WaterVal

Streamlining Technology Validation

Reverse osmosis and nanofiltration

Validation protocol

About Australian WaterSecure Innovations Ltd

Australian WaterSecure Innovations Ltd (trading as WaterSecure) was established in 2016 to oversee the implementation of national research outcomes, including the WaterVal™ program, one of the flagship outcomes developed by the Australian Water Recycling Centre of Excellence (the Centre), an independent research organisation established in 2009 by Commonwealth funding.

About WaterVal™

WaterVal™ is a framework that provides national consistency in the validation of water treatment technologies for the water industry. The framework, jointly developed by the Centre, regulators, water utilities, researchers and the private sector, is underpinned by protocols and agreed methods to validate pathogen removal by treatment technologies. The framework and protocols are applicable to a broad range of water sources, and give effect to key objectives of the *Australian guidelines for water recycling* and the *Australian drinking water guidelines.*

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1. Background and scope

Reverse osmosis (RO) and nanofiltration (NF) membranes are widely recognised as being capable of achieving high log reductions values (LRVs) for pathogens, including viruses, bacteria and protozoa. However, the performance of individual RO/NF systems can vary significantly, and high LRVs cannot be arbitrarily credited to any system. Instead, each RO/NF membrane treatment process should be systematically validated to determine its associated LRV.

This protocol has been prepared as part of WaterVal to provide a method for validating polyamide (PA) RO/NF spiral wound membranes (other types of membranes are specifically excluded) by focusing on the removal of viruses and determining:

- the LRV that can be achieved based on challenge testing using a suitable surrogate
- the maximum LRV that can be consistently verified by monitoring a suitable membrane integrity indicator.

This document is consistent with the WaterVal Validation *Protocol template* (AWRCE 2015), which provides a recommended approach to validation that is based on the following nine elements:

- identification of the mechanisms of pathogen removal by the treatment process unit
- identification of target pathogens and or surrogates that are the subject of the validation study
- identification of factors that affect the efficacy of the treatment process unit in reducing the target pathogen
- identification of operational monitoring parameters that can be measured continually and are related to the reduction of the target pathogen
- identification of the validation method to demonstrate the capability of the treatment process unit
- description of a method to collect and analyse data to formulate evidence-based conclusions
- description of a method to determine the critical limits, as well as an operational monitoring and control strategy
- description of a method to determine the LRV for each pathogen group in each specific treatment process unit performing within defined critical limits
- provision of a means for revalidation or additional onsite validation where proposed modifications are inconsistent with the previous validation test conditions.

This protocol focuses on the validation of LRVs for microbiological pathogens and does not include validation of chemical removal.

2. Identification of pathogen removal mechanisms

High-pressure membranes, such as RO/NF membranes, are comprised of three layers. The first (top) layer is a semipermeable membrane made of polyamide (PA), which is pH resistant, rough, slightly negatively charged and has hydrophilic properties. This layer is responsible for the passage of water and the rejection of dissolved species and pathogens. The second layer is comprised of nanoporous polysulfone serving as structural support for the first layer. The final layer is a nonwoven polyester fabric that gives stiffness and further support to the membrane.

High-pressure membranes use three primary removal mechanisms: size exclusion, charge repulsion and, to a lesser extent, adsorption. The main pathogen rejection mechanism of RO/NF membranes is by size exclusion, however the physicochemical properties of the membrane, the surface properties of the pathogen being removed (electrostatic and hydrophobic interactions) and the solution environment (Antony et al. 2012) can influence removal. An intact RO/NF membrane system can reject virus particles as small as 16 nm (e.g. poliovirus). For such a system, this would suggest that bacteria, protozoa and helminths are also rejected by size exclusion, as these are considerably larger than viruses.

The main risk to the integrity of an RO/NF system is the potential for the active membrane layer to be compromised by physical defects or more general system failures, including compromised seals and O-rings, leaks, or faulty connectors between membranes.

2.1. Size exclusion

RO membranes possess polymer material–free void spaces that form a continuous and interconnected network (Kamide & Iijima 1994, Košutic et al. 2000, Meares 1976). The size of these spaces follows a log normal distribution (Košutic et al. 2000) that is characterised by the molecular weight cut-off (MWCO). The MWCO of a membrane is determined by the molecular weight of a solute that the membrane removes by 90% (Van der Bruggen et al. 1999).

The MWCO of RO/NF membranes is in the range of 100–300 Dalton (Da) for organic molecules with a rejection of 90% or greater for inorganic ionic solutes (Wilf 2010). However, the MWCO provides an estimate of the sieving effect only, and does not take into account the hydrophobicity, charge and geometry of the molecule, which can also affect rejection (Kiso et al. 2001).

The principal mechanism to remove compounds with a molecular weight greater than the MWCO is size exclusion. By extension, this applies to particles (including viruses, bacteria and protozoa) that are considerably larger than these compounds.

2.2. Charge repulsion

The presence of carboxylate functional groups in the PA membrane layer results in a negatively charged membrane surface when used in typical water recycling and desalination applications where the pH ranges between 5 and 9. Therefore, RO/NF membranes can remove negatively charged solutes by charge repulsion.

Viruses also possess a pH-dependent surface charge that can potentially result in charge repulsion (Michen & Graule 2010). However, the isoelectric point (IEP) of viruses can vary significantly – from pH 2 to pH 9 for animal-based viruses and from pH 1.9 to pH 7.4 for bacteriophage. While the primary mechanism of virus and larger pathogen removal is size exclusion, charge repulsion may occur, although to a lesser extent.

2.3. Adsorption/diffusion

Hydrophobic interactions can occur during filtration, resulting in the adsorption of hydrophobic (nonpolar) compounds onto the surface of the hydrophobic membrane. However, the number of adsorption sites is limited in PA membranes, so this removal mechanism is not as common as size exclusion and charge repulsion.

The influence of adsorption on rejection is considered time-dependent (Kimura et al. 2003, Nghiem et al. 2002, Shan et al. 2009). In the first step, rejection increases due to the adsorption of compounds onto the membrane causing an overestimation of retention until the membrane reaches its equilibrium, when adsorption equals desorption on the feed and permeate side. A change in operating conditions can cause desorption of the compounds (Chang et al. 2003). The sorption–diffusion model has been used to describe the adsorption of a compound onto the membrane and its transport by diffusion (Wiesner & Buckley 1996, Williams et al. 1999). Other constituents in the feedwater can also impact negatively or positively on a compound's rejection. The interaction between two molecules can improve their rejection by size exclusion or charge repulsion mechanisms (Jin et al. 2010). Conversely, a molecule may improve the adsorption of another molecule onto the membrane and its diffusion through it, reducing the rejection (Tödtheide et al. 1997).

