



# BACTERIOPHAGE

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Viruses that infect bacterial cells are called bacteriophage. There are a variety of these viruses, called coliphage, which infect many subspecies of *Escherichia coli*. These phages are commonly present in wastewater in relatively large numbers as compared to enteric animal viruses. Their source is the feces of humans and animals.

## *Male-Specific Coliphage*

A variety of coliphage are called "male specific" because they infect the bacteria via the pili (small appendages on the bacterium's surface) and bacteria with these appendages are called "male". Certain of these male specific phage, MS-2 for example, have the same shape and size of small enteroviruses (25 nm) and contain single stranded RNA. It has been noted that they demonstrate the same or greater resistance to environmental factors, including disinfection, as do the most resistant animal enteroviruses.

## *Somatic Coliphage*

Those coliphage that infect the bacteria via the cell membrane are called somatic coliphage. These are also present in wastewater but because of the similarity of many of the male coliphage with animal enteric viruses the latter are of more interest as surrogates for the presence of animal viruses.

## **SURROGATE FOR ENTERIC VIRUS?**

With the relative abundance of coliphage in wastewater; their ease in detection; and, their apparent equivalency to the survival characteristics of important enteric viruses, there is a growing interest in the use of these coliphage to monitor the capability and reliability of a treatment process to reduce enteric viruses to acceptable levels. They appear to be particularly useful in evaluating the ability of new or

innovative water and wastewater treatment processes to remove viruses. In those instances where the *in situ* coliphage concentration is too low for the demonstration of orders of magnitude change the treatment process can be challenged with the addition of high concentrations of cultured coliphage. BioVir has been involved in a number of successful challenge studies of UV and membrane systems throughout the country.

## **HAZARD**

Bacteriophage only infect bacteria and so do not pose any public health risk.

## **DETECTION**

In the laboratory, these viruses are detected by the formation of plaques on "lawns" of susceptible *E. coli*. This procedure is easier and less costly than the use of tissue culture as employed with animal viruses.

In many instances the detection of male specific coliphage is desirable. Unfortunately some *E. coli* hosts are also susceptible to somatic coliphage. In some instances the numbers of these phage in a given sample are equal to the number of male specific phage. It is important to select the correct bacterial host when conducting these assays.

## **ANALYTICAL METHOD(S)**

There are a number of methods available for the detection of coliphage in water samples. All of the methods rely, to one degree or another, on the ability of virus to infect its host and cause lysis of the bacterial cell. This lysis is evidenced by the formation of plaques (a clearing) in a "lawn" of host cells growing in



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a nutrient agar. The most common assay method employs the inoculation of the bacterial lawn with a known volume of sample and enumerating the resultant plaques. The results are reported as plaque forming units (Pfu) per volume of water sample inoculated. With this method the volume of sample that can be conveniently used is limited.

In cases where larger volumes are required an MPN method can be used. In this instance the sample is set up much in the same fashion as the coliform MPN except that each tube of broth contains an inoculum of a susceptible *E. coli* strain. As the tubes containing the various dilutions of sample water are incubating the *E. coli* multiplies and supports the reproduction of coliphage that are present in the sample. In essence this magnifies the numbers present to easily detectable levels. At the end of the incubation period the presence or absence of coliphage in each tube is confirmed by spotting a small amount of the tube contents on to a "lawn" of host bacteria. The MPN value is determined in the same manner as in the coliform test except that a positive tube is one that contained coliphage as evidenced by the "spot" tests.

There are also presence absence methods which are analogous to the MPN method except that a single large sample volume (1L for example) is enriched by the addition of growth medium and host bacteria, incubated and the presence or absence of coliphage determined by "spot test". The EPA draft methods 1601 and 1602 are directed to the detection of coliphage in ground water using large volume samples.

Results are usually obtained within 24 - 48 hours of sample inoculation depending on the assay method employed.

## SAMPLING

Sampling is no more complicated than sampling for coliforms. A 100 mL sample is generally sufficient and adherence to a 24 holding time is recommended.

## FURTHER INFORMATION

For more information regarding Virus sampling, detection and current regulation, please call BioVir Laboratories at 1-800-GIARDIA (442-7342).