World Health Organization
REGIONAL OFFICE FOR Europe

Revision of Annex I of the Council Directive on the Quality of Water Intended for Human Consumption (Drinking Water Directive)

Background paper on microbiologically safe water and microbiological parameters

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1 Introduction

The Council Directive 98/83/EC on the Quality of Water Intended for Human Consumption, the Drinking Water Directive (DWD, in this document: Directive), was published in November 1998 and (when excluding water offered for sale in bottles or containers) lists two microbiological parameters in Annex I Part A and three microbiological parameters in Part C.

In accordance with Article 11 of the Directive, "*at least every five years the Commission shall review Annex I in the light of scientific and technical progress and shall make proposals for amendments, where necessary*". Recital 16 of the Directive further acknowledges that "*the standards in Annex I are generally based on the World Health Organisation's Guidelines for Drinking-water Quality*".

The parametric values of the parameters covered in Annex I were primarily based on the second edition of the World Health Organization (WHO) Guidelines for Drinking-water Quality (in this document: Guidelines) published in 1993. Since the publication of the Directive there have been two further editions of the Guidelines with a number of changes to guideline values in the light of new scientific evidence. The latest, fourth edition of the Guidelines was published in 2011; WHO undertakes regular updates on a rolling basis, and the first addendum to the fourth edition of the Guidelines is to be published in 2016.

2 Comparison of the approach to microbiologically safe drinking-water in the Directive and the WHO Guidelines

2.1 General obligation

A straightforward comparison of the microbiological parameters in Annex I of the Directive with the Guidelines does not do justice to the paradigm change in assessing the microbiological safety of drinking-water introduced since the third and fourth edition of the Guidelines. Therefore, this section aims to give a brief overview of the rationale for a new, risk-based paradigm as the backbone of the Guidelines with an emphasis on microbiological safety.

The general principle in the Guidelines is:

"Safe drinking-water, as defined by the Guidelines, does not represent any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages."

This is essentially the same principle as stated in the general obligation of Article 4 of the Directive:

"(…) Member States shall take the measures necessary to ensure that water intended for human consumption is wholesome and clean. For the purposes of the minimum requirements of this Directive, water intended for human consumption shall be wholesome and clean if it (…) is free from any micro-organisms and parasites and from any substances which, in numbers or concentrations, constitute a potential danger to human health."

It could be argued that reference to the "potential danger" in the Directive is stricter than the "significant risk" referred to in the Guidelines. However, it is important to consider how this general principle is translated into practical requirements. This will be done in the following sections, with the focus on microbial safety.

2.2 Control of excreta-related, enteric pathogens

The Directive's general obligation is translated into minimum water quality requirements for microbiological parameters set out in Annex I Part A. These parameters are *Escherichia coli* (*E. coli*) and enterococci. The minimum water quality requirement is defined as their absence in 100 ml of water.

E. coli and enterococci themselves do not constitute a danger to human health. They are present in high numbers in the intestinal tract of all healthy people. The rationale for setting minimum quality requirements for these two parameters is their indicator function: both parameters reliably indicate the presence of human or (warm-blooded) animal excreta in water.

The contamination with excreta is still the most significant and frequently occurring health risk through drinking-water exposure. Excreta may harbour enteric pathogens and contamination of drinking-water with these pathogens can cause illness when the water is consumed.

The indicator concept was developed already more than 100 years ago: Schardinger proposed in 1892 that, since *E. coli* (then called *Bacterium coli*) was a characteristic component of the faecal flora, its presence in water could be taken as *"an indication of the presence of faecal pollution and therefore of the potential presence of enteric pathogens".*

There is a wide range of bacterial, viral, protozoal and helminth pathogens that may be present in excreta and for which there is evidence that they can occur in drinking-water and cause waterborne disease (i.e. bacteria such as *Campylobacter, Salmonella, Shigella, EHEC;* viruses such as norovirus, enterovirus, adenovirus, rotavirus, Hepatitis A and E virus; parasitic protozoa such as *Cryptosporidium* and *Giardia;* helminths such as *Ascaris*). Cost and complexity in pathogen testing would not allow for an approach aiming at assaying water for all possible enteric pathogens. As the common source of all of these pathogens is faecal pollution, for microbiological safety, the aim has been a universal microbial indicator of faecal contamination. This approach has been markedly different from health-associated chemical parameters, where a more individual parameter approach is used.

