

Chapter 7: Virological compliance

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7.1 Introduction

No maximum acceptable values (MAVs) have been set for human viruses in the *Drinking-water Standards for New Zealand 2005 (revised 2008)*. It is likely that a MAV or MAVs will be established in a future edition. This chapter foreshadows such developments.

In the absence of any MAVs for viruses in the current DWSNZ it should be understood that if they are specifically sought in drinking-water, they should not be detected. If detected, advice should be sought from the relevant health authorities.

Neither the Australian Drinking Water Guidelines (NHMRC, NRMCC 2011) nor the WHO (2011) Guidelines include a guideline value for any viruses. Health Canada (2011) (<http://www.hc-sc.gc.ca/ewh-semt/water-eau/index-eng.php>) has specified a treatment goal of a minimum of 4 log reduction and/or inactivation of enteric viruses.

In the US, the Surface Water Treatment Rule (SWTR) (54 FR 27486, June 29, 1989) applies to all PWSs using surface water or ground water under the direct influence of surface water as sources. It established MCLGs of zero for *Giardia lamblia*, viruses, and *Legionella*, and includes the following treatment technique requirements to reduce exposure to pathogenic microorganisms:

- 1 filtration, unless specific avoidance criteria are met
- 2 maintenance of a disinfectant residual in the distribution system
- 3 removal and/or inactivation of 3-log (99.9%) of *Giardia lamblia* and **4-log (99.99%) of viruses**
- 4 maximum allowable turbidity in the combined filter effluent of 5 NTU and 95th percentile turbidity of 0.5 NTU or less for plants using conventional treatment or direct filtration (with different standards for other filtration technologies)
- 5 watershed protection and source water quality requirements for unfiltered PWSs.

There are more than 140 different types of human enteric viruses that may contaminate potable source waters. These include several important groups: Hepatitis A virus, Hepatitis E virus, norovirus, enterovirus and adenovirus, that have been associated with waterborne illness and are capable of causing severe, and in some cases fatal, infections. Individual datasheets have been prepared for the more important viruses.

Viruses are obligate intracellular parasites, which means they cannot grow or multiply outside their host. Viruses simply consist of a nucleic acid genome (either ribonucleic acid [RNA] or deoxyribonucleic acid [DNA]) surrounded by a protein capsid and, in some cases, a lipoprotein envelope. These viruses are very small, ranging from 20–80 nm (0.02–0.08 micrometres) in diameter; see Figure 4.2 in Chapter 4 to gain a perspective of their size.

Human enteric viruses are present in the gut, respiratory tract and occasionally urine of an infected person, and are discharged with body wastes into wastewater and the environment. Infected people do not always show signs of illness (they are asymptomatic) but they will still produce viruses in their wastes. Specific viruses or strains of viruses are not always present in a community at any one time, but representatives of the large groups (eg, adenovirus or enterovirus) are generally present on most occasions.

Enteric viruses may be found in high numbers in domestic wastewater. Recent New Zealand studies have shown adenovirus and enteroviruses to be present in concentrations greater 10,000 infectious virus units per litre of wastewater (DRG 2002). The number of viruses in wastewater varies with the level of infection in the community but, in general, human viruses will always be present in wastewater, averaging 100–1,000 infectious viruses/L, occasionally reaching very high levels of >10,000 infectious virus/L (Lewis et al 1986). Wastewater treatment processes that do not include a disinfection step may be inefficient in removing or inactivating viruses (<90 percent removal) so viruses may be found in the raw water.

Human enteric viruses cannot multiply in the environment once outside the host. The viruses are characterised by the ability to survive (ie, retain capability to cause infection) for days, weeks or longer, in the environment depending on the type of water, season and other factors (Hunter 1997). In general, as the temperature increases, the survival time decreases. Exposure to UV light (including sunlight) also shortens the survival time of viruses. In a Finnish study (Kauppinen et al 2017) viral genomes of noro- and adenoviruses could be detected in contaminated water samples after being stored at 4°C up to 1277 and 1343 days, respectively.

A large proportion of the human viruses present in source drinking-waters will normally be removed or inactivated by well-operated standard drinking-water treatment processes.

Routine monitoring for viruses in treated water and source water is currently impractical in most situations in New Zealand because of the high cost of sampling and analysis, and problems of detection of a full range of the viruses occurring.

Not all viruses pose a health risk to humans. For example, a pesticide product, *carpovirusine* (see PMEP), contains the active ingredient codling moth granulosis virus (CpGV). This substance appears on the NZFSA's complete database of Agricultural Compounds and Veterinary Medicines (ACVM) as at 2009 (see <https://eatsafe.nzfsa.govt.nz/web/public/acvm-register> and select entire register). It is also approved in many other countries to control codling moth on apples and pears.

National Guidelines for separation distances based on virus transport between on-site domestic wastewater systems and wells have been developed (ESR 2010a). Previous studies using bacterial rather than viral transport as the basis for guidelines is a shortcoming for two main reasons. The survival characteristics of viruses favour their transportation over long distances in aquifers, and their high infectivity means they can cause disease, even though their numbers may have been substantially reduced during transport. The final separation distances given in the Guidelines are based on rotavirus and hepatitis A virus. Rotaviruses were selected because they are among the most infectious viruses and are shed at the greatest concentrations by infected individuals. Many tables are provided, covering a range of vadose zone and saturated zone conditions.

WHO (2006) discusses studies and findings on avian influenza pertaining to water resources, water supplies, sanitation (human excreta, sewerage systems and health care waste) and hygiene. There is a datasheet for influenza viruses.