As with charge repulsion, while the primary mechanism of virus and larger pathogen removal is size exclusion, there may be some adsorption effects.

2.4. Impact of membrane ageing on virus adsorption

The rejection efficiency is expected to vary as a function of ageing because of physicochemical changes of the membrane surface. Surfaces of aged RO/NF membranes become more negatively charged, more hydrophobic and rougher compared with new membranes. Considering the negative charge of MS2 bacteriophage (MS2) (above pH 3.9) and its hydrophobicity, it is expected that aged membranes will have greater charge and hydrophobic repulsion, but this has not been experimentally verified. However, adsorption of more MS2 was observed on aged membranes. This is attributed to the increasing surface roughness that overcomes the charge and hydrophobic effects. Therefore, adsorption will have a greater contribution to the removal mechanism in aged membranes, compared with new membranes.

3. Identification of target pathogens or surrogates

The target pathogen is the pathogen that is the subject of challenge testing as part of the validation study (as described in Section 6.1) and is the most resistant to the treatment process. Generally, a target pathogen should be selected from each pathogen group. However, as size exclusion is the primary mechanism for pathogen removal by RO/NF, removal of bacteria and protozoa will be much higher than viruses, therefore viruses are the target pathogens for validation of RO/NF.

It is unlikely for RO/NF systems to be validated solely for the removal of bacteria or protozoa, therefore this validation protocol focuses on the removal of viruses with LRVs demonstrated for viruses applying equally to bacteria and protozoa.

To minimise the potential for severe membrane fouling, the feedwater to RO/NF systems undergoes extensive pre-treatment before it is introduced to the RO/NF process. As a result, pathogens removed by pre-treatment are generally not present in sufficient quantities to conduct validation procedures. In addition, because of the high removal rates typically achieved by RO/NF membranes, these organisms are generally not detectable in the permeate of an intact membrane system, preventing the calculation of realistic LRVs. As a consequence, indigenous pathogens cannot be used for validation.

Spiking large quantities of pathogens in feedwater is not a realistic option. Using surrogates, either already present in RO/NF feedwater or added artificially as part of a challenge test, is the preferred option. In the *Australian guidelines for water recycling* (NRMMC, EPHC & NHMRC 2008), a surrogate is defined as a 'parameter or combination of parameters that can be used to assess the quality of water; a specific contaminant, group of contaminants or constituent that signals the presence of something else'. A surrogate is a challenge organism, particulate or chemical that is a substitute for the target pathogen of interest. An 'ideal surrogate' should have characteristics that are likely to affect removal efficiency and that are similar to those of the target pathogen, while a 'conservative surrogate' should have characteristics that may result in a lower removal efficiency relative to the target organism. In general, it is necessary to use a conservative surrogate unless there are data to support the use of an ideal surrogate (US EPA 2005).

Several surrogates are suited for validating RO/NF systems, including MS2 bacteriophage (MS2) and fluorescent dyes such as Rhodamine-WT (R-WT). These microbial and nonmicrobial surrogates are considered appropriate, based on a range of criteria, including:

- sensitivity, with LRVs comparable to, or more conservative than, the smallest viruses
- opportunity for real-time detection (excluding MS2) to identify membrane system defects
- relatively low cost
- ability to implement a full-scale challenge test.

3.1. MS2 bacteriophage

MS2 is one of the most widely used surrogates in virus removal challenge tests for RO/NF membranes because of its small size (ca 27 nm), low IEP, ability to be cultured at high concentrations, ease of enumeration by a standard plaque assay and lack of pathogenicity (Golmohammadi et al. 1993, Michen & Graule 2010, UNESCO CMST 2009). In addition, based on a low IEP compared with other viruses, its negative surface charge at near neutral pH (6–8) favours electrostatic repulsion by negatively charged membranes and prevents aggregation, avoiding higher apparent LRVs resulting from cluster formation (AWPRC 1991, Langlet et al. 2008, Michen & Graule 2010). Rejections of MS2 of up to 6-log have been demonstrated (Lozier et al. 2003).

Incorporating a regular MS2 challenge test at full scale is impractical, and, considering the cost and time required to culture and plate sufficient quantities of the phage (24–48 h using a plaque assay), this surrogate cannot be used for real-time integrity testing. MS2 can, however, be used as a benchmark for other monitoring strategies by comparing its rejection with other nonmicrobial surrogates and membrane integrity indicators.

3.2. Fluorescent dyes

3.2.1. Rhodamine WT

Rhodamine WT (R-WT) is a nonreactive and nontoxic tracer chemical approved in Australia and by the United States Environmental Protection Agency for use in drinking water (Zornes et al. 2010). R-WT and MS2 removals by RO treatment have been shown to be correlated (Lozier et al. 2003) and R-WT is therefore considered an appropriate nonmicrobiological alternative to MS2.

This dye has a molecular weight of 487 g/mol, which is considerably larger than the typical MWCO of RO/NF membranes. R-WT (pKa = 5.1) is negatively charged at pH values observed in water treatment applications, and is removed by charge repulsion and size exclusion. R-WT can be quantified relatively easily using fluorescence measurement techniques. LRVs as high as 4-log have been obtained using pulse spiking (Surawanvijit et al. 2015).

3.2.2. Other dyes

Other fluorescent dyes may be considered as surrogates subject to evidence being provided that their removal by RO/NF treatment correlates with the removal of MS2 under relevant operating conditions.

Uranine is an example of a dye which could be considered as an alternative to R-WT provided the correlation of its removal by RO/NF with MS2 removal is demonstrated. Uranine is the di-sodium salt of fluorescein, and is a nonreactive and nontoxic tracer dye (Behrens et al. 2001, Smart & Laidlaw 1977). Its molecular weight, at 332 $g.mol^{-1}$, is lower than that of R-WT, but it is also well rejected by RO/NF membranes, as reported in a recent study where the dye was used as a pulsed marker for a new membrane integrity monitoring test protocol (Surawanvijit et al. 2015). Rejections of uranine by RO/NF greater than 4-log have been demonstrated (Frenkel et al. 2014).

Fluorescent dyes attached to antiscaling compounds have been considered as potential surrogates however further evidence is required that their removal can be correlated with MS2 removal. Primarily used to quantify antiscalant levels in a range of water systems, including RO/NF (Kelle Zeiher et al. 2003), these dyes could be used in membrane integrity monitoring. Two studies in advanced water treatment plants used such tracers to monitor RO membranes and showed rejection greater than 4-log (MWH 2007, Steinle-Darling et al. 2015).

4. Influencing factors

Factors influencing the performance of the RO/NF process and its ability to remove pathogens and surrogates need to be considered when validating RO/NF systems. For example, changes in membrane properties, process conditions and the composition of feedwater may influence to a different extent the rejection of both pathogens, surrogates or water quality parameters used to monitor membrane integrity (referred to herein as membrane integrity indicators as detailed in section 5.1). The influence of these factors may therefore impact the final LRV which can be demonstrated.