E. coli is, undoubtedly, the most commonly used microbial parameter for testing drinking-water quality. Its use has led to significant improvement in the safety of drinking-water world-wide and is had been adopted already in the first edition of the Guidelines and virtually in all national drinking-water quality standards globally. One of the main reasons for their success was and is the ease of the assay.

Even though the use of *E. coli* and enterococci for water quality monitoring has led to significant improvements in drinking-water safety, the use of these microbial indicators has serious shortcomings. These have been summarized as "*too little and too late*" (WHO/OECD, 2001).

"Too late"

A major shortcoming for public health protection is that monitoring the microbial safety of drinking-water is reactive, in the sense that any incident or breakdown in the water supply system can occur many hours and sometimes days before it is detected by monitoring of any of the microbial indicator parameters. The water has been consumed before the microbial test results are reported. This is related to the nature of the microbial testing which currently requires at least a day to produce a result. It is also related to the monitoring strategy which frequently focuses on finished drinking-water as it leaves the treatment works and in the distribution system, rather than on source water to identify peak events in time to take appropriate response measures. Also, the sampling frequencies typically stipulated for compliance monitoring in distribution networks suggest that contamination events in the distribution network have a low probability of being detected, let alone that timely corrective actions can be taken. Water utilities are expected to be in control of the water quality they supply and demonstrate due diligence. The use of *E. coli* and enterococci for end-product monitoring with methods that produce results slowly is not sufficient. Illustrative is the observation that many waterborne outbreaks are first "detected" by consumer complaints about water quality changes (see overview of outbreaks in France (Therre et al., 2008) and for instance the large outbreaks through municipal water supplies in Finland in 2007 (Miettinen et al., 2012) and in Belgium in 2010 (Braeye et al., 2015)).

"Too little" – control of transmission of enteric viruses and protozoa

The use of *E. coli* and enterococci as bacterial indicators of faecal pollution has proved successful in preventing the spread of waterborne bacterial pathogens from faecal origin, such as cholera and typhoid.

Already since the 1960s it has been recognised that enteric viruses, such as hepatitis A, enteroviruses, noroviruses, rotaviruses, can be transmitted through drinking-water (see also Table 7). Virus contamination of water also originates from pollution with human excreta. However, the nature of viruses is very different from that of bacteria. They are much smaller than bacteria (i.e. 20 – 80 nm in comparison to 0.5 – 2.0 µm) and therefore less likely to be removed during filtration or soil passage. Also, their resistance to disinfection is typically higher than bacteria, ranging from high resistance of hepatitis A virus to chlorination to the very high resistance of adenovirus to UV irradiation. The occurrence of outbreaks of viral illnesses associated with drinking-water which was in compliance with *E. coli* standards indicates that *E. coli* is an inadequate parameter to assess the virological safety of treated drinking water.

Since the 1990s, a further challenge was identified with the drinking-waterborne outbreaks of intestinal illness due to parasitic protozoa, mainly *Giardia* and *Cryptosporidium*. As with viruses, large waterborne outbreaks have occurred, also in the European Union (EU), without any indication from *E. coli* testing that water quality was compromised (see early *Cryptosporidium* outbreaks in England and Wales (Badenoch, 1990), large *Cryptosporidium* outbreaks in Sweden (Widerstrom et al., 2014) and Ireland (Pelly et al., 2007)). *Giardia* and particularly *Cryptosporidium* are far more robust in the environment, and survive much longer in water and resist chemical disinfection far better than the indicator bacteria. Several Member States have responded to this shortcoming by explicitly targeting *Cryptosporidium,* or both protozoa and viruses, in their national drinking-water regulation.

2.3 Control of Legionella pneumophila that may grow in the drinking water system

Another class of pathogenic micro-organisms can be present in drinking-water, and have been associated with illness through drinking-water (see also Table 7). Unlike the enteric pathogens discussed above, these opportunistic pathogens are not from faecal origin but naturally occur and grow in (specific) water environments. They include *Legionella pneumophila*, non-tuberculous mycobacteria and others.

Legionellosis is a serious illness that is caused from inhalation of water droplets (aerosols) from warm water systems in which *Legionella pneumophila* can multiply. Legionellosis is, unlike gastro-intestinal infections, almost exclusively waterborne. The reported incidence in the EU/EEA is 11.4 per million Europeans, which amounts to about 6,000 cases per year with a fatality rate of 10% (ECDC, 2014). The reported incidence as well as the reporting system vary significantly between EU Member States from 0.1 to 40 per million. Due to under-diagnosis and under-reporting, the true incidence may be considerably higher than what is reported.

