Testing Drinking Water for Coliphage as a Fecal Quality Indicator

Advances in Rapid Coliphage Detection

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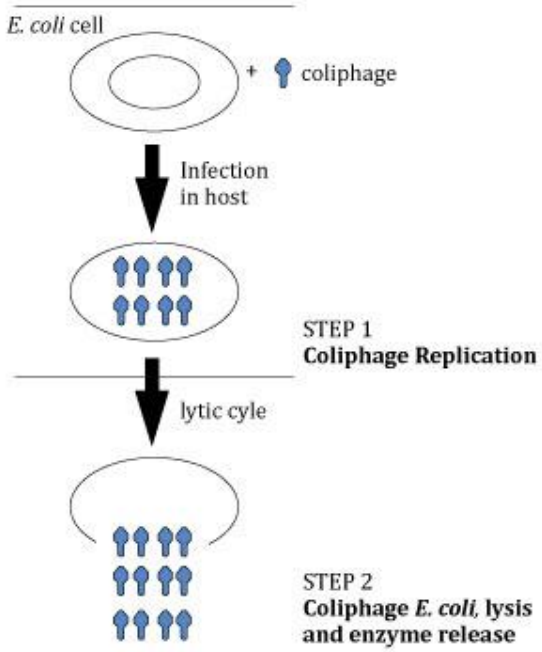
Drinking water production includes a series of processing steps designed to reduce the risk of chemical and biological contamination(1, 2). The most common source of biological contamination is sewage pollution from human or animal waste. Raw sewage may contain pathogenic bacteria and viruses that cause illness but it also contains a higher prevalence of innocuous fecal indicators, such as enteric bacteria and bacteriophages (3). These are used as fecal indicators to assess water processing quality and integrity based on the assumption that by controlling the indicators, one controls for the less prevalent pathogens. The most common fecal indicators used in water testing for regulatory compliance are coliform and *E. coli* bacteria. In 2006, fecal *enterococci* bacteria and coliphage (bacteriophage in the coliform group of bacteria) were listed as equivalent fecal markers to *E. coli* in the Ground Water Rule (GWR) (4).



←Figure 1.

Coliphage infection of *E. coli* produces multiple progeny that are released during the lytic cycle after replication. The multiple progeny can then re-infect and replicate to produce a detectable reaction between 4-6 hours after first infection.

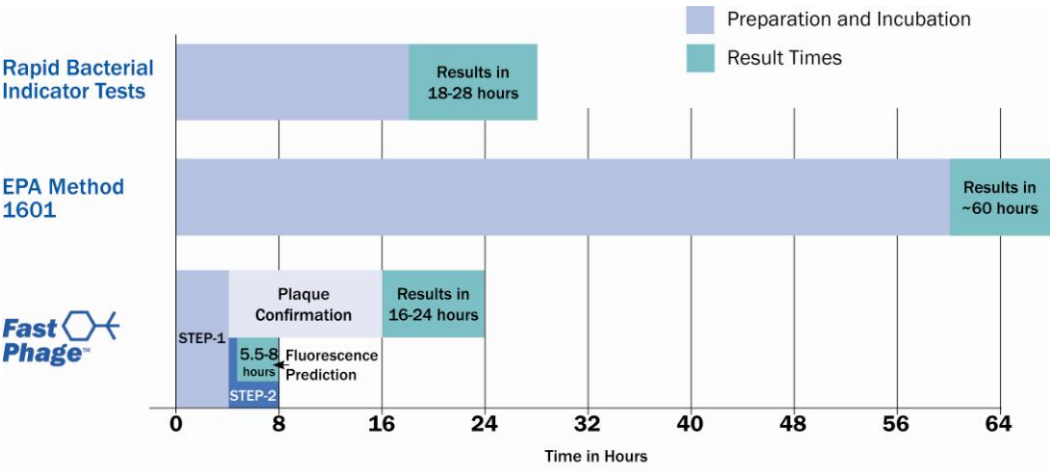
Coliphage were added to the GWR as an equivalent indicator to *E. coli* based on 20 years of epidemiological data showing that over 50% of waterborne illnesses in the US were viral in origin (5). Coliphage are bacterial viruses and some types are structurally similar to pathogenic enteric viruses. They contain either a protein outer coat and DNA or either single- or double-stranded RNA in the interior (6). Some male specific coliphage are morphologically similar to enteric viruses and have similar chemical resistance to water disinfection. They are shed in fecal material and do not replicate naturally in water systems without the presence of coliform bacteria, making them a useful indicator of water pollution and they can also be detected quickly making them a good indicator for measuring viral risk in the water supply.