7.2 Health significance of human viruses in drinking-water

Section 11.2 of WHO (2004 and 2011) begin with:

Viruses associated with waterborne transmission are predominantly those that can infect the gastrointestinal tract and are excreted in the faeces of infected humans (enteric viruses). With the exception of hepatitis E virus, humans are considered to be the only source of human infectious species. Enteric viruses typically cause acute disease with a short incubation period. Water may also play a role in the transmission of other viruses with different modes of action. As a group, viruses can cause a wide variety of infections and symptoms involving different routes of transmission, routes and sites of infection and routes of excretion. The combination of these routes and sites of infection can vary and will not always follow expected patterns. For example, viruses that are considered to primarily cause respiratory infections and symptoms are usually transmitted by person-to-person spread of respiratory droplets. However, some of these respiratory viruses may be discharged in faeces, leading to potential contamination of water and subsequent transmission through aerosols and droplets. Another example is viruses excreted in urine, such as polyomaviruses, which could contaminate and then be potentially transmitted by water, with possible long-term health effects, such as cancer, that are not readily associated epidemiologically with waterborne transmission.

Hepatitis A virus, Hepatitis E virus, norovirus, enterovirus adenovirus and rotavirus may occur in drinking-water where they are present in the source water and when water treatment does not remove them completely. Very few human enteric viruses (1–50 virus particles depending on type) are required to produce an infection in a susceptible person (Hunter 1997; Teunis et al 2008). The symptoms generally attributed to enteric viruses are gastroenteritis and diarrhoea but they can also cause hepatitis, respiratory, central nervous system, liver, muscular and heart infections. Some waterborne viruses have also been associated with some forms of diabetes, chronic fatigue syndrome and dementia (Nwachuku and Gerba 2004; Ashbolt 2004; Klemola et al 2008). The major groups of viruses contaminating water are discussed below but may not represent all the viruses likely to be transmitted by water. It is reasonable to expect that further important groups of waterborne viruses will be detected in the future and that these will most likely cause atypical waterborne disease (Nwachuku and Gerba 2004; Ashbolt 2004). In general, viruses are host specific, so viruses that infect animals or plants do not usually infect humans, although a small number of enteric viruses have been detected in both humans and animals. There are more than 140 enteric viruses known to infect humans.

Norovirus: this group of caliciviruses includes the Norwalk and Norwalk-like viruses. Noroviruses are currently subdivided into seven genogroups (GI to GVII), which are composed of more than 40 distinct genotypes. Members of this group are strongly associated with waterborne outbreaks in many parts of the world. Symptoms of infection are self-limiting and include vomiting, diarrhoea and nausea over 24–48 hours. Norovirus is quite prevalent in New Zealand and is responsible for a large proportion of viral gastroenteritis reported to health authorities (ESR 2004). This virus is one of the easiest to link to a common source outbreak as the symptoms occur rapidly after contact with the virus (approximately 24 hours). More than 200 people developed acute gastroenteritis in July 2006 when sewage contaminated the water supply (which had inadequate treatment) at a South Island ski resort. The illness was caused by norovirus genogroup GI-5 (Hewitt et al 2007).

Hepatitis A and E: Hepatitis A and E have a relatively low occurrence in New Zealand (ESR 2004) but induce quite significant symptoms including fever, malaise anorexia and jaundice. The disease caused by these viruses is essentially clinically indistinguishable and is generally self-limiting but has a 1 to 2 percent mortality rate. The infectious doses for these viruses are relatively low (10–100 viruses) and symptoms do not occur until 10–50 days after infection. Internationally Hepatitis A and E outbreaks have frequently been associated with water (Kasorndorkbua et al 2005; Meng 2005; Vasickova et al 2005; Kuniholm et al 2009). The other hepatitis viruses appear not to be transmitted via the faecal–oral route.

Enteroviruses and adenoviruses: these two different groups represent the viruses (over 300) that are most commonly found in contaminated surface water (Ashbolt 2004). These viruses produce a very broad range of symptoms including respiratory, skin and eye, nervous system, liver, heart and muscular systems. Gastroenteritis with vomiting and diarrhoea is a less common outcome of infection with these viruses and is limited to only a few adenovirus and enterovirus types. Reported waterborne outbreaks of these viruses, other than in swimming pools, are very infrequent. It is not clear whether lack of reporting is because the dominant symptoms produced by these viruses are not those traditionally associated with water or food borne disease, or because such outbreaks are indeed rare (Hunter 1997). Enteroviruses include the polioviruses, echoviruses and coxsackieviruses A and B. Adenovirus is the most UV resistant virus known Linden and Sobsey 2005). Coxsackie B5 (CB5) is an enterovirus known to be highly resistant to chlorine in wastewater of varying turbidity, and Adenovirus 2 is a respiratory virus known to be highly resistant to monochloramine, in wastewater of varying turbidity (Keegan et al 2012).

Rotavirus: rotavirus has been detected in sewage, rivers and lakes, and treated drinking-water. Transmission occurs via the faecal-to-oral route. Cases of infection tend to be sporadic but several waterborne outbreaks have been reported (Gratacap-Cavallier et al 2000; Parashar et al 2003; Amar et al 2005). Rotaviruses are responsible for a large proportion of severe episodes of diarrhoea in small children and infants, and they also cause gastroenteritis in the elderly. They are responsible for as much as 50 percent of the gastroenteritis in hospitalised paediatric patients during the cooler months of the year in temperate climates (Parashar et al 2003). Acute infection is characterised by the abrupt onset of severe watery diarrhoea with fever and vomiting. Dehydration and metabolic acidosis may develop, resulting in death if untreated. Children aged 6 to 24 months are the most severely affected. Rotaviruses are ubiquitous, infecting over 90 percent of all children up to three years of age internationally (Pérez-Vargas et al 2006; Brooks et al 2007). New Zealand, however, does not have a human rotavirus surveillance programme so the prevalence of rotavirus and its disease burden cannot be estimated.