Legionella may be transmitted via non-drinking-water systems, such as cooling towers, spa pools and fountains, and outbreaks via these routes are reported. Most of the *Legionella* cases are not outbreak-associated but sporadic cases (92%). Most (73%) of the infections are community acquired, 19% is associated to travel and 8% to healthcare settings (ECDC, 2014). Outbreaks have been associated to plumbing systems for drinking-water in buildings. In the United States of America (USA), *Legionella* outbreaks via drinking-water are on the rise in the last 15 years (Beer et al., 2015). In Europe, the number of reported legionellosis cases increased in the period 2000 – 2007 and remained stable relatively since at around 11.4 cases per million (ECDC, 2014). Because legionellosis is generally a severe disease, the costs associated with legionellosis are substantial. For the USA, with an estimated 13000 hospitalizations due to legionellosis, the direct healthcare

costs amount to 434 million US$ per annum (Collier et al., 2012). A study on legionellosis among pneumonia cases in Germany indicated that *Legionella pneumophila* was a leading cause of community-acquired pneumonia (von Baum et al., 2008). While around 900 legionellosis cases are reported through the infectious disease reporting system, von Baum et al. estimate 15000-30000 cases of legionellosis annually in Germany.

Legionella pneumophila and the other opportunistic pathogens are a particular control challenge and require multi-stakeholder involvement (water supplier and building owner) for adequate control. *Legionella pneumophila* has been found throughout the water distribution system: from the mains supply to the consumer's showerhead. It has been found in the plumbing system of many types of buildings, including hospitals, hotels and homes. *Legionella pneumophila* resides in the biofilm and appears to proliferate inside amoebae that feed on the biofilm. Control of proliferation of *Legionella pneumophila* in water networks and plumbing systems require adequate control of microbial growth in these systems, particularly control of conditions that favour proliferation of *Legionella pneumophila* (such as elevated temperatures). Adequate control of *Legionella* requires control of the water treatment (removal of nutrients for biofilm growth), distribution network (materials, residence time, possibly disinfection residual) and in-house plumbing system (temperature, materials). The main points for control of proliferation of *Legionella pneumophila* lie within the buildings' plumbing systems.

The parameter for the general bacteriological quality of drinking-water in the Directive (heterotrophic plate counts (HPC)) is not directly related to *Legionella pneumophila* or other opportunistic pathogens, and meeting the HPC standard in the Directive is not sufficiently protective against *Legionella pneumophila* and other opportunistic pathogens (see also the discussion of the HPC standard later in this paper). So, the current Directive does not offer adequate protection against *Legionella pneumophila* (or other opportunistic pathogens that may grow in drinking-water systems).

Many EU Member States have implemented codes for *Legionella* control and prevention as part of their occupational safety regulations, under EU Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work (EU-OSHA, 2011). The Directive requires the employer to develop a risk assessment scheme, thus reducing the exposure to biological agents such as *Legionella.* Several Member States have standards or guidelines for risk assessment and risk management of *Legionella* in building plumbing systems, mostly focussed on warm water systems (and other water systems, such as cooling towers). Some Member States (Germany, Netherlands) have embedded *Legionella* control by risk assessment and monitoring of warm water systems in the national drinking-water legislation.

2.4 Conclusion: current parameters in the Directive are insufficient to ensure drinking-water safety

The shortcomings of the current parameters in the Directive listed above have a very important implication: they imply that end-product testing of drinking-water for faecal indicator bacteria and HPC testing provide insufficient safeguards to public health. With the current state of knowledge about microbiological safety of drinking-water, the view that the general obligation of Article 4 of the Directive is fulfilled with only the current point-of-compliance testing requirements for *E. coli*, enterococci and HPC can no longer be maintained. Legionellosis is almost exclusively waterborne and the health burden and cost in the EU is significant and largely preventable. The occurrence of

outbreaks of legionellosis and intestinal illness via drinking-water systems undermines the confidence of EU citizens in the safety of their water supply.