Factors that may influence rejection characteristics (Victorian Department of Health 2013) include:

- membrane properties (such as MWCO, surface charge and hydrophobicity)
- operating conditions (such as permeate flux, cross-flow velocity and recovery)
- feedwater properties (such as pH, temperature, ionic strength and organic content)
- Process and membrane failures.

4.1. Membrane type

The rejection of viruses and surrogates by membrane processes is known to vary with the type and properties of the RO/NF membrane, and previous studies have demonstrated the effect of membrane composition (cellulose acetate or PA) on virus rejection by RO/NF membranes (Adham et al. 1998, Lovins et al. 2002). The rejection of surrogates and membrane integrity indicators is also dependent on the membrane composition (Pype et al. 2015, Surawanvijit et al. 2015). Given that the two main mechanisms of rejection by high-pressure membranes are size exclusion and charge repulsion, intrinsic membrane properties such as MWCO and surface charge will affect these mechanisms and subsequently the rejection of virus, surrogates and membrane integrity indicators.

4.2. Operating conditions

In high-pressure systems, feed pressure, cross-flow velocity, permeate flux and recovery are important process parameters that can influence the removal of contaminants for a given membrane system (Doederer et al. 2014). Models that can predict changes in salt rejection, for example, as a function of different operating conditions, are commonly used to optimise membrane systems. Less is known about the influence of operating conditions on the rejection of surrogates and membrane integrity indicators and therefore on their ability to conservatively demonstrate the integrity of the system.

A study into the influence of operating conditions on the rejection of surrogates and membrane integrity indicators (Pype et al. 2015) showed that MS2 removal was unaffected under typical operating conditions. Indicative impacts of changes in operating conditions on the LRV of surrogates and membrane integrity indicators are summarised in Table 1 and described in further detail in each of the following sections. No normalisation took place in this research study. In practice however, operating parameters are generally normalised to compensate for changing feedwater temperature.

Table 1 Impact of influencing factors on the removal of surrogates (MS2 and R-WT) and membrane integrity indicators (sulfate, Dissolved Organic Matter (DOM) and EC). Adapted from Pype et al. (2015)

Influencing factor	Impact of influencing factor on LRV of surrogates and membrane integrity indicators				
	MS2	R-WT	Sulfate	DOM	EC
Increasing permeate flux	No impact	No impact	LRV ↗	No impact	LRV ↗
Increasing cross-flow velocity	No impact	No impact	LRV ↗	Variable	LRV ↗
Increasing recovery	No impact	LRV ↘	LRV ↗	LRV ↗	LRV ↗
Increasing feedwater pH from 3 to 5	No impact	No impact	LRV ↗	No impact	LRV ↗
Increasing feedwater pH from 5 to 8	No impact	LRV ↗	No impact	No impact	LRV ↗
Increasing feedwater pH from 8 to 10	na[a]	LRV ↘	LRV ↘	No impact	LRV ↘
Increasing feedwater temperature	No impact	LRV ↘	LRV ↗	LRV ↘	LRV ↘
Change in ionic strength between 8 mM and 23 mM	No impact	No impact	No impact	No impact	No impact
Change in DOC between 4.5 mg/L and 14 mg/L	No impact	No impact	No impact	No impact	No impact

LRV ↗ indicates that the change in influencing factor results in an increase in LRV
LRV ↘ indicates that the change in influencing factor results in a decrease in LRV
[a] Not applicable as a pH of 10 and above affects the viability of MS2

4.2.1. Permeate flux

Permeate flux is a plant-specific parameter that depends on capacity and production objectives. Permeate flux is directly related to feed pressure, which decreases along the length of a spiral-wound module due to friction. At higher permeate flux, the diffusive flux of solute is lower that the water flux, which causes an increase in water permeability and in solute rejection (Doederer et al. 2014, Ochando-Pulido et al. 2012, Spiegler & Kedem 1966). Above a certain feed pressure limit, the solute rejection reaches a plateau due to the transport of salt with water (Filmtec 1998).

Lozier et al. (2003) demonstrated that a change of feed pressure (up to 20 bar) did not adversely impact virus rejection. The rejection of MS2, R-WT, sulfate, DOM and EC was studied as a function of permeate flux between 10 and 40 L/m^2.h, at constant cross-flow velocity (0.1 m/s) and temperature (~22 °C) using a flat-sheet system (Pype et al. 2015). In such a system, changes in permeate pressure, permeate recovery and thus in solute concentration along the cell are negligible. Table 1 summarises the influence of permeate flux on the rejection of surrogates and membrane integrity indicators. MS2, R-WT and DOM rejections do not significantly change as a function of permeate flux. As can be expected, sulfate and EC rejections increase with permeate flux, plateauing at a flux above 30 L/m^2.h due to transport of salt with water.

4.2.2. Cross-flow velocity

Concentration polarisation (CP) is a phenomenon caused by the formation of a layer on the membrane surface where the concentration of solute is higher than in the bulk solution (Amjad 1993). This layer increases the osmotic pressure at the membrane surface resulting in a decrease of the water flux, and the increased concentration gradient causes an increase in solute passage. Eventually, CP can lead to scaling when the surface concentration exceeds the maximum level of solubility.

Along the length of a spiral-wound module, cross-flow velocity decreases due to a reduction in turbulence, resulting in an increase in CP. This phenomenon is reduced when mixing occurs at the membrane which can be achieved by working at high cross-flow velocities or by using and optimising turbulence promoters (Williams

2003).

The rejection of MS2, R-WT, sulfate, DOM and EC was studied as a function of cross-flow velocity in the range of 0.05 to 0.2 m/s, at constant permeate flux (20 L/m^2.h) and temperature (~22°C) using a flat-sheet system (Pype et al. 2015). Table 1 summarises the influence of cross-flow velocity on the rejection of surrogates and membrane integrity indicators.

When increasing cross-flow velocity, the rejection of MS2 or R-WT does not significantly change whereas the rejection of sulfate and salt tends to increase due to a reduced CP. With regard to DOM, a higher cross-flow velocity reduces the potential for organic fouling (Mattaraj et al. 2010), the latter being associated with an increase in DOM rejection. Therefore, an increase in cross-flow velocity results in a lower DOM rejection although this effect is insignificant for smooth membranes which experience less organic fouling.

4.2.3. Recovery

Recovery, defined as the ratio of permeate flow to feedwater flow, can have an impact on the removal of contaminants (Chellam & Taylor 2001). All conditions being equal (same cross-flow velocity), increasing recovery involves an increase in permeate flow rate by increasing feedwater pressure. There is always some salt passage through the membrane but as feedwater pressure is increased, this salt passage is increasingly overcome as water is pushed through the membrane at a faster rate than salt can be transported (Filmtec 1998).