Astroviruses: astroviruses are members of the *Astroviridae* family. They are divided into eight serotypes (HAst1-8); novel types continue to be discovered. Astroviruses comprise two genogroups (A and B) capable of infecting humans. Astrovirus infection typically results in diarrhoea lasting 2–3 days, with an initial incubation period of 1 to 5 days. Infection generally results in milder diarrhoea than that caused by rotavirus and does not lead to significant dehydration. Other symptoms that have been recorded as a result of astrovirus infection include headache, malaise, nausea, vomiting and mild fever. Healthy individuals generally acquire good immunity to the disease, so reinfection is rare. Astrovirus infections generally peak during winter and spring.

Ebola virus: Ebola virus is not likely to be found in New Zealand so a datasheet has not been prepared. WHO (2014 and 2016) provided the following. Ebola virus belongs in the Filoviridae family. Ebola is transmitted through direct contact with blood or bodily fluids of an infected person or animal, and is not transmitted through air, water or sewage. The characteristics of the Ebola virus suggest that it is likely to be relatively fragile in the environment in comparison with the enteric viruses that commonly cause diarrhoeal disease. To date, there is no evidence for transmission of Ebola viruses via drinking-water contaminated by faeces or urine. The virus is unlikely to survive for extended periods outside of the body. Higher temperatures (room temperature or above) are likely to increase the speed at which the virus dies off in the environment. There is no evidence to date that Ebola has been transmitted via sewerage systems, with or without wastewater treatment. Current thinking is that Ebola survives in water for only a matter of minutes. Once in water, the host cell will take in water in an attempt to equalise the osmotic pressure, causing the cells to swell and burst, thus killing the virus. WRA (2014) states that based on current knowledge, water supplies which receive an adequate level of treatment and disinfection to remove the common human enteric viruses are not likely to pose any risk of transmission of the Ebola virus – even if the raw water contains faecal contamination from Ebola cases. At a WHO Workshop results of a study on the persistence of Ebola virus in sterilised wastewater were presented: the virus titre declined by approx. 99% after 1 day with declines thereafter lower than this.

Health Canada (2017) discusses some potential emerging viruses in drinking water.

Virus infections resulting from correctly treated water have not been reported in New Zealand (ESR 2004), although internationally such outbreaks are recognised (Hunter 1997; Hrudehy and Hrudehy 2007). Human viruses have been reported to occur at very low levels (0.1–1/100 L) in conventionally treated drinking-water in many countries (Vivier et al 2004) including New Zealand (Kim 2005).

Estimations of viral disease risk using standard risk assessment techniques with a high infectivity virus predict the surprisingly high annual risk of infection of between 1:3 and 1:25 from conventionally treated drinking-water contaminated by viruses at low levels (~1 virus per 100 litres) (Gerba and Rose 1992).

Viruses and phages are also discussed in Chapter 5: General Microbiological Quality, sections 5.3.4 and 5.4.4. More detailed discussion appears in the datasheets.

7.3 Occurrence of human viruses in source waters

The New Zealand freshwater microbiology study (Till et al 2002) is the most significant study of human virus's occurrence in surface water in New Zealand to date. This study carried out in collaboration between the Ministries for the Environment, Agriculture and Forestry, and Health tested recreational water locations on 25 rivers and lakes every two weeks for 15 months.

Human adenovirus and/or enterovirus were detected, by qualitative molecular methods, in more than 50 percent of the 275 samples collected. These data suggest that human virus occurs quite frequently in surface waters and in a wide range of source water locations and types.

Subsequent culture based studies of virus occurrence in the Waikato River showed that adenovirus and enterovirus levels are generally low, less than 5 per 100 L, but on some occasions may be as high as 10 per 100 L (Watercare Waikato River Monitoring studies 2003–2004).

Studies using sensitive qualitative molecular-based virus detection methods suggest that adenovirus occurrence may be 10 times higher than this level on some occasions in the Waikato River (Kim et al 2005) although it is not clear whether all of these viruses are able to produce infections.

International data collated by WHO suggest that typical surface source waters may contain 0–10 viruses per litre (WHO 2004).

A UK study (DWI 2013) of the occurrence of adenovirus (AdV), norovirus (NV), plus several chemical determinands in raw waters found none of the chemical and microbiological parameter measurements gave useful indications of the levels of viruses. But there was strong evidence of an association between NV and AdV in raw water. However AdV was frequently detected in the absence of NV.

Health Canada (2017) reports studies where enteric viruses have been found in groundwater samples that did not contain indicator bacteria. For example, a study of private wells in the US found that 8% of the wells tested by polymerase chain reaction (PCR) were positive for one or more enteric viruses, however, none of the contaminated wells contained indicators of faecal contamination (ie, *E. coli*, enterococci, coliphages), and only 25% of the virus-impacted wells were positive for total coliforms.

7.4 Risk management

Potential for disease outbreaks associated with human virus contamination of source waters, and the potential to carry over to treated drinking-water is recognised throughout the developed world. Approaches to controlling the risks are largely through protection of source water quality by control of human activity in reservoir catchments, and through adequate treatment and disinfection of drinking-water. It is now well accepted that bacterial indicators such as *E. coli* are not necessarily adequate surrogates of viral occurrence. Human viruses tend to be more resistant to environmental stresses and water treatment mechanisms than are bacterial indicators, so the absence of the indicator may not equate with absence of the virus contaminant.

7.4.1 International approaches

The paucity of knowledge on the specific occurrence of human viruses in source waters, and the problems of virus detection and regular monitoring, mean that most guideline documents include only the qualitative requirement that, if tested for, human viruses should not be detected in treated drinking-water.

Where virus guidelines or standard requirements are in place these are stated either in terms of virus occurrence, or as water treatment plant virus removal efficiency. Such values are either derived from acceptable levels of health risk or, pragmatically, reflect virus detection capability.

Recent standard and guideline recommendations have moved towards risk-based evaluation of water treatment requirements. The USEPA Surface Water Treatment Rule includes a virus treatment requirement and requires that treatment of both filtered and unfiltered water sources is sufficient to remove or inactivate 99.99 percent (4 log) of viruses (USEPA, SWTR). This requirement is principally based on the acceptable (USEPA 1994) level of waterborne illness in a community (one case per 10,000 consumers) and the likely level of viruses in surface water.¹ Recent US proposals for surface water disinfection (USEPA 2003/2006a) use the adenovirus group as the target virus.