3 Microbiologically safe drinking-water in the WHO Guidelines for Drinking-water Quality

3.1 The Safe Drinking-Water Framework

The second edition of the Guidelines was in a similar position: The recognition that end-point testing for indicator bacteria and HPC was insufficiently protective of public health has been a major driver for the paradigm change in the Guidelines towards a preventative risk assessment and risk management framework: "*The most effective means of consistently ensuring the safety of a drinking water supply is through the use of a comprehensive risk assessment and risk management approach that encompasses all steps in water supply from catchment to consumer.*" This is now the backbone of the third and fourth editions of the Guidelines. The Guidelines are based on the "Safe Drinking-Water Framework" which provides a conceptual framework to assess water quality hazards and manage associated risks. The framework is depicted in Figure 1 and comprises of three key components:

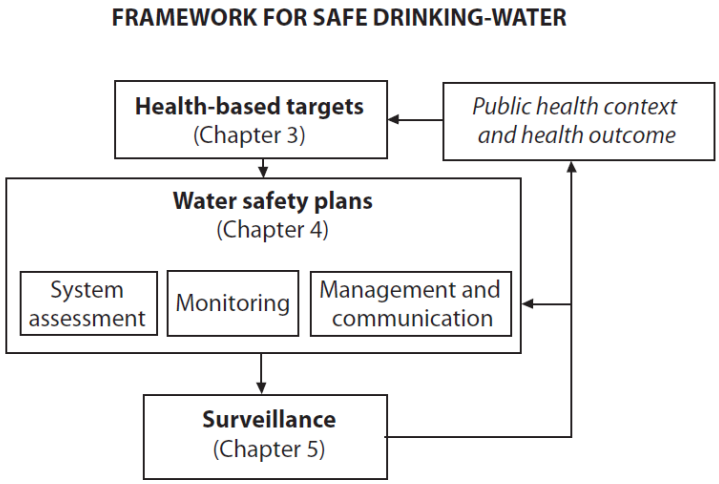

Figure 1. WHO Guidelines framework for safe drinking-water

1. Health-based targets: these are the targets for safe drinking-water supply systems. These should be set by high-level health authorities and take into account the overall public health situation and contribution of drinking-water to disease. Health-based targets can be health outcome targets or water quality targets, treatment performance targets or prescribing the use of specified technologies.
2. Water Safety Plans (WSPs) comprising:
   - A system assessment to determine whether the drinking-water supply (from source to treatment to point of consumption) as a whole can provide water of a quality that meets the health-based targets.
   - Operational monitoring of control measures in the drinking-water supply that are of particular importance in securing drinking-water safety.
   - Management plans documenting the system assessment and monitoring plans and describing actions to be taken in normal conditions and incident conditions, including upgrade and improvement, documentation and communication.

3. A system of independent surveillance that verifies that the above are operating properly.

Although the framework was developed with an emphasis on the control of waterborne infectious diseases, it also serves as basis for the control of toxic chemicals, other health hazards as well as technical or aesthetical water quality issues.

The central point of the framework is the WSP, which is the responsibility of the owner of the water system. WHO has published step-by-step guidance documents for creating water safety plans for piped water supply systems (WHO, 2009), for water supply systems for small communities (WHO, 2012; WHO Regional Office for Europe, 2014) and also for control of *Legionella* in water systems (WHO, 2007). In the other areas of WHO water guidelines (i.e. for recreation and for reuse of wastewater), WHO has also applied the Safe Water Framework and adopted similar approaches, such as sanitation safety plans. For a comprehensive description of the WSP approach, the reader is referred to the Guidelines (chapter 4) and guidance documents above.

In this paper, we will zoom in on the system assessment (risk assessment and prioritization), monitoring (operational monitoring, inspection and verification) elements of the WSP, and discuss the aspects related to microbiological safety. The risk-based approach for microbiological safety in the Guidelines is a holistic approach that systematically assesses the risks throughout the drinking-water supply chain from the catchment to the consumer's tap and identifies how these risks are addressed by appropriate control measures, i.e. ranging from catchment protection measures, engineered treatment processes to hygiene and design protocols for maintenance of networks and plumbing systems (Figure 2). The risk-based approach implies to move away from overreliance on end-product testing towards focussing on prevention and adequate control of risks through developing good understanding of possible risks and controlling sources, treatment, infrastructure and processes.