A study of distribution system infiltration suggests that coliphage monitoring could be a useful tool for tracking and controlling low levels of contamination, particularly for chloraminated water systems (7). In beach water studies, the presence of FRNA coliphages was found to have a 0.99 predictive quality relative to the presence of adenovirus (8). In ground water systems, primarily made up of negative samples, there was however, poor correlation between all indicators, including coliphages and human adenovirus as detected by PCR (not necessarily viable bacteria) (9). More studies of at-risk drinking water systems are needed to relate the coliphage indicator to the incidence of viruses in drinking water.

It is recognized that some ground water supplies are more prone to viral contamination than others. Public health officials agree that bacterial indicators and viral indicators provide different information about the water processes and that it is possible to detect viruses without bacteria and bacteria without viruses. The most comprehensive risk assessment of water is to test for both bacterial and viral indicators because they give complementary information. The National Drinking Water Advisory Council recommended during the Ground Water Rule comment period, that both bacterial and viral indicators should be included in water safety testing as each indicates a different microbial risk (4). Testing for both indicators would give a more complete picture of ground water quality. However, due to cost constraints and the complexity of earlier coliphage methods, requiring testing for both indicators was previously not considered economically feasible (10).

Current drinking water regulations mandate testing for fecal contamination using bacterial indicators: total coliform and *E. coli* (11). Since the the 1970's when the Total Coliform Rule (TCR) required coliform and *E. coli* testing, there have been numerous method developments to quicken, simplify and lower the cost of coliform and *E. coli* testing. This has stimulated point-of-use testing, quickening corrective response to bacterial contamination to less than 48 hours in some cases. Traditional coliphage testing has been more complicated and expensive than *E. coli* methods. The official method coliphage qualitative assessment, EPA Method 1601, uses multiple steps and reagents and takes over 48 hours or longer for a result (12). Due to the complexity of the protocol and labor involved it was unlikely that water municipalities or regulators would choose to perform a coliphage test unless there was an expected high risk of viral contamination. A shortage of certified and confirmatory laboratories that can confirm coliphage positive tests is another reason why coliphage testing has been a less popular alternative to coliform and *E. coli* testing. With the recognition in the GWR that viral indicators are an equivalent indicators to *E. coli*, there are now efforts to simplify and quicken coliphage methods as was seen with *E. coli* diagnostic tests under the TCR.

Figure 2. Timeline of enzyme-substate coliform/*E. coli* tests compared to conventional coliphage assay EPA Method 1601 and Fast Phage.



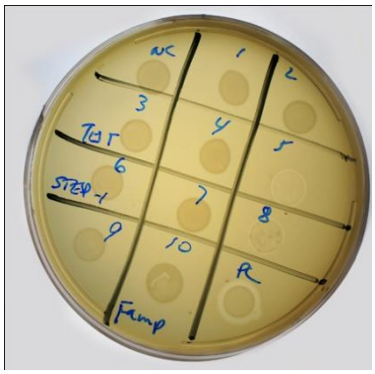
Fast Phage is a new commercially available coliphage testing kit for drinking water (13). The presence/absence format is a modified form of Method 1601 involving an enrichment culture in host *E. coli* followed by detection steps (see Figure 2). One of the detection steps involves a fluorescent indicator that gives a result in the same working day as the sample was tested, i.e. in 8

hours or less (see Figure 3). The test also includes a traditional confirmation step onto an *E. coli* lawn, equivalent result to Method 1601 (15,16) (see Figure 4). The entire testing process takes as little as 16 hours. The same-day predictive result allows for a rapid remedial response. Reagents are packaged ready-to-use to avoid time-consuming preparation. Costs are consistent with rapid coliform/*E. coli* screening tests. It has been demonstrated by 5 separate laboratories that the Fast Phage method can be validated using the Tier 1 guidelines of EPA Method 1601 to demonstrate it is an acceptable modification of the performance based system method (15). Equivalence between Method 1601 and Fast Phage was further verified by four water test laboratories in a national Tier 2 study using shared waste water to spike a variety of ground waters following an EPA approved protocol (16).