The WHO Guidelines recognise that water treatment requirements will differ for different communities, and propose a risk-based approach for setting performance targets for surface water treatment plants (WHO 2004/2017).

The risk-based approach takes into account a broad range of factors including virus occurrence and infectivity, water type, community health status and treatment characteristics. Such an approach requires a detailed knowledge of the water supply, water treatment performance and community activities and health status.

Approaches to managing viruses in treated water also recognise that the greatest health risk to a community occurs when water treatment conditions are atypical such as when source water condition is unusual, very high levels of virus occur, or through poor performance, or even failure, within the water treatment process.

Health Canada (2017) proposes a guideline:

The proposed guideline for enteric viruses in drinking water is a health-based treatment goal of a minimum 4 log removal and/or inactivation of enteric viruses. Depending on the source water quality, a greater log reduction may be required. Methods currently available for the detection of enteric viruses are not feasible for routine monitoring. Treatment technologies and watershed or wellhead protection measures known to reduce the risk of waterborne illness should be implemented and maintained if source water is subject to faecal contamination or if enteric viruses have been responsible for past waterborne outbreaks.

¹ In the Drinking-Water Contaminant Candidate List 3 – Draft in the Federal Register: 21 February 2008, 73(35); <http://www.epa.gov>.

7.4.2 Virus removal by current water treatment processes

Reduction of virus numbers in water as a result of treatment can occur through either virus removal or virus inactivation. Each virus type may react somewhat differently to particular water treatment methods, but the bulk of research to-date suggests that some broad generalisations can be made. WHO (2004a) discusses treatment processes suitable for pathogen control.

Virus removal can occur by physical association of a virus with other particles. Virus association with particles may be enhanced by addition of coagulants to form a floc, which can then be removed by settlement and/or filtration. The extremely small size of viruses means that they are unlikely to be removed if they are not associated with other particles. Water treatment processes such as flocculation, sand filtration, microfiltration and ultrafiltration, and prolonged standing in reservoirs, will result in physical removal of particle-associated viruses. Only reverse osmosis and dialysis membranes have pore sizes small enough to trap virus particles that are not associated with larger particles or flocs.

The effectiveness of virus removal is affected by those factors that act against particle association or floc formation including water condition and pH (LeChevallier and Au 2004).

Virus inactivation occurs through disruption of the external protein coat (capsid), modification of specific surface sites needed for infection (host receptor recognition sites) or major change to the nucleic acid (RNA or DNA). Disinfectants such as chlorine, chlorine dioxide, and ozone will cause disruption of the virus coat and of the exposed nucleic acids (Shin and Sobsey 2003, Tree et al 2003). Ultraviolet light in the range of 200–310 nm (antimicrobial range) will disrupt the nucleic acids by causing cross-linking, leaving viruses unable to replicate.

Viruses can differ greatly in their ability to resist various disinfectants. For example, it is widely regarded that double-stranded DNA viruses such as adenoviruses are more resistant to UV disinfection than single-stranded DNA viruses or RNA viruses.

Viruses can also be inactivated by prolonged holding in reservoirs that are exposed to sunlight, elevated temperature and extremes of pH, eg, lime treatment (Sobsey 1989). Different virus types and strains will show different levels of resistance to chemical or physical inactivation. Adenoviruses are considered to be the most resistant virus group to many disinfection treatments, because of their structure and nucleic acid makeup, and have been used by the USEPA as a model virus for designing UV criteria for surface water treatment (USEPA 2003/ 2006a).

The potential for virus inactivation by disinfectants is reduced by the presence of other particles or organic matter that will consume disinfectants or of light adsorbing or blocking materials that reduce UV penetration (LeChevallier and Au 2004).

Repair of disinfection damage is unlikely to occur in viruses as they do not appear to have repair mechanisms. It has been suggested that some viruses (such as the double stranded DNA adenoviruses) may be able to repair their DNA, and if there is no damage to the virus coat, they are still able to infect a host cell (Nwachuku and Gerba 2004).

Water treatment plants will normally include both virus removal and virus inactivation processes that act as multiple barriers.

Studies conducted in the 1980s using cell cultures to detect cytopathic effects (CPE) to assay infective viruses indicated that well-operated conventional water treatment removed 2-logs of viruses, and disinfection with chlorine could readily achieve a further 2-logs reduction. These studies also indicated that finished water produced by well-operated conventional treatment and disinfection was usually free of infective viruses as judged by examination of large volumes by the cell culture/CPE assay method. A target of 4-logs of virus reduction for surface waters was adopted by the USEPA in 1989 and has become the de facto benchmark for water treatment in other developed nations even if not formally stated in their standards or guidelines. More recent research indicates that the definition of a 'well-operated' conventional water treatment needs to be reassessed. Studies indicated that control of the coagulation/sedimentation/filtration process is critical for pathogen removal, and that implementation of an operational target of 0.2 NTU or less for turbidity for individual filtration units is needed to ensure optimum virus removal (2.0 to 2.5 logs). Operation at higher turbidity levels, but still well below the traditionally accepted figure of 1 NTU, may achieve only minimal virus removal (less than 0.5 log), and greatly diminishes the effectiveness of the filtration barrier. The target of 0.2 NTU or less also provides enhanced removal of other pathogen classes (taken from the executive summary of *Strategic Review of Waterborne Viruses*, Occasional Paper 11, CRC for Water Quality and Treatment).