The rejection of MS2, R-WT, sulfate, DOM and EC was studied as a function of recovery in the range 6 to 21% at constant permeate flux (20 L/m^2.h), cross-flow velocity (0.1 m/s), temperature (~22°C) and pH (~7) using a single 4" spiral-wound module system (Pype et al. 2015). Table 1 summarises the influence of recovery on the rejection of surrogates and membrane integrity indicators.

MS2 rejection was not significantly impacted by increases in recovery whereas R-WT tended to be less well rejected. The removal of membrane integrity indicators slightly increased with recovery. A higher recovery leads to an increase in solute concentration at the membrane surface which can facilitate the formation of a cake layer (fouling), consequently reducing the passage of organic matter and resulting in an apparent increase in DOM rejection.

4.3. Feedwater properties

Feedwater properties such as feedwater pH, temperature, ionic strength and organic content can also influence the removal of contaminants for a given membrane system (Doederer et al. 2014), however a study into the influence of key feedwater properties on rejection (Pype et al. 2015) showed that MS2 removal was unaffected under typical operating conditions. The impact of changes in feedwater properties on the LRV of surrogates and membrane integrity indicators is summarised in Table 1 and described in further detail in each of the following sections.

4.3.1. Feedwater pH

The pH of the feed to the RO system can influence the rejection of charged contaminants. Depending on the feed solution pH, the amine, hydroxyl and carboxylic functional groups of membranes can ionise, which affects their properties and impacts rejection. In addition, some studies have shown that pH could also impact the size of membrane cavities due to changes in electrostatic interaction (López-Muñoz et al. 2009; Donose et al. 2013).

For particles such as viruses, it has been suggested that an IEP close to the pH of the aqueous solution can improve virus rejection by size exclusion due to an increase in virus-virus and virus-impurity aggregation when viruses are in this zwitterionic form (equal positive and negative charge around the virus) (Herath et al. 1999).

The rejection of MS2, R-WT, sulfate, DOM and EC was studied as a function of pH in the range of 3 to 10, at constant permeate flux (20 L/m^2.h), cross-flow velocity (0.1 m/s) and temperature (~22°C) using a flat-sheet system (Pype et al. 2015). Table 1 summarises the influence of feedwater pH on the rejection of surrogates and membrane integrity indicators.

No measureable impact was observed on the rejection of MS2 due to its very high log rejection in all conditions and the limited sensitivity of the measurement method. R-WT is negatively charged at pH > 5, resulting in an increase in removal at higher pH as the charge repulsion mechanism becomes more significant. As expected, based on the heterogeneous nature of DOM, no measurable difference was reported. Generally salt rejection increases from pH 3 to 8 although the specific rejection of sulfate was not significantly influenced by pH, until pH 10, where both salt and sulfate rejections were reduced.

4.3.2. Feedwater temperature

An increase in feedwater temperature can lead to an increase in the size of membrane cavities due to the relaxation of the PA in the membrane layer (Ben Amar et al. 2007, Sharma et al. 2003, Sharma and Chellam, 2006).

The rejection of MS2, R-WT, sulfate, DOM and EC was studied as a function of feedwater temperature in the range of 15 to 35°C, at constant permeate flux (20 L/m2.h), cross-flow velocity (0.1 m/s) and pH (~7) using a flat-sheet system (Pype et al. 2015). Table 1 summarises the influence of feedwater temperature on the rejection of surrogates and membrane integrity indicators.

An increase in temperature had a negative impact on the rejection of salt (or EC), R-WT and to a lesser extent DOM by increasing their diffusivity through the membrane. MS2 rejection did not appear to be impacted. The rejection of sulfate increased with temperature (Pype et al. 2015).

4.3.3. Feedwater ionic strength and organic content

Both the ionic strength and organic content of feedwater can have an impact on rejection by RO/NF membranes. It has been previously shown that an increase in ionic strength can increase the size of membrane cavities (Bargeman et al. 2005) and that viruses have the capacity to aggregate with organic contaminants (Herath et al. 1999), both of which are potentially able to affect rejection (Huang et al. 2012b).

The rejection of MS2, R-WT, sulfate, DOM and EC was studied as function of feedwater composition by varying the ionic strength between 8 and 23 mM and varying DOC between 4.5 and 14 mg/L, at constant permeate flux (20 L/m^2.h), cross-flow velocity (0.1 m/s), temperature (~22°C) and pH (~7) using a flat-sheet system (Pype et al. 2015).

Varying the feedwater composition in terms of ionic strength and organic content did not appear to have a significant impact on the rejection of surrogates and membrane integrity indicators within the ranges studied.

4.4. Process and membrane failures

High pressure membrane elements are complex systems that are comprised of multiple components including membrane leaves, connecting tubes, caps and O-rings. Imperfections in these components, such as glue-line leaks and cracks in O-rings or permeate tubing, can compromise the integrity of the element and allow the passage of untreated feedwater. Defects can also occur during membrane installation, such as rolled O-rings, or during operation such as delamination of membrane sheets.

Due to the conservative nature of surrogates and membrane integrity indicators, any significant process or membrane failure considered herein and able to negatively impact membrane integrity would be expected to lead to a reduction in the LRV of these surrogates or indicators, thereby alerting of any potential impact on the rejection of viruses and other pathogens.

4.4.1. System leaks

Leaks can occur during operation due to the failure of seals or O-rings and the impact on pathogen removal will depend on the characteristics of the failure (type, location and extent). It would be expected that the probability of a faulty O-ring/interconnector increases with an increasing number of modules per pressure vessel and an increasing number of pressure vessels. In case of major leaks, also referred to as catastrophic loss of integrity, where feedwater directly bypasses the membrane and enters the permeate stream, the resulting contamination will be directly proportional to the amount of feedwater entering the permeate stream. Lozier et al. (2003) reported that a cracked O-ring may not systematically impact the rejection of MS2 and R-WT. The presence of a physical defect in an individual element (such as a hole or faulty O-ring) will have a greater impact if the element is in a trailing position, rather than in a lead position, in a train due to the higher feedwater contaminant concentrations at this location (Lozier et al. 2003).

4.4.2. Membrane defects

There is a lack of consensus on the impact of microscopic membrane defects (such as small holes) on pathogen rejection but it has been shown that the influence of a hole in a membrane on virus rejection depends primarily on the hydrodynamics (flux and hole flow) which are principally functions of transmembrane pressure, water temperature and membrane resistance (Pontius et al. 2011, Sorber et al. 1972). It has been suggested that a hole in a membrane can be recovered or repaired by compounds present in feedwater (Lozier et al. 2003). The presence of a physical defect in an individual element will have a greater impact if the element is in a trailing position rather than in a lead position in a train due to the higher feedwater contaminant concentrations at this location (Lozier et al. 2003).