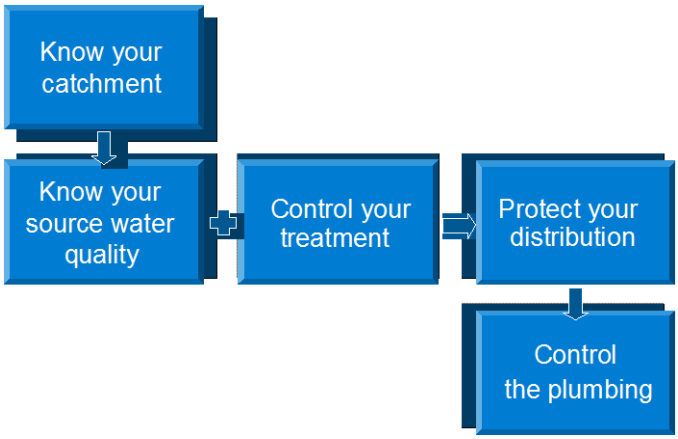

Figure 2. Source-to-tap approach for drinking-water safety management

Enteric pathogens (pathogens of the intestinal tract) are spread via the contamination of water with faeces of infected humans or animals. For this class of pathogens the key control strategy is the multiple barrier approach: to install sufficient barriers in the water supply system to

1. prevent them from entering source water (catchment protection);
2. remove or inactivate them by water treatment processes; and
3. protect the distribution network against ingress.

Opportunistic pathogens such as *Legionella pneumophila* are able to multiply in water networks and plumbing systems under certain conditions. For this class of pathogens the key control strategy is to avoid conditions that are favourable for multiplication of these pathogens. For *Legionella pneumophila*, key favourable conditions are warm water temperatures and presence of biofilm and amoebae in the network or plumbing system.

The risk based-control strategies under the Guidelines for these classes of pathogens are addressed separately in the following paragraphs. It is not the intent of these paragraphs to describe the risk-based control in detail, but to give an overview of the risk-based control strategy, especially the assessment of hazards and risk factors and how they are controlled in the water system (system assessment) and how the efficacy of the control measures for pathogens in the water system is monitored (operational monitoring) and verified (verification monitoring).

3.2 Risk-based control of enteric pathogens

System assessment – risk factors

The source of this class of pathogens is the excreta of man and (for several of these pathogens) warm-blooded animals, such as farm animals, but also wild mammals and birds. Risk factors for contamination of drinking-water with this class of pathogens are:

- Contamination of source water with excreta from man, livestock, wildlife;
- Contamination of source water with (treated) domestic sewage, run off of manure, leaching of septic tanks and manure storage;
- Events leading to peak contamination of source waters, such as heavy rains, snowmelt and flooding;
- Insufficient protection of groundwater sources;
- Insufficient integrity of groundwater wells;
- Insufficient treatment, treatment failure or periods of suboptimal or poor treatment performance, allowing breakthrough of pathogens;
- Accumulation of pathogens in the treatment chain (such as via filter backwash water);
- Ingress of pathogens via open storage or openings, leaks etc. in the treatment plant;
- Ingress of pathogens in storage reservoirs or the piped network (leaks, low/no pressure events, repairs, cross-connections etc.).

System assessment – control measures

The first element of the control strategy is catchment protection. Groundwater from confined aquifers is well-protected by natural barriers for pathogens, that do not survive the long travel times (decades) of the water in the soil or cannot pass the very fine-grained soil layers, such as clay. Producing drinking-water from such well-protected sources requires no treatment to remove pathogens, but does require adequate protection of the catchment, of the abstraction wells and the water treatment, storage, transport and distribution to ensure that no excreta can enter the water.

In contrast, surface water in agricultural or urban areas is contaminated by pathogens via discharges of sewage treatment plants, sewer overflows, seepage of septic tanks, run-off of animal manure etc. The more abundant these sources, the more the surface water will become contaminated with pathogens. Many surveys of pathogens in European surface waters show

enteric pathogens are very frequently to always present in surface waters. For these source waters, catchment protection is still a very important control point that reduces (peak) pathogen concentrations and hence pathogen risks.

Contaminated source waters need treatment that removes/inactivates pathogens to a level that is so low that consumption of the treated water does not pose any significant health risk. So, the more contaminated the source water, the more treatment is needed. In the system assessment the required treatment is established, based on the contamination level of the source water (including its vulnerability to peak events). This element of the system assessment requires information about the occurrence and concentration of relevant pathogens in the catchment and source water.

The system assessment is expanded in the Guidelines. Figure 3 is taken from the Guidelines and shows how the required level of pathogen removal/inactivation (performance target) by water treatment increases as the concentration of selected pathogens in source water increases. The performance target is expressed as the $\log_{10}$-reduction in the concentration of pathogens that the treatment process as a whole should achieve to produce safe drinking-water.