Waterborne outbreaks of viral disease have been recorded mainly in groundwater systems where no disinfection or treatment to remove pathogens has been routinely practised. In instances where viral outbreaks have occurred in disinfected water supplies, investigations have revealed either a failure in disinfection, or unusually high levels of contamination in source waters which overwhelmed the disinfectant dose being applied. Therefore, provided that adequate control and monitoring of treatment and disinfection processes is being implemented, well-operated conventional water treatment and disinfection will provide an effective barrier against such outbreaks. Epidemiological studies of the possible contribution of pathogens in conventionally treated drinking-water to endemic gastroenteritis have given mixed results; however a study of robust design conducted in the USA found no evidence that waterborne pathogens made a detectable contribution to gastroenteritis in a city served by a stringently operated conventional water supply system (taken from the executive summary of *Strategic Review of Waterborne Viruses*, Occasional Paper 11, CRC for Water Quality and Treatment).

Virus removal and inactivation efficiencies for a range of water treatment processes are reviewed in the WHO (2004) Guidelines (Chapter 7), and by LeChevallier and Au (2004). WHO (2015) stated that viruses are inactivated at temperatures between 60°C and 65°C, but more slowly than bacteria. However, as shown for poliovirus and hepatitis A, as temperatures increase above 70°C, a greater than 5 log inactivation (99.999% reduction) is achieved in less than 1 minute. Based on these results, it is considered that the process of heating water to a rolling boil, as recommended in the *WHO Guidelines for Drinking-water Quality* (WHO 2011), is sufficient to inactivate pathogenic bacteria, viruses and protozoa. After the water has reached a rolling boil, it should be removed from the heat, allowed to cool naturally, without the addition of ice, and protected from post-treatment recontamination during storage. If turbid water needs to be clarified for aesthetic reasons, this should be done before boiling.

If a treatment process meets the turbidity requirements specified in the *Guidelines for Canadian Drinking Water Quality* the following may apply (Health Canada 2011):

Treatment process	Virus removal log credit
Conventional filtration	2.0
Direct filtration	1.0
Slow sand filtration	2.0
Diatomaceous earth filtration	1.0
Microfiltration	No credit

Ultrafiltration membranes typically remove viruses to greater than a 3-log level. Nanofiltration and reverse osmosis membranes are typically considered to be non-porous and represent a physical barrier to viruses. Because any breach in the integrity of the membranes would allow viruses to pass through the filter, direct integrity testing should be conducted. Health Canada (2013) gives no virus log credits for microfiltration, but for UF, NF and RO log credits are based on demonstration and challenge testing, verified by direct integrity testing.

The ESR is conducting a long-term research project for the Ministry of Health with the aim to generate evidence about the concentrations and risks to public health of viruses in river water. The 2009–2010 report presented data gathered from water samples taken from the Waikato River in Waikato and the Oreti River in Southland to put together a quantitative microbial risk assessment. This provided a statistical estimate of the risk of infections arising from various viruses following consumption of drinking-water, and enabled an estimate of the amount of treatment required to reduce the risk of viral-related waterborne disease. Key findings were:

- Enteric viruses were detected in essentially all river water samples taken from the Waikato River at Huntly and the Oreti River at Branxholme, with most water samples containing three or more virus types. Adenovirus (AdV), norovirus-GII (NoV GII) and rotavirus (RoV) were the most frequently detected.
- The risk of infection is assumed to be independent for each virus.

- The project predicts that daily consumption of untreated river water will cause most of the population of Huntly or Invercargill to become infected by each of the enteric viruses over the course of one year. This prediction gives a baseline from which to assess the amount of virus treatment required. Water treatment significantly reduced the number of people predicted to become infected.
- For Waikato River water, if 4-log-removal/inactivation of viruses were achieved at the treatment plant, and it is assumed that all the viruses in the source water were infective, it was predicted that 200–300 people in Huntly would become infected with RoV and with AdV during one year. However, if only 10 percent of the viruses in the intake water were infective then the number of infected people would drop to about 30 in one year.
- Similarly, for the Oreti River water, if 4-log-removal/inactivation were achieved at the treatment plant, and it is assumed that all the viruses in the source water were infective, it was predicted that 1000–3500 people in Invercargill would become infected with RoV and with AdV during one year. If only 10 percent of the viruses in the intake water were infective, the number of infected people would fall to 150–350 in one year.
- An assessment for NoV is not yet possible; however if the values are of a similar magnitude to those of AdV and RoV, then some of the outbreaks of NoV seen in the community could arise from drinking-water treated to achieve at least a 4-log removal of viruses.
- The study has indicated that even at low concentrations, waterborne viruses may have an effect on public health, and that in the absence of water treatment achieving 4-log removal or inactivation of viruses, the effect on health would be significant.

No analyses were carried out to determine whether either the Huntly or Branxholme treatment plants operate sufficiently to reduce the viral concentration, or whether the level of disease predicted by the study could be seen in the community by medical professionals; it could be present as 'background' gastroenteritis.

Linden and Sobsey (2005) studied the efficacy of UV disinfection on some waterborne pathogenic viruses and bacteriophages. A conclusion was that the inactivation of human enteric viruses and bacteriophages by UV irradiation is proven not predictable by the type and size of the virus or its nucleic acid genome. Overall, bacteriophage MS2 was a good conservative surrogate for representing the UV inactivation of many viruses, with the exception of adenovirus. These data will be helpful for utilities and regulatory officials to evaluate the efficacy of UV irradiation for viruses and plan for levels of disinfection that will help ensure public health in drinking water.

Hijnen et al (2006) reviewed information about inactivation credits of UV radiation for viruses, bacteria and protozoan (oo)cysts in water. In the group of pathogenic micro-organisms, viruses are generally more resistant than *Cryptosporidium*, *Giardia* and the bacterial pathogens. Adenovirus 40 is the most UV resistant waterborne pathogen known. *Acanthamoeba* is also very resistant. Bacterial spores, especially environmental spores of *Clostridium* are also resistant to UV, with k-values that are comparable to the Adenoviruses. They summarised the doses required to achieve a range of log credits for a wide range of micro-organisms. The yellow highlighting in the following table indicates the microorganisms that will not achieve 4-log inactivation by UV appliances

that deliver the standard 40 mJ/cm². That includes several types of adenovirus, and Calicivirus canine. Rotavirus SA-11 and Calicivirus feline are marginal.