4.4.3. Membrane fouling

In addition to installation and manufacturing defects, the membrane active layer can change during operation. For example, the surface charge can change with the presence of foulants on or inside the membrane, and cavities from small defects can be blocked by foulants and scalants, all of these events being able to potentially impact permeate quality. Virus-impurity coagulation has been considered by Huang et al. (2012) who demonstrated that in the presence of organic matter and on a fouled membrane, the LRV of viruses can increase due to the formation of virus-impurity aggregates (increasing of the particle size) and the subsequent obstruction of membrane cavities.

4.4.4. Membrane ageing

Ageing is known to alter the physicochemical properties of the membrane active layer and can occur naturally over time or be accelerated by exposure to chemicals such as hypochlorite and cleaning agents. Damage to the active PA layer can result in a decrease in salt rejection however laboratory scale experiments have shown that, depending on operating conditions, this may not systematically be correlated to a change in virus removal efficiency (Pype et al. 2015). A typical polysulfone supporting layer is a nanoporous structure with surface pores of approximately 15 nm which is smaller than the smallest virus (~ 16 nm). Therefore, although a loss of integrity of the active membrane layer can result in an increase in permeate conductivity, virus removal would not be expected to change significantly.

5. Operational monitoring parameters

Operational monitoring parameters are used to measure the performance of the treatment process unit, and relate to the removal performance of the target pathogen (treatment efficacy). Continuous monitoring of operational parameters provides assurance that the system is under control, and alerts operators and control systems when treatment efficacy is reduced to an unacceptable level. This would trigger corrective actions to prevent unsafe recycled water being delivered to the end user.

In theory, every factor that may affect the efficacy of the treatment process should have an operational monitoring parameter. However, in practice, it is often possible to select a few key operational monitoring parameters that effectively demonstrate efficacy.

In ideal situations it may be possible to continuously monitor a parameter in feedwater and permeate to demonstrate the integrity of the treatment process and validate that the RO/NF system is achieving the required pathogen removal. This validation protocol provides guidance on the type of parameters that may be considered.

5.1. Indirect integrity monitoring

Indirect integrity monitoring is defined as monitoring a filtrate water parameter that is indicative of the removal performance, as per section 1.3.4 of the US EPA *Membrane filtration guidance manual* (US EPA 2005). Ideally, such monitoring would be performed on a continuous basis; however, this may not be achievable, or practical, for some parameters, in which case daily integrity monitoring may be appropriate.

Indirect integrity monitoring of RO/NF membranes uses water quality parameters such as EC, TOC, DOM, sulfate or R-WT as measures of membrane integrity. These parameters are referred to herein as membrane integrity indicators. Control limits must be established that are used to indicate the presence of an integrity breach rather than being a definitive measure of performance (Victorian Department of Health 2013).

Indirect integrity monitoring should confirm the validated log reduction, and it may include the monitoring of the following parameters:

- EC
- TOC
- sulfate
- DOM
- R-WT (or other approved fluorescent dye).

5.1.1. Electrical conductivity monitoring

Electrical conductivity (EC) is used to measure ionic content, which is usually dominated by monovalent ions considering their prevalence in most waters. Online continuous monitoring of the EC of both feedwater and permeate is easy to implement and is therefore commonly used to monitor the integrity of RO/NF membranes. Most small and large-scale plants integrate EC monitoring devices during plant installation for continuous or intermittent monitoring because of their low cost and ease of operation.

The sensitivity of this technique is, however, limited to a maximum of 2 LRV because of the limited rejection of monovalent ions by RO/NF membrane systems and the relatively low EC in feedwater. For wastewater treatment, the influent EC will vary significantly between plants and may also vary within a plant depending on a range of factors, including seasonal variations and wastewater volumes and source (e.g. domestic or industrial).

Spiking feedwater with inert salts, including NaCl and sulfate compounds, can improve existing conductivity

profiling when the feedwater salt concentrations are low and target LRVs cannot be demonstrated. Spiking salt is relatively simple and inexpensive, can be performed online using existing EC instrumentation and is recommended by manufacturers under various protocols, including the pulse integrity testing by Dow Filmtec (Jons et al. 2005). The salt concentration is usually 2000 ppm of sodium chloride (NaCl) for RO/NF membranes, and 2000 ppm of magnesium sulfate ($MgSO_4$) for NF membranes. Spiked salt testing results are correlated with online EC results and MS2 LRVs (Pype et al. 2015), and can be used to benchmark against the initial specifications or rejection performance of the membranes and assess potential ageing or impairment.

5.1.2. Total organic carbon monitoring

Online measurement of TOC is also commonly integrated into treatment systems as an online indirect integrity monitoring technique based on the continuous monitoring of the organic carbon load in the influent and permeate streams. Measurement of TOC in permeate samples using laboratory-scale equipment is challenging because of inherently low concentrations, and online instrumentation is preferred. A typical analyser measures TOC by initially liberating any inorganic carbon present as carbon dioxide (CO_2), which is then detected. Any remaining carbon in the sample is assumed to be TOC, which is then oxidised to CO_2 and detected. Compared to conductivity monitoring, this type of instrument requires a higher level of operator involvement in terms of training, calibration and maintenance.

TOC has been reported to be sensitive to changes in membrane performance (Clark 2001). Online TOC monitoring can typically be used to validate LRVs below 3-log (Kumar et al. 2007). A representative full-scale RO/NF plant implementing online TOC monitoring was able to consistently demonstrate LRVs higher than 2 logs (Zornes et al. 2010).

5.1.3. Dissolved organic matter monitoring

Dissolved organic matter (DOM) is a heterogeneous mixture of aromatic and aliphatic hydrocarbon structures containing different functional groups. In the past decade, the use of excitation–emission matrix (EEM) fluorescence has been widely studied to analyse DOM in aquatic samples (Chen et al. 2003, Hambly et al. 2010, Her et al. 2008, Leenheer & Croue 2003, Peiris et al. 2010a, 2010b, Singh et al. 2009). Integrity monitoring of the RO process and validation of between 1.9 and 2.7 LRVs was demonstrated using DOM rejection analysed by EEM (Pype et al. 2013, Singh et al. 2009).

DOM can be measured using commercially available portable instruments. Singh et al. (2012) demonstrated the possibility of using a specific excitation–emission wavelength pair of the peak C area ($\lambda_{Ex}/\lambda_{Em}$ = 340/426 nm) for online membrane monitoring (1.7 LRV). Fluorescence EEMs can be obtained in a few minutes and used to identify the presence of DOM, which may indicate a membrane integrity issue. Paired grab samples of feedwater and permeate can also be tested offline in a suitable fluorescence spectrophotometer, and can demonstrate from 1.9 to 2.7 log by using a specific region of the EEM (Pype et al. 2013).