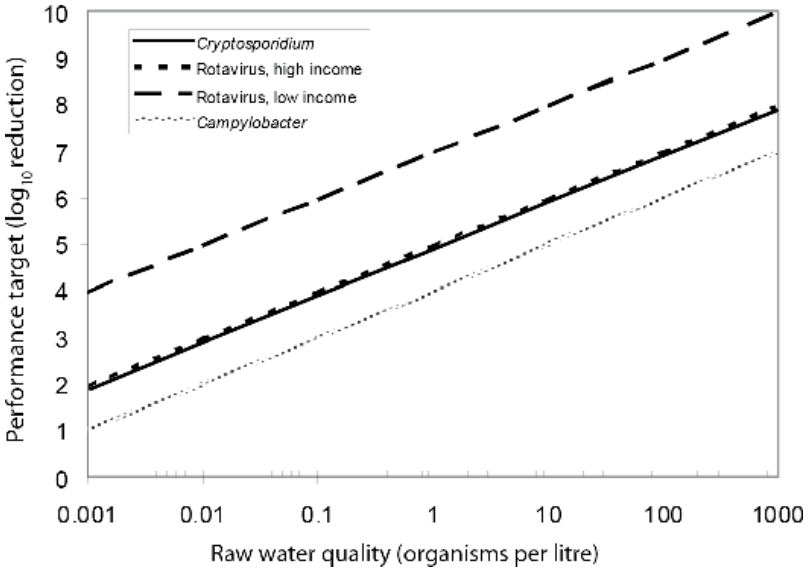

Figure 3. Performance targets for specific bacterial, viral and protozoan pathogens in relation to raw water quality (to achieve 10^{-6} DALY per person per year) (WHO, 2011)[1]

In many situations, multiple treatment processes are required to produce safe drinking-water. This is also part of the multiple barrier approach that is in the core of the WSP approach. Multiple barriers provide redundancy. The combination of physical removal by a (coagulation) filtration process and inactivation of pathogens by a disinfection process provides is a widely proven concept. The Guidelines provide information on the ability of different water treatment processes

[1] There are two other concepts of the Guidelines embedded in this figure: a health-based target and reference pathogens. The health-based target is to define when drinking-water is safe. The Guidelines propose the use of a tolerable disease burden via drinking-water (expressed in Disability Adjusted Life Years or DALYs) as metric to define "safe" and use 10^{-6} DALYs as the safe level. Appendix 1 describes DALYs and the origin of the 10^{-6} DALYs as safe level, as well as how this is translated to the treatment performance targets given in Figure 3. The concept of reference pathogens is described in Appendix 2. Figure 3 shows the reference pathogen example used in the Guidelines, with a bacterial pathogen (*Campylobacter*) a virus (rotavirus) and a protozoan parasite (*Cryptosporidium*).

to remove pathogenic bacteria, viruses and protozoan parasites (Table 1). There is also a guidance document on pathogen removal by water treatment processes (WHO, 2004; currently under revision).

The treated water is distributed through a piped distribution network, and the high quality drinking-water needs to be protected against the ingress of excreta or contaminated sewage, surface water or other contaminated sources. Key control measures are the structural integrity (no leaks) in combination with the hydraulic integrity (continuously under pressure), strict hygiene during construction and repair and the presence of a disinfectant residual (optional in systems that receive sufficient protection through the other control measures).

Table 1. Removal that can be achieved by water treatment processes[2]