Log inactivation	Required fluence			
	1	2	3	4
<i>Bacillus subtilis</i> ^a	56	111	167	222
Adenovirus type 40	56	111	167	b
<i>Clostridium perfringens</i> ^d	45	95	145	b
Adenovirus type 2,15,40,41	42	83	125	167
<i>Acanthamoeba</i> ^c	40	71	119	167
Adenovirus a (no type 40)	25	50	d	b
Calicivirus canine	10	21	31	41
Rotavirus SA-11	10	20	29	38
Calicivirus feline	9	19	28	38
Coxsackie virus B5	8	17	25	34
<i>Streptococcus faecalis</i> ^a	9	16	23	30
<i>Legionella pneumophila</i> ^d	8	15	23	30
Poliovirus type 1	7	15	22	30
<i>Shigella sonnei</i> ^d	6	13	19	26
<i>Salmonella typhi</i> ^a	6	12	17	51
Hepatitis A	6	11	17	22
Calicivirus bovine	5	11	16	21
<i>E. coli</i> O157 ^d	5	9	14	19
<i>E. coli</i> ^a	5	9	14	18
<i>Cryptosporidium</i> USEPA ^c	3	6	12	e
<i>Giardia</i> USEPA ^c	2	5	11	e
<i>Campylobacter jejuni</i> ^d	3	7	10	13
<i>Yersinia enterocolitica</i> ^d	3	7	10	13
<i>Legionella pneumophila</i> ^d	3	6	8	11
<i>Shigella dysenteriae</i> ^d	3	5	8	11
<i>Vibrio cholera</i> ^d	2	4	7	9

a = environmental spp.; b = Log inactivation max <4 log; c = no correction for environmental spp. (research needed); d = corrected for environmental spp.; e = no value due to tailing.

UV disinfection can be achieved using low pressure (LP) lamps, which emit UV light at essentially a single (monochromatic) wavelength (~254 nm), or medium pressure (MP) lamps, which emit radiation across a broader (polychromatic) spectrum. Ultraviolet light-emitting diodes (UV-LEDs) are an emerging technology for UV water treatment. A review of published studies on the application of UV-LEDs concluded that a standard method for the UV-dose determination of UV-LEDs is needed to reduce the inconsistent and incomparable dose-response data currently available in the literature. From Health Canada (2017).

7.5 Sampling, testing and data interpretation

The determination of virus removal efficiency within a water treatment plant, or occurrence in treated water, is dependent on the ability to reliably detect and enumerate the viruses. Determination of the health risk that viruses pose to the community using the water further depends on the ability to demonstrate or infer that the viruses detected are capable of causing human infection.

Virus detection and enumeration: No single method allows detection of all virus types and strains. Traditionally, viruses have been concentrated from water samples using filtration or adsorption based techniques with subsequent detection by culture in a permissive human or primate cell line. Many of the virus concentration techniques were developed using poliovirus or other enterovirus types and it is unclear how effectively these work for other virus types, particularly norovirus. Most virus concentration and detection methods recover less than 50 percent of the viruses present in a sample (Haramoto et al 2005).

Virus concentration from large volumes of water is laborious and time consuming and adds significantly to the cost of virus analysis. Not all virus types are culturable in cell lines, with norovirus and hepatitis E virus not able to be cultured routinely and are not detectable using traditional culture-based methods. Some virus cell culture-lines are susceptible to several virus types, for example BGM cells will permit enterovirus, adenovirus and reovirus to grow (Lee and Jeong 2004), thus if all these are present in one sample, they cannot be separated based on cytopathic effect alone.

Viruses (culturable and non-culturable) can be detected at very low levels using polymerase chain reaction (PCR) based molecular methods that target novel DNA or RNA sequences in the genetic information of the virus. Virus assay using PCR can target individual viruses or groups of viruses, and multiple analyses are required to investigate all the relevant viruses in a particular sample (Greening et al 2002). Recent advances in real-time PCR have made these methods both rapid and quantitative and potentially quite routine. PCR based methods use only a small amount of the original sample in the assay, so compared with culture methods that typically use considerably more of the original sample, PCR molecular methods are around 10-fold less sensitive than culture based methods for virus detection (Lewis et al 2000). PCR-based methods are very utilitarian, offering the advantages of rapid turn-around time, detection of currently unculturable viruses, and lower assay costs than traditional culturing methods (Lee and Jeong 2004). However, they are still generally too expensive to be used routinely.

DWI (2013) discusses analytical techniques for adenovirus and norovirus.

Virus sampling strategies: Relatively few viruses are needed for an infection to occur in a susceptible person, so low numbers of viruses must be quantified in relatively large volumes of finished water. For example, if source waters contain 5000 viruses per 100 L it would be necessary to sample and analyse at the very least 200 L of finished water to demonstrate a 4-log reduction in viruses. Typically, source water sample volumes should be 10–100 L, partially treated waters 50–200 L, and finished, disinfected water sample volumes 100–200 L.

The current cost of virus analysis may make regular monitoring beyond the means of many groups responsible for drinking-water treatment.

Specific short-term studies of virus occurrence and inactivation/removal within a plant are feasible but should be designed carefully to allow adequate interpretation of the data.

Determination of virus infectivity: Molecular methods for virus detection do not specifically show whether viruses are still infectious. Detection of viruses using a cell-culture based technique shows that the viruses are infectious and pose a risk of illness to water consumers. Infectivity of a virus can however be inferred for certain RNA viruses (norovirus, enteroviruses, Hepatitis A and E) from molecular detection data where the viruses are subjected to chemical disinfection, but not UV disinfection (Greening et al 2002). Virus viability is inferred whenever virus nucleic acid is detected because the nucleic acids (single stranded RNA) are extremely susceptible to degradation in the environment.