←Figure 3. UV 366 nm light image of negative and positive test.

<u>Left:</u> Negative = Turbid and not fluorescent =absence of coliphage <1 pfu (plaque forming unit) /100 ml

<u>Right:</u> Positive = Fluorescent and possibly less turbid= presence > 1 pfu coliphage/100 ml



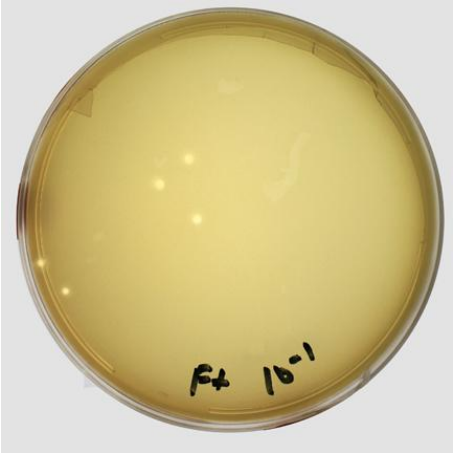
←Figure 4. Step 1 Plate- Conventional plaque confirmation involve spotting the *E. coli* cultures in water sample on a lawn of *E. coli* seeded agar. Zones of inhibition observed in samples 5, 8 and PC (positive control) indicate positive coliphage samples.

The fluorescence detection aspect of Fast Phage may also be conducted in a most probable number (MPN) format that takes less than 6 hours. In this format the sample is not pre-enriched but is added along with host *E. coli* to fluorescent media. The sample is then immediately divided into a MPN compartment design and incubated. Fluorescent subdivisions are counted for MPN approximation of pfu/sample, see Figure 5. The somatic method gives results comparable to plaque enumeration methods such as EPA Method 1602 and double-layer agar techniques, see Figure 6 (13). While coliphage is not a regulated indicator in source water, MPN determination in a 5 to 6 hour time period could be valuable in performing risk assessment of fecal pollution in water sources and its implied risks to water quality. This has potential application to waters used in produce production, e.g. irrigation and process waters, and in fish farming.

←Figure 5.

<u>Top:</u> Fluorescent view of TEMPO® 4 ml MPN format. 5 of 16 positive top wells, 15 of 16 fluorescent positive middle wells and 16 of 16 positive bottom wells give a calculated MPN of 140 pfu/ml.

<u>Bottom:</u> Fluorescent view of 2 Quant-Tray® 2000 MPN format. Two 100 ml samples with 45^4 = 116 pfu/100 ml and 19^5 = 29.8 pfu/100 ml



←Figure 6.

DAL (Double Layer Agar) Plate – Plaques observed in a soft agar layer of seeded *E. coli* is a conventional method used to quantitate coliphage in 100-500 µl water samples.

Continuous monitoring for fecal contamination in water is another Fast Phage application made possible using coliphage as the contamination indicator. Host *E. coli* can be brought into a continuous exponential growth state in a simple chemostat that continuously feeds a water/media mixture at a rate equal to the bacteria generation. If coliphage are in the water, the steady state of the *E. coli* is disrupted by the faster growing coliphage and their multiple progeny produced by the viral infection. A rapid fluorescence response is seen when coliphage enter the system. When coliphage are absent, the test sample volume continuously grows to liter volumes, providing greater assurance that water contamination risk is low (13). In addition, when the water sample is continuously drawn from a flowing distribution source, the sample is a dynamic representation of the entire flow through the system. Continuous samples, which are steadily drawn over time, can detect heterogeneous contamination events that might be missed by batch sampling at a particular time. Larger volume testing and improved sample representation can give added assurances that water is safe and processes are functional.

Coliphage testing is a viral indication assessment of water that provides additional and complementary information to bacterial indicator testing and gives added assurances that water processes are functional. When water supplies are at risk of fecal contamination, a same-day test enables fast detection and corrective response to these risks. Additionally, coliphage has shown correlation to fecal infusion into distribution systems and could be a useful tool in leak diagnosis and effective emergency repair.

Water municipalities are under increasing demands with aging infrastructure, population encroachment on the water supplies, storm-related pollution and reduced budgets. Despite these problems they must meet increasing regulatory water quality criteria and provide pure and safe water to consumers. Technological tools that simplify water analysis and perform broad risk assessment, such as Fast Phage, will help municipalities meet those challenges.

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