Treatment process	Enteric pathogen group	Minimum removal (LRV)	Maximum removal (LRV)	Notes
Pretreatment				
Roughing filters	Bacteria	0.2	2.3	Depends on filter medium, coagulant
Storage reservoirs	Bacteria	0.7	2.2	Residence time > 40 days
	Protozoa	1.4	2.3	Residence time 160 days
Bank filtration	Viruses	> 2.1	8.3	Depends on travel distance, soil type, pumping rate, pH, ionic strength
	Bacteria	2	> 6	
	Protozoa	> 1	> 2	
Coagulation, flocculation and sedimentation				
Conventional clarification	Viruses	0.1	3.4	Depends on coagulation conditions
	Bacteria	0.2	2	
	Protozoa	1	2	
High-rate clarification	Protozoa	> 2	2.8	Depends on use of appropriate blanket polymer
Dissolved air flotation	Protozoa	0.6	2.6	Depends on coagulant dose
Lime softening	Viruses	2	4	Depends on pH and settling time
	Bacteria	1	4	
	Protozoa	0	2	
Filtration				
Granular high-rate filtration	Viruses	0	3.5	Depends on filter media and coagulation pretreatment
	Bacteria	0.2	4.4	
	Protozoa	0.4	3.3	
Slow sand filtration	Viruses	0.25	4	Depends on presence of schmutzdecke, grain size, flow rate, operating conditions (mainly temperature, pH)
	Bacteria	2	6	
	Protozoa	0.3	> 5	
Precoat filtration	Viruses	1	1.7	If filter cake is present
	Bacteria	0.2	2.3	Depends on chemical pretreatment
	Protozoa	3	6.7	Depends on media grade and filtration rate
Membrane filtration: microfiltration, ultrafiltration, nanofiltration reverse osmosis	Viruses	< 1	> 6.5	Varies with membrane pore size (microfilters, ultrafilters, nanofilters and reverse osmosis filters), integrity of filter medium and filter seals, and resistance to chemical and biological ("grow-through") degradation
	Bacteria	1	> 7	
	Protozoa	2.3	> 7	

[2] Removal is expressed in Log_{10}-removal values (LRV). Guidelines Table 7.7.

Primary disinfection[a,b]			
Chlorine	Viruses	2 (Ct_{99} 2–30 min·mg/l; 0–10 °C; pH 7–9)	Turbidity and chlorine-demanding solutes inhibit this process; free chlorine × time product predicts efficacy; not effective against *Cryptosporidium* oocysts. In addition to initial disinfection, the benefits of maintaining free chlorine residuals throughout distribution systems at or above 0.2 mg/l should be considered
	Bacteria	2 (Ct_{99} 0.04–0.08 min·mg/l; 5 °C; pH 6-7)	
	Protozoa	2 (Ct_{99} 25–245 min·mg/l; 0–25 °C; pH 7–8; mainly *Giardia*)	
Chlorine dioxide	Viruses	2 (Ct_{99} 2–30 min·mg/l; 0–10 °C; pH 7–9)	
	Bacteria	2 (Ct_{99} 0.02–0.3 min·mg/l; 15–25 °C; pH 6.5–7)	
	Protozoa	2 (Ct_{99} 100 min·mg/l)	
Ozone	Viruses	2 (Ct_{99} 0.006–0.2 min·mg/l)	Viruses generally more resistant than bacteria
	Bacteria	2 (Ct_{99} 0.02 min·mg/l)	
	Protozoa	2 (Ct_{99} 0.5–40 min·mg/l)	Depends on temperature; *Cryptosporidium* varies widely
UV	Viruses	4 (7–186 mJ/cm²)	Excessive turbidity and certain dissolved species inhibit process; effectiveness depends on fluence (dose), which varies with intensity, exposure time, UV wavelength
	Bacteria	4 (0.65–230 mJ/cm²)	
	Protozoa	4 (< 1–60 mJ/cm²)	

Ct, product of disinfectant concentration and contact time; LRV, log_{10} reduction value
[a] Chemical disinfection: Ct values are given that achieve 2 LRV.
[b] UV irradiation: UV dose range is given that achieves 4 LRV.
Sources: Chevrefils et al. (2006); Dullemont et al. (2006); Hijnen, Beerendonk & Medema (2006); see also the supporting document *Water treatment and pathogen control* (Annex 1).

Monitoring – operational

Operational monitoring is used to determine whether the control measures in the system are actually functioning to control the risk factors. Some of the control measures have to be embedded in the design or in hygiene protocols and operational monitoring is a site inspection to verify that hygiene protocols during mains repair works are strictly adhered to, that the structural integrity of a storage reservoir is intact or an abstraction well is constructed in a manner that prevents short circuiting of pathogens that are deposited near the well to the aquifer.

Other control measures are more prone to variation and need to be monitored regularly (ideally continuously) to determine that they are functioning properly, for example:

- River level, flow, turbidity to determine peak contamination events;
- Dose of coagulant (aid);
- Filter effluent turbidity to determine effective particle (and pathogen) removal;
- Residual dose of chlorine or ozone after contact chambers to determine efficacy of disinfection;
- UV fluorescence, UV transmission, flow to control the efficacy of UV disinfection;
- Particle counts of effluent of ultrafiltration.