Interpretation of virus detection and occurrence data: Where viruses are detected in finished drinking-water the response to the data should be based, in consultation with relevant health authorities, on a risk evaluation incorporating the type and number of virus detected, the reproducibility of the result, and the health status and vulnerability of the community.

7.6 C.t values

A C.t value is the product of the residual concentration (C mg/L) of the disinfectant after the contact time (t minutes) required to cause a specified level of inactivation in a micro-organism. The C.t value is a measure of the exposure to the disinfectant and has the unit mg.min/L. Further discussion appears in Chapter 15: Disinfection Processes, section 15.2.1 (C.t values) and section 15.2.9 (measuring the contact time).

A range of C.t values is given in Appendix C of the Disinfection Profiling and Benchmarking Technical Guidance Manual (USEPA 1999), including C.t tables for disinfection of viruses by various disinfectants. These tables are referenced to AWWA (1991) in the text of USEPA (1999) and in CRC (2005). The 1991 data were carried out using hepatitis A virus and are derived from experiments conducted by Sobsey and co-workers in the late 1980s (Sobsey et al 1988). Subsequent publications continue to use the USEPA 1991 tables because research on disinfectant contact time has apparently not been revisited.

The USEPA Surface Water Treatment Rule (SWTR) required (inter alia) that treatment of both filtered and unfiltered sources remove or inactivate 4 log (99.99 percent) of viruses. This requirement was enacted in 1989. The 1991 tables were developed to assist water suppliers assess the degree of disinfection of viruses being achieved at their water treatment plants.

USEPA's LT2ESWTR (2003/2006a) includes a table showing the C.t values for disinfecting viruses using UV light. The proposed UV doses for inactivation of viruses were based on the dose-response of adenovirus because, among viruses that have been studied, it appears to be the most UV-resistant and is a widespread waterborne pathogen. Health effects of adenovirus are described in Embrey (1999).

It is doubtful that this same approach was used in developing the 1991 tables; viruses are simply referred to collectively, and 'viruses' were not defined in the 1991 information provided. Some viruses require a much higher C.t value than others. Nor is it explained whether the data relate to studies in single virions or cell-associated virions – the latter require a higher C.t value.

Table 7.1 shows the UV doses that water suppliers must apply to receive credit for up to 4 log inactivation of viruses. This is taken from Table IV – 21 in USEPA (2003), Table IV.D-5 in USEPA (2006a), and Table 1.4 in USEPA (2006b). The UV dose requirements in Table 7.1 account for uncertainty in the UV dose-response relationships of the target pathogens but do not address other significant sources of uncertainty in full-scale UV disinfection applications. These other sources of uncertainty are due to the hydraulic effects of the UV installation, the UV reactor equipment (eg, UV sensors), and the monitoring approach. Due to these factors, the USEPA requires water suppliers to use UV reactors that have undergone validation testing, see Chapter 8. Clearly, UV disinfection is impractical for attempting to achieve 4-log inactivation of adenovirus; treatment plants using UV disinfection for protozoa inactivation would achieve both bacterial compliance and 4-log virus inactivation simply by chlorination. The data in Table 7.1 are based on adenovirus studies; dose requirements for hepatitis A, coxsackievirus B5, poliovirus type 1 and rotavirus SA-11 indicate that a dose of around 40 mJ/cm² is generally adequate for 4-log inactivation (Health Canada 2011).

Table 7.1: UV dose requirements for virus inactivation credit

Log credit	Virus ¹ UV dose (mJ/cm ²)
0.5	39
1.0 (90% removal)	58
1.5	79
2.0 (99% removal)	100
2.5	121
3.0 (99.9% removal)	143
3.5	163
4.0 (99.99% removal)	186

¹ Based on adenovirus studies.

Tables 7.2, 7.3, 7.4, 7.5 have been taken from Appendix C of USEPA (1999) and copied from the 1991 publication, where they refer to 'undefined viruses'. Health Canada (2011) states that the data for ozone are based on poliovirus 1, and the rest is based on hepatitis A virus; and the C.t values incorporate a safety factor. The units are mg.min/L. Although ozone is clearly the most effective disinfectant, it does not leave a residual. Chlorine is more potent than chlorine dioxide, and chloramine is all but ineffective.

A free available chlorine content of 0.20 mg/L after 30 minutes retention time is equivalent to a C.t value of 6. Based on Table 7.2, this would achieve 4 log inactivation of viruses at 10°C. To achieve 4 log inactivation at 5°C, which requires a C.t value of 8.0, the minimum retention time should be 40 minutes (0.20 mg/L after 40 minutes retention time; C.t = 8.0). If that retention time cannot be achieved, the residual free chlorine content should be increased to 0.30 mg/L (0.30 mg/L after 30 minutes retention time; C.t = 9.0).

Table 7.2: C.t values for inactivation of viruses by free chlorine, pH 6–9

Log inactivation	1°C	5°C	10°C	15°C	20°C	25°C
2	5.8	4.0	3.0	2.0	1.0	1.0
3	8.7	6.0	4.0	3.0	2.0	1.0
4	11.6	8.0	6.0	4.0	3.0	2.0

Table 7.3: C.t values for inactivation of viruses by chloramine

Log inactivation	1°C	5°C	10°C	15°C	20°C	25°C
2	1243	857	643	428	321	214
3	2063	1423	1067	712	534	356
4	2883	1988	1491	994	746	497

Table 7.4: C.t values for inactivation of viruses by chlorine dioxide, pH 6–9

Log inactivation	1°C	5°C	10°C	15°C	20°C	25°C
2	8.4	5.6	4.2	2.8	2.1	1.4
3	25.6	17.1	12.8	8.6	6.4	4.3
4	50.1	33.4	25.1	16.7	12.5	8.4