5.1.4. Sulfate monitoring

Sulfate (SO_4^{2-}) is a divalent anion that is bigger than most monovalent ions and naturally present in feedwater. The sulfate concentration naturally present in feedwater or permeate may be lower than can be measured by the detection method, in which case sulfate – as $MgSO_4$ – can be spiked in feedwater. LRVs above 2 logs can generally be demonstrated (Pype et al. 2015).

At present, continuous online monitoring of sulfate is not readily available at the sensitivities required. Sulfate is typically monitored offline using analytical techniques such as ionic chromatography (IC). Online IC systems are available but the cost of installation is usually prohibitive for typical treatment plants. The sensitivity of offline sulfate monitoring is sufficient to demonstrate up to 3 LRV (Kruithof et al. 2001), depending on the concentration of sulfate in feedwater.

In lieu of continuous monitoring, offline sulfate monitoring may be used, provided the sampling frequency is justified based on the specific design of the water recycling system and the associated risk assessment. Such discrete monitoring is usually in addition to online EC monitoring, which is required to identify any sudden change in membrane performance or loss of integrity.

5.1.5. Rhodamine WT (or other dyes) monitoring

R-WT and other dyes are considered for their suitability as virus surrogates and discussed in more detail in section 3.2. Using commercially available fluorescence measurement instruments, these chemicals can be monitored continuously or semi-continuously within the treatment process to demonstrate integrity and performance and can therefore also be considered membrane integrity indicators.

5.2. Operational envelope

Operational parameters that need to be continuously monitored in RO/NF systems to ensure that the operation stays within the validated envelope relate to the performance of the membrane. Parameters that should be monitored include:

- permeate flux
- feed pressure
- feedwater pH
- feedwater temperature
- recovery
- factors that influence feedwater composition such as ionic strength and DOC.

Critical limits or ranges should be set for each operational parameter, indicating when the treatment process is outside the validated range and appropriate corrective actions are required to bring the system back into the validated range and/or cease supply of potentially inadequately treated water.

Control systems may provide access to a broader range of operating parameters; the recommended set above is considered a minimal requirement.

6. Validation method

The objective of validation is to demonstrate the pathogen log reduction capability of the treatment process. In most circumstances, it is expected that the RO/NF system would have been challenge tested by the manufacturer prior to installation in order to demonstrate the achievable virus removal. If challenge testing has not been performed prior to installation, this protocol allows for the possibility of such challenge testing being conducted as part of the validation process in a pilot or full-scale facility, as per section 6.1.

The maximum theoretical LRV that can be demonstrated using a specific membrane integrity indicator is determined by the removal performance of the membrane for the particular parameter. However, the actual LRV that can be demonstrated might be lower than this theoretical LRV, depending on both the limit of detection in permeate and the parameter concentration in feedwater. In situations where the parameter concentration in feedwater is the limiting factor, spiking (implemented as discrete pulses or continuous addition) can be considered to demonstrate higher LRVs (Jons et al. 2005). The validation monitoring described herein is to be maintained during normal operations, with the validated LRV set as a critical limit.

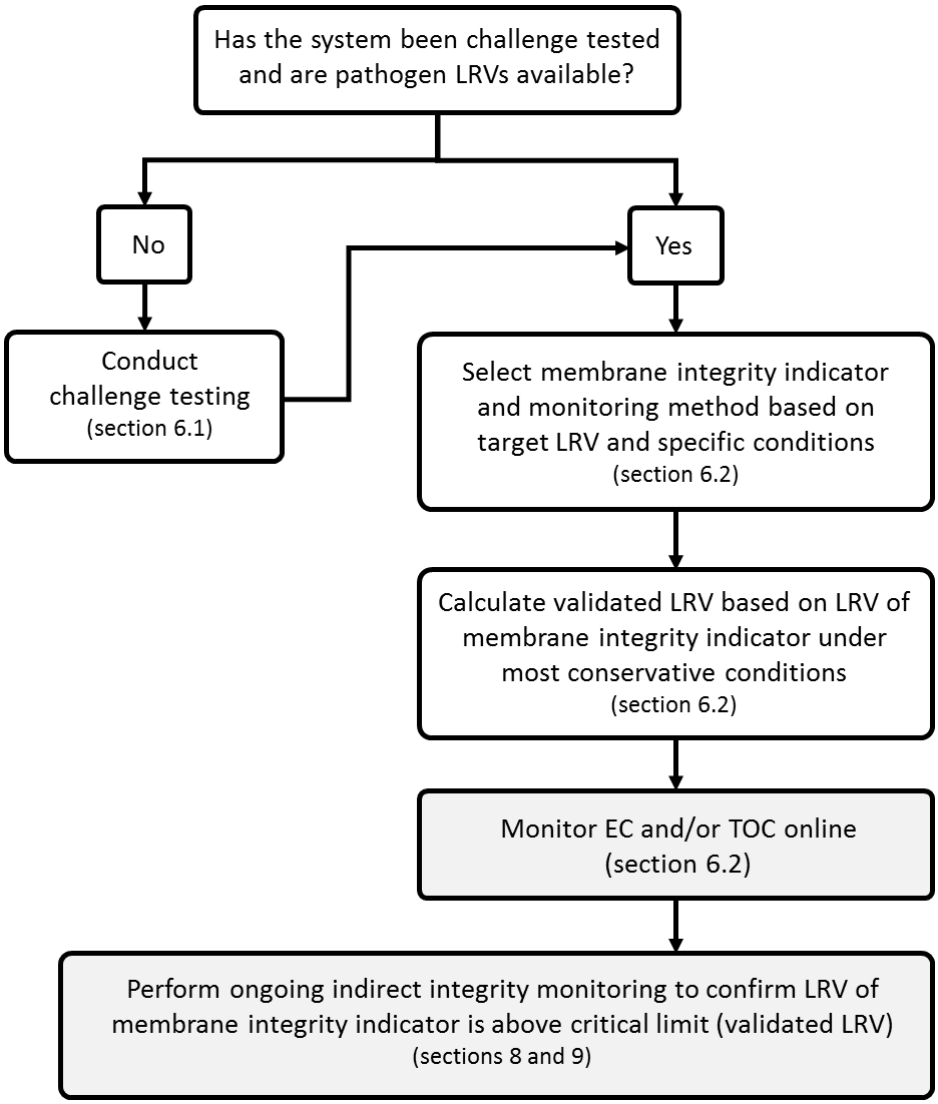

Figure 1. Overview of RO/NF validation method

The validation method, as summarised in Figure 1, is as follows:

