# Phages Demystified

## A key parameter in recycled water testing

## A New Indicator

**Water quality in Australia has long been measured in part by determining the presence or absence of coliforms. Coliforms are a type of bacteria known as an indicator organism: its presence indicates that other pathogenic organisms of fecal origin may be present. While coliforms, and particularly E. coli, remain an important measure of the sanitary quality of foods and water, a 'new' parameter has recently stepped to centre stage as Queenslanders look more and more to recycling water.**

Coliphages, known simply as phages, have risen up from anonymity because their presence is an indication of the presence of enteroviruses—viruses that infect the human intestinal tract—and hence are a required testing parameter in the Queensland Water Recycling Guidelines for A+ quality.

## Bacteria and Viruses

Before we jump straight into the coliphages, let's review some of our basic high school biology. We all recall that bacteria are single-celled micro-organisms that are ubiquitous in every habitat on Earth. Many bacteria are essential for our survival, being responsible for such crucial activities as fixing Nitrogen from the atmosphere into a useable form, and breaking down dead plants and animals, thus enriching the soil. But many bacteria can also be harmful to us, such as the infamous E. coli, among many others.

Viruses are another bag altogether. A virus is, arguably, not actually a living thing—although it does have some life-like characteristics. Rather, a virus is more like a tiny piece of information, in the form of DNA, that can invade a host cell and change its functions, often causing illness or disease. Most viruses prefer a specific type of host cell—it might be human cells (such as the Hepatitis A virus), bird cells (such as the virus that causes Bird Flu), or bacterial cells.

However, a virus can exist just fine without a host for long periods of time, just on its own, waiting patiently for an unsuspecting host to meander by. This is a crucial point when considering wastewater treatment processes, because certain processes will kill all the bacteria, but the viruses can survive those processes. Hence the need for a different indicator organism to ensure that no viruses exist in a water sample.

(For more information about the processes used to treat recycled water, see our Position Paper on Recycled Water, available in the Resource Centre at www.simmondsbristow.com.au.)

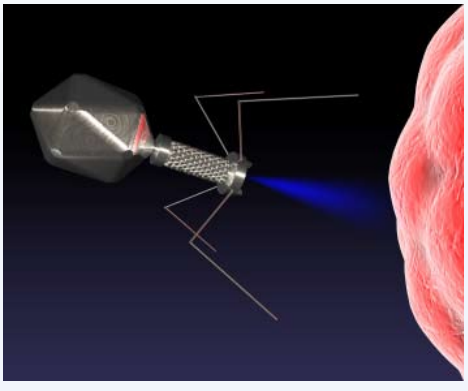
## What is a Coliphage?

That brings us to the coliphage. Viruses that specifically invade bacterial cells are called bacteriophages. Coliphages are bacteriophages that infect coliform bacteria. So the coliphages themselves are not specifically harmful to humans, because they prefer bacterial cells; however they are a useful indicator for the presence of enteroviruses because the two types of viruses have identical survival characteristics.

What's more, coliphages are excreted in large numbers by humans and other warm-blooded animals, meaning phages are abundant in raw sewage, with more than 100,000 per 100ml of raw sewage. This ample ratio makes them easy and cheap to detect and culture.

The relative abundance of coliphages in wastewater, the ease of detection and their equivalence to the survival characteristics of enteroviruses make them a useful indicator organism in evaluating treatment processes for wastewater.

In a nutshell, if you get rid of all the coliphages in raw sewage, you can be certain you've also taken out all the enteroviruses. You can also be sure that you've gotten rid of the bacteria, because phages are hardier, and withstand treatment processes much better than bacteria. The opposite is emphatically not true: it is very possible to have a negative result for coliform bacteria, but phages (and also enteroviruses) can still exist.



A 3D computer rendering of a bacteriophage

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### Why test for phages?

Recycled water that is contaminated with phages is not suitable for use, because the water may be infected with harmful enteroviruses. (In fact, the same could be said of non-recycled water, or any water for that matter. But other water sources—such as rain water or dam water—have journeyed through our planet's lengthy and complex water cycle and so have a very low risk of containing enteroviruses or coliphages.) This poses a possible but serious health risk even if used for irrigation. The presence of pathogenic human viruses in water has been responsible for outbreaks of such illnesses as gastroenteritis and Hepatitis A in many parts of the world.

Because enteroviruses are more resistant to disinfection (such as chlorination) than coliform bacteria, merely testing for faecal coliforms or E. coli is not enough; the absence of these bacteria does not guarantee the absence of enteroviruses, and hence does not eliminate the potential transmission of viral diseases.

Enteroviruses are sensitive to UV exposure and other treatment technologies, as are coliphages; hence, to adequately detect enteroviruses in recycled water it is essential to test specifically for phages.

The Queensland Water Recycling Guidelines stipulate that A+ recycled water should have a phage count of <1 pfu/100mL. Specifically, the guidelines recommend two coliphages in particular be tested: the somatic coliphage, and the male specific (F+) coliphage.

### How is the testing done?

Phage testing is not a common parameter in most laboratories. In fact, detection of human enteric viruses in waters and wastewaters is beyond the capabilities of most water laboratories.

Traditionally, to detect human enteroviruses, cell culture techniques were used, which is difficult, time-consuming and labour-intensive.

The utilisation of coliphages as an indicator organism means detecting enteroviruses is now becoming relatively inexpensive and quick, although phage testing methods are continually being refined and updated.

Phage testing is usually done using a culture based, pour plate method. This means a volume of the water sample is placed on a specially made medium that is designed to encourage any coliphages present to 'reproduce' successfully (or infect more and more cells). Actually, a host bacterium is added to the plates, usually a strain of E.coli, in which the phages replicate if they are present. If there are phages present, clear zones called plaques appear on the plates which can be interpreted in a similar way to growth of bacteria on a plate. No phages, no plaques seen.

Assessments in different laboratories vary in sensitivity, using anywhere from two to eight plates for detection. Some laboratories will have a higher limit of reporting depending on how much sample, i.e. 10mL or 100mL, is used to conduct the test. The test results are normally available within 24 hours.

The use of bacterial (such as E. coli) and viral (such as coliphages) indicators provides a high degree of assurance regarding the safety of recycled water, and a properly designed risk assessment will guarantee protection of human and environmental health.

*References*

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Testing in the laboratory.

Clear zones, called plaques, indicate the presence of phages.

Phage investigation in the Microbiology Laboratory.