Table 7.5: C.t values for inactivation of viruses by ozone

Log inactivation	1°C	5°C	10°C	15°C	20°C	25°C
2	0.9	0.6	0.5	0.3	0.25	0.15
3	1.4	0.9	0.8	0.5	0.40	0.25
4	1.8	1.2	1.0	0.6	0.50	0.30

Several studies show that iodine solutions are effective virucides. Viruses are more resistant than bacteria to iodine disinfection, typically requiring higher C.t.s than bacteria and in some cases much higher C.t.s at low pH levels (eg, 4–5), where hypiodous acid (HOI) is not present, and at cold water temperatures (eg, 5°C). Most studies evaluated the virucidal efficacy of iodine solutions against f2 virus and poliovirus. Data indicate 2-log inactivation at near neutral to alkaline pH levels (6–10) and various water temperatures (5–30°C) occurred at C.t.s of 15–75 mg-min/L with the higher C.t.s occurring at lower pH levels and colder water temperatures. Iodine resin studies show at least 4-log inactivation of various viruses at pH levels above 3.0 with low turbidity water for both triiodide and pentaoidide resins. From USAPHC.

DWSNZ section 4.3.2.1 (bacterial compliance) allows *E. coli* monitoring to be substituted by online FAC monitoring provided the chlorine contact time is more than 30 minutes, and the pH is <8, turbidity <1 NTU. This represents a C.t value of 6 or more. The DWSNZ also assume that drinking-water that meets the bacterial compliance requirements should be free from infective viruses. That means that when the water temperature falls below 10°C, either the FAC level or the contact time, or both, need to be increased. During an outbreak of norovirus at Cardrona in 2012, samples were found to contain viruses in the absence of *E. coli* when the chlorine dose was inadequate.

Some new research has been reported in DWI (2010). Baseline disinfection experiments were performed in pH 7 and pH 8 demand-free reagent grade water with 0.2 mg/L free chlorine or 1 mg/L monochloramine at 5°C. These baseline experiments were performed using several human adenoviruses (HAdV2, HAdV40, and HAdV41), two coxsackieviruses (coxsackievirus B3 [CVB3] and coxsackievirus B5 [CVB5]), two echoviruses (echovirus 1 [E1] and echovirus 11 [E11]) and murine norovirus (MNV, studied as a surrogate for human norovirus). The most resistant representative of each virus type for each disinfectant was selected for additional virus disinfection experiments using three distinct types of source water collected from drinking water treatment plants. Experiments were performed in source water at pH 7 and 8 using 0.2 and 1 mg/L free chlorine or 1 mg/L and 3 mg/L monochloramine at 5 and 15°C. Free chlorine and monochloramine disinfection experiments were then performed for aggregated preparations of HAdV2 in source water from one drinking water treatment plant. Viral titres before and after disinfection were determined by virus-specific plaque assays. The efficiency factor Hom model was used to calculate C.t values (disinfectant concentration in mg/L x exposure in min) required to achieve 2-, 3-, and 4-log₁₀ reductions in viral titres.

In all water types, chlorine and monochloramine disinfection were most effective for MNV, with 3-log₁₀ C.t values at 5°C ranging from <0.02 to 0.03 for chlorine and 53 to 111 for monochloramine. Chlorine disinfection was least effective for CVB5 for all water types, with 3-log₁₀ C.t values at 5°C ranging from 2.3 to 7.6. Monochloramine disinfection was least effective for HAdV2 and E11, depending on pH and water type. At 5°C, 3-log₁₀ C.t values for HAdV2 ranged from 1044 to 3308, while those for E11 ranged from 814 to 2288. Overall, chlorine was much more effective than monochloramine, and disinfection proceeded faster at 15°C and at pH 7 for all water types. C.t values for chlorine and monochloramine disinfection of aggregated HAdV2 were 2 and 1.4 times higher than for monodispersed HAdV2, respectively.

The results from this project indicate that a C.t value of 10 (or 20, if incorporating a 2x factor of safety for aggregated virus) may be needed to achieve a 4-log₁₀ inactivation of CVB5 with free chlorine at 5°C, pH 8, which is above the C.t value of 8 recommended in the USEPA's Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources (Guidance Manual) to achieve a 4-log₁₀ inactivation with chlorine at 5°C, pH 6–9. The Guidance Manual recommended C.t value of 8 included a safety factor of 3x to account for potential virus aggregation. However, C.t values for the study viruses, including CVB5, were below the 2-log₁₀ C.t value of 12 reported by the World Health Organization as an expected performance level for chlorine disinfection of viruses in water at 5°C, pH 7–7.5.

Some more recent data was presented by Keegan et al (2012). Although it was a recycled wastewater study, the findings are still relevant to drinking water; the less than expected protective effect of turbidity was interesting:

Table 1E: Free available chlorine C.t values (mg/L.min) for 4-log inactivation of Coxsackie B5 (CB5) virus calculated by determining the integral of residual chlorine vs time for wastewater of various turbidities and pHs at 10°C

pH	C.t value, 0.2 NTU using 6.5 mg/L chlorine	C.t value, 2 NTU using 6.87 mg/L chlorine	C.t value, 5 NTU using 6.87 mg/L chlorine	C.t value, 20 NTU using 9 mg/L chlorine
7	5.4	5.5	6.0	25.8
8	15.5	15.7	21.9	34.5
9	24	26	28	52

Table 2E: Preformed monochloramine C.t values (mg/L.min) for 4-log inactivation of adenovirus 2 calculated by determining the residual monochloramine vs time for wastewater of various turbidities and pHs at 10°C

pH	C.t value, 2 NTU using 15 mg/L monochloramine	C.t value, 5 NTU using 15 mg/L monochloramine	C.t value, 20 NTU using 16 mg/L monochloramine
7	3,082	3,337	3,757
8	3,949	4,426	5,900
9	6,746	9,096	10,718

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