- In all cases, supply pre-installation challenge testing data demonstrating virus LRVs that can be achieved by the specific RO/NF system under relevant operating conditions and using appropriate target pathogens or surrogates as described in section 3.
- Irrespective of the validation methodology selected, monitor EC and/or TOC removal online in feedwater and combined permeate to allow the detection of any catastrophic breach of integrity.
- Select a water quality parameter to be used as a membrane integrity indicator based on the target LRV and on a range of factors, including feedwater properties, system design, operating envelope, and analytical equipment or methods available. The validated LRV is defined as the minimum LRV that can be demonstrated continuously or near real-time for the validated operating envelope using the parameter selected from EC, TOC, DOM, sulfate or an approved fluorescent dye such as R-WT. Guidance on membrane integrity indicators, associated monitoring methods and LRVs that can in principle be demonstrated using such parameters is provided in section 6.2 and Table 2.
- Where the LRV is limited by the membrane integrity indicator concentration in feedwater, consider regular or continuous spiking as an option to demonstrate a higher LRV (although this is not recommended for TOC or DOM as this could impact system performance and water quality).
- Preferably, monitor the membrane integrity indicator online to calculate the actual LRV and demonstrate that it is higher than the validated LRV. However, if the appropriate instrumentation is not available, a daily laboratory test is acceptable provided EC and or TOC removal is monitored continuously, as prescribed above.

6.1. Challenge testing

The objective of challenge testing prior to installation is to demonstrate virus LRVs that can be achieved by the specific RO/NF system under relevant operating conditions and using appropriate target pathogens or surrogates as described in section 3. Based on the implementation of an indirect integrity monitoring strategy as part of the validation method as described above and in section 6.2, there is no requirement for post commissioning challenge testing.

Challenge testing should be consistent with section 3 of the US EPA *Membrane filtration guidance manual* (US EPA 2005). The process for challenge testing is summarised as follows:

- Select the target pathogen or surrogate
- Determine test operating conditions
  - select modules for testing. The modules tested should be identical to those used in the full scale installation.
  - define operating envelope to be validated based on the relevant influencing factors, including maximum operating flux and recovery. For surrogates whose removal is temperature sensitive, such as R-WT, temperature compensation must be considered.
  - define characteristics of the challenge testing solution
- Calculate removal efficiency.

6.1.1. Challenge testing solution

The challenge testing solution should be of a high quality, as particulate matter in the feedwater can increase removal of smaller contaminants. High-quality water, or 'particle-free water' with a low concentration of suspended solids (e.g. membrane filtrate), should be used as the matrix for the challenge solution, minimising the potential for formation of a fouling layer during the challenge test that would increase removal of the challenge organism or surrogate.

No oxidants, disinfectants or other pre-treatment chemicals should be added to the test solution, unless necessitated by process requirements. The water quality of the matrix for the test solution should not interfere with the introduction, dispersion or measurement of the surrogate. For a tracer dye test, mass balance calculations must be carried out based on the feed, filtrate and concentrate streams, to assess the potential for adsorption or other loss of the surrogate.

If a microbial challenge particulate is used, it may be necessary to add buffers or other materials to maintain the viability of the organisms. When using a challenge organism, there should be no residual disinfectant in the feedwater that could lead to inactivation.

The challenge test solution should be characterised with respect to basic water quality parameters, such as pH, temperature, turbidity, total dissolved solids, ionic strength and DOC/TOC, and any others that are critical to the test or interpretation of the results.

6.1.2. Challenge testing operating conditions

Both the seeding and sampling methods selected for challenge testing, as well as the hydraulic configuration of the system, affect the design of the test apparatus which should be operated under representative hydraulic conditions

The operating envelope is defined as a set of operating limits (minimum, maximum or range, where appropriate) for key operating parameters as defined in Section 5.2. The most conservative conditions, or boundary conditions that will lead to the most conservative LRV, need to be applied during challenge testing. Therefore, the validation sampling program should be conducted at the:

- lowest permeate flux
- lowest cross-flow velocity
- lowest pH
- lowest recovery
- highest temperature.

In addition to parameters listed as significant, other potential influencing factors should be documented during the validation study. Membrane parameters, including supplier, model number, configuration, nominal MWCO, membrane material, planned chemical cleaning frequency and replacement schedule, should be documented for each validation study.

6.2. Selection of membrane integrity indicator

The validated LRV is determined as the minimal LRV that can be consistently demonstrated by indirect integrity monitoring during operations, within the validated operating envelope, and using one of the following membrane integrity indicators: EC, TOC, DOM, sulfate or an approved fluorescent dye such as R-WT. Guidance on membrane integrity indicators, associated monitoring methods and the LRVs that can, in principle, be demonstrated using such parameters is provided in Table 2.

Table 2 Potential ranges of LRVs demonstrated using different membrane integrity indicators and monitoring methods

Membrane integrity indicator	Monitoring method	LRV			
		1	2	3	4
Electrical conductivity	Feed and permeate online monitoring – spiking[a] option if feed limited				
Total organic carbon	Feed and permeate online monitoring – no spiking				
Dissolved organic matter	Feed and permeate daily grab sample and analysis – no spiking				
Sulfate	Feed and permeate daily grab sample and analysis – spiking[a] option if feed limited				
Fluorescent dye	Feed and permeate monitoring of spiked[a] fluorescent dye (online monitoring or grab sample)				

[a] Spiking can be implemented as either discrete pulses or continuous addition

The selection of the parameter will take into account a number of aspects as follows:

- LRV targeted for the RO/NF system and LRV that can be theoretically demonstrated using a particular parameter.

  As an example, based on the removal mechanisms and on the intrinsic performance of RO/NF membranes, EC is not expected to be able to be used to demonstrate LRVs greater than 1.5-log under most operating circumstances, whereas higher LRVs can in principle be demonstrated with R-WT. The LRV which could be theoretically demonstrated will also be affected by system design and operating parameters such as recovery and membrane type which need to be taken into account when selecting a membrane integrity indicator.

- Feedwater concentration.

  This affects the actual LRV (as opposed to the theoretical LRV) which can be demonstrated for a RO/NF system. Some membrane integrity indicators may be able to be spiked in feedwater, either on a continuous or regular basis (e.g. daily), in order to increase the actual LRV being demonstrated.

- Analytical method and equipment.

  The limit of detection of a particular parameter in permeate also directly affects the actual LRV which can be demonstrated and the availability of instrumentation for sensitive online detection needs to be taken into account when selecting a membrane integrity indicator. When online analysers are not available, daily testing using laboratory-based methods may be appropriate (e.g. sulfate monitoring for which up to 3 log removal credits can be demonstrated in some circumstances);

Based on influencing factors as described in Section 4, within the validated envelope, the minimum removal of membrane integrity indicators (i.e. validated LRV) would be expected under the following conditions:

- low recovery (apart from R-WT for which a high recovery is more appropriate) and low permeate flux
- low feedwater pH
- high feedwater temperature (apart from sulfate for which a low temperature is more appropriate).

