, D.C., which has picnic facilities and game areas. After passing through the zoo, Rock Creek empties into the Potomac River well above Haines Point.

**Sampling procedure.** Sterile 500-ml bottles were filled with about 350 to 400 ml of water at the sampling site. The samples were kept cold during transport to the laboratory and were processed within 2 h of collection.

**Bacteriological methods.** TC analyses were carried out on 100-ml samples (diluted when necessary) by the standard membrane filter procedure by using *M*-Endo Agar LES (Difco). FC assays were performed by the membrane filter technique by using *M*-FC Agar (Difco) and incubating for 22 h at 44.5 °C (±0.2 C). All media used were prepared the previous day and stored at 4 C overnight. *M*-Endo Agar LES was used rather than *M*-Endo Medium (Difco) because the metallic sheen on the coliform colonies was more pronounced on the *M*-Endo Agar LES (Difco). This greatly facilitated counting. Agar was used instead of broth for both FC and TC procedures only as a matter of convenience. It was found that both agar and broth gave comparable colony counts.

The bacteriophage counts were carried out in the standard manner by using the following protocol. (i) Shake water sample vigorously (25 or 30 times). (ii) Pipette a 1.0-ml sample into 3.0 ml of fluid semisolid media (at ~45 C), add 0.1 ml of an 18-h culture of host cells, mix by Vortex or inversion, and pour onto a fresh, dry agar plate. (iii) Prepare 10, 50, or 100 such

1.0-ml samples (depending upon the level of contamination of the water sample and the counting precision desired). (iv) Incubate the plates overnight at 37 C in an inverted position. (Plaques may be counted sooner if desired.) Between 10 and 50 1-ml samples were plated for each water sample tested.

**RESULTS**

The results of the water assays were plotted as a log-log plot of total phage versus fecal coliforms for all waters (Fig. 1). From Fig. 1, it appears that a linear equation, \( \log \theta = 0.0065 + 0.866 \log FC \), may be fitted through the data points, even though some individual points are dispersed somewhat widely from the best fit line. To test for the significance of the apparently linear relation, a correlation coefficient was calculated by using standard statistical techniques. A correlation coefficient of unity implies an exact relation between variables, whereas a coefficient near zero implies that there is no relation. A coefficient of 0.95 was calculated for the representation given by the above equation. This result implies a very high probability (greater than 999 in 1,000) that a correlation does exist.

Semilog plots of total phage, total coliforms, fecal coliforms versus sampling dates for a few specific waters are given in Fig. 2 and 3. Figure 3 suggests that the ratio of phage to bacteria is about 0.7:1, regardless of the level of contamination. This ratio (0.7:1) holds over a range of five orders of magnitude of fecal coliform populations ranging from 0.1/ml to over 6,000/ml. Although the best line through the data supports this ratio, it must be remembered that individual data points were sometimes an order of magnitude away from this average. A similar
relationship is true for total coliforms versus phage, with the least square fit approximately 1 phage per 25 total coliforms.

Note in Fig. 1 that the correlation was obtained over a range of 0.1 to over 6,000 fecal coliforms/ml. This represents a concentration variation of over four orders of magnitude. The deviation of the data points from the best fit line is similar, regardless of the concentration, which indicates that the correlation is equally meaningful at both low and high coliform levels.

Figures 2 and 3 show variations of about one order of magnitude within the same water source. In some cases, the change in coliform count varied by this much in a single week. Note that the phage population varies in almost exactly the same manner as the coliform count.

Various linear, logarithmic, and polynomial relationships were examined, considering the following variables: (i) number of fecal coliforms, (ii) number of total coliforms, (iii) total coliforms minus fecal coliforms, (iv) number of phage giving large plaques, medium plaques, small plaques, (v) total number of bacteriophage—large, medium and small plaques, (vi) Potomac River flow, (vii) precipitation on sampling dates, and (viii) precipitation on dates immediately preceding the sampling. The data were examined for (i) each water separately, (ii) all waters taken together, and (iii) all waters except Manassas sewage. Phage were related to coliforms. Phage and coliforms were related to precipitation, river depths, and sampling dates. Although several interesting trends were noted among precipitation, sampling dates, and numbers of phage and coliforms, they were not strong enough to warrant detailed investigation.

In addition to the above, the possibility of improving this correlation by considering a lead-lag relationship was studied. This was done by normalizing the absolute levels of phage and coliform and considering the possible effect of an imbalance between them. That is, it is conceivable that a high bacteria count at some given time could result in a high rate of lysis by phage. This in turn would result in a high rate of phage replication and loss of some bacteria by lysis. Thus, at some later time, the phage population might be abnormally high and the bacteria count might be relatively low. It was determined, however, that no correlation represented an improvement over that obtained by relating phage and coliform counts taken at the same time.

The results of these correlations were not as good as that obtained by correlating phage and coliform counts, ignoring observable external influences. Thus, although the external events,
such as precipitation, may have affected the absolute levels of contamination, they did not appear to have any significant effect upon the ratio of phage to coliforms, either fecal or total.

DISCUSSION

A technique has been developed wherein detection of coliphage has been used as a quantitative indication for coliform bacteria. The method requires as little as 2 or as many as 8 h to obtain results, depending upon the level of contamination. The quantitative relationship between coliphage and coliforms in natural waters has been established. Results show that the use of coliphage as an indicator will provide presumptive coliform counts that agree with actual counts.

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