In all cases, EC and/or TOC removal needs to be monitored online in feedwater and combined permeate to allow the real-time detection of any catastrophic breach of integrity.

7. Data collection and analysis

The data collected during the validation testing program must be representative and reliable. To ensure that quality data are collected:

- appropriate sampling methods and techniques must be consistent with the *Standard methods for the examination of water and wastewater* (Rice et al. 2012)
- National Association of Testing Authorities (NATA)–accredited methods must be used, where available. Where NATA accredited methods are not available, the laboratory must
    - demonstrate that the method used is consistent with a standard method, where this is available
    - document the method used for the analysis
    - retain documentation and appropriate quality assurance data
    - engage independent expert(s) to peer review and endorse the method
- field and laboratory equipment must be maintained and calibrated
- limits of detection must be appropriately measured
- all procedures must be completed by qualified personnel and be subject to quality assurance or quality control procedures.

The monitoring program for the validation study must ensure that the data collected are relevant and sufficient for a statistically valid analysis. The raw data and their analysis must be appended to the validation report. If data are excluded from the analysis, the rationale must be provided.

In analysing data, validation uncertainty needs to be taken into account, including biases and errors in measurements, laboratory equipment, experimental design and analytical techniques. The measurement of uncertainty must be included, to the extent practicable, when attributing an LRV to the treatment process unit.

Under the ISO standard to which NATA accredits laboratories – ISO/IEC 17025-2005: *General requirements for the competence of testing and calibration laboratories* – accredited laboratories are required to estimate the uncertainty associated with the results they produce (known as the measurement of uncertainty). Measurement of uncertainty data must be provided when reporting analytical results. This information will show the variability in the analytical data and will assist in formulating evidence-based conclusions.

Furthermore, during validation testing, all equipment must be carefully selected and calibrated to minimise uncertainty. Measurements must be traceable to a registered standard method, where this is available.

Increasing the sample number and/or sample volume, and using more accurate and precise measuring devices will provide the best estimate of the capability of a treatment process unit to remove or inactivate pathogens.

8. Critical limits and operational monitoring

Operational monitoring is necessary to ensure adequate control over the system and to continuously confirm that the system is operating within the validated operational envelope. Where operational parameters are found to be outside the validated operating envelope, the log inactivation may not be achieved, resulting in the supply of water that is not fit for use. Action should be taken to bring the system back into the envelope and/or stop the supply of potentially unsuitable water.

A critical limit is a value that must be met to ensure that a critical control point effectively controls a potential hazard; it is a limit that separates acceptability from unacceptability.

8.1. LRV

The validation methodology described under this validation protocol relies on indirect integrity monitoring by measuring the LRV of a specific water quality parameter or membrane integrity indicator to demonstrate that it is consistently above the validated LRV credited to the system (section 6.2), thereby demonstrating integrity of the system. Therefore the validated LRV is to be set as a critical limit so that corrective actions are taken in instances when the actual parameter LRV falls below the validated LRV.

The LRV is to be calculated based on the ratio of the parameter concentration in combined permeate versus combined feedwater as per Section 9. This is independent of the performance monitoring which may occur within the RO/NF system, for example between trains, vessels or individual modules. Such performance monitoring is considered normal practice and is required to identify localised losses of performance or integrity which may affect the overall removal performance of the system and generally involves EC monitoring.

8.2. Validated operating envelope

Based on the fact that the RO/NF system is being validated for a defined set of operating and feedwater quality conditions, specific critical limits should be set on all operating parameters which may affect removal performance as described under Section 5.2. Operating outside this envelope would invalidate the LRVs established under this protocol.

9. Method to determine the LRV for each pathogen group

The USEPA *Membrane Filtration Guidance Manual* (USEPA 2005) identifies the following equation for calculating LRV:

$$LRV = \log (C_f) - \log (C_p)$$

where:

LRV = log removal value demonstrated during a challenge test

C_f = combined feedwater concentration of the challenge pathogen or surrogate

C_p = combined permeate concentration of the challenge pathogen or surrogate

Feedwater and permeate concentrations must be expressed in identical units (i.e. based on equivalent volumes) in order to yield a valid LRV. If the challenge pathogen or surrogate is not detected in the permeate, then the term C_p is set equal to the detection limit.

A single LRV is calculated for each module challenge tested. The overall removal efficiency demonstrated during challenge testing is called $LRV_{C\text{-}test}$. The approach to determining the LRV results should be consistent with the USEPA *Membrane Filtration Guidance Manual* (USEPA 2005):

- where fewer than 20 modules are tested, the lowest representative LRV achieved among the various modules tested will be the adopted as $LRV_{C\text{-}test}$
- where more than 20 modules are tested, the 10th percentile of the representative LRVs among the various modules will be adopted as $LRV_{C\text{-}test}$.

10. Triggers for revalidation

Processes should be revalidated when variations occur that may affect the performance of processes (e.g. impacts of changes to primary or secondary treatment processes on downstream filtration or disinfection). Any new processes should be tested using benchtop, pilot-scale or full-scale experimental studies to confirm that the required results are produced under conditions specific to the individual water supply system.

Significant changes to operations include:

- design modifications
- control philosophy or operating parameters
- membrane replacement with a different model to the one validated
- changes to the intended use requiring a higher water quality (higher LRVs)
- continual breaches of the critical limit
- changes to the operating envelope outside which the process is validated for.

Glossary and abbreviations

CP	concentration polarisation
DOC	dissolved organic carbon
DOM	dissolved organic matter
EC	electrical conductivity
EEM	excitation emission matrix
IEP	isoelectric point
LOD	limit of detection
LRV	log reduction value A $\log_{10}$ reduction value is used in the physical–chemical treatment of water to characterise the removal or inactivation of microorganisms such as bacteria, protozoa and viruses (1-$\log_{10}$ = 90% or a 10-fold reduction, 3-$\log_{10}$ = 99.9% or a 1000-fold reduction, and so on). LRV = $\log_{10}$ (N_0) – $\log_{10}$ (N), where N_0 = concentration of infectious microorganisms before treatment and N = concentration of infectious microorganisms after treatment.
$LRV_{C\text{-}test}$	The log removal value as determined from challenge testing.
MS2	MS2 bacteriophage
MWCO	molecular weight cut-off
NATA	National Association of Testing Authorities
NF	nanofiltration
PA	polyamide
RO	reverse osmosis
R-WT	rhodamine WT
TDS	total dissolved solids
TOC	total organic carbon
US EPA	United States Environmental Protection Agency

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