Monitoring – verification

Verification monitoring is used to verify, independent from the operational monitoring, that the control measures effectively control the risk of enteric pathogens. This is where the current water quality standards for *E. coli* and enterococci are used: water intended for human consumption should contain no faecal indicator organisms. Water that does contain *E. coli* is contaminated with

excreta form humans or warm-blooded animals and hence there is potential presence of pathogens in the water.

The Guidelines give guideline values for *E. coli* in water intended for drinking, for treated water entering the distribution system and water in the distribution system: *E. coli* must not be detectable in any 100 ml sample (same value as in the Directive for drinking-water at the point of use). Again, as discussed in section 2.2, the absence of *E. coli* in water does not necessarily imply that pathogens are also absent. Hence the paradigm change described above.

Monitoring for *E. coli*, however, provides a high degree of assurance (certainly against enteric bacteria) because of their large numbers in polluted waters. The Guidelines indicate that it may be desirable to include more resistant indicators to verify the control measures are effective against viruses and protozoa, such as bacteriophages and/or bacterial spores.

3.3 Risk-based control of Legionella pneumophila

System assessment - hazard identification

Legionella pneumophila is a member of the genus *Legionella. Legionella* bacteria are typical water-inhabitants. They are ubiquitous in natural and man-made water systems world-wide. *Legionella pneumophila* is the species that is the causative agent of legionellosis, a severe pneumonia, and Pontiac fever, that is a relatively mild, flu-like illness. *Legionella pneumophila* typically inhabits warm water systems, with optimum growth temperatures of 37-42˚C. As the temperature is below 20-25˚C, *Legionella pneumophila* does not grow in water systems as it is outcompeted by other micro-organisms. Therefore, the recommendation is to keep cold water systems below 25˚C and preferably below 20˚C. At temperatures above 50˚C, *Legionella pneumophila* does not grow in water systems, but can survive. At 60˚C, *Legionella pneumophila* is rapidly inactivated (the time for 90% reduction of viable bacteria is 2 minutes). That is the basis for the recommendation to keep the input water in warm water systems above 60˚C to prevent *Legionella pneumophila* growth.

System Assessment – Risk factors

Factors that can lead to proliferation of, or exposure to, *Legionella pneumophila* in drinking-water distribution networks and in building plumbing systems include (adapted from WHO, 2007):

- poor treated water quality and treatment failures, particularly the removal of nutrients for growth of microbes;
- distribution system problems, such as stagnation, dead zones and low flow rate;
- construction materials that contribute to microbial growth and biofilm formation (particularly hemp (i.e. in shower hoses) and natural rubber (i.e. in O-rings));
- inefficient or ineffective disinfection (particularly when biofilms and amoeba are present to protect *Legionella pneumophila* from disinfectants);
- water temperature of 25–50 °C (even in small areas of the system);
- presence of biofilms (and amoeba);
- aerosol production (showerheads, nebulizers, toilet flushing etc.).

It is important to investigate whether the combination of factors present in the system is likely to lead to the proliferation of legionellae. These factors are strongly interrelated, with temperature being a key factor.

System assessment – control measures

The focus of attention to control *Legionella pneumophila* risks should be on the combination of risk factors, in line with the multiple barrier approach that forms the core of the WSP approach. Systems will need to be assessed individually, with particular attention to system sites and appendages where aerosol formation may occur.

- Sufficient removal of nutrients for microbial growth to produce water with no or very limited growth of microbes (biostability). Some source waters are naturally biostable. For source waters with high natural organic matter content, several (combinations of) treatment processes have been shown to produce biostable water;
- Design and operate distribution networks and plumbing systems to limit stagnation, remove dead zones;
- Use construction materials that do not promote microbial growth;
- Use disinfecting agents that are more effective for control of *Legionella pneumophila* in biofilms, such as monochloramine;
- Keep water temperature of cold water systems <25˚C and if possible <20˚C and of warm water systems >50 °C (even in small areas of the system). For circulating warm water systems, this implies that the temperature of the water leaving the heater should be 60˚C or higher. For non-circulating warm water systems, the piping to connect the heater to the tap should be as short as possible;
- Reduce presence of biofilms (and amoeba). The basic control measure is to remove nutrients (see above); regular cleaning and disinfection may also help to reduce biofilm presence.

Monitoring - operational

Operational monitoring is used to determine whether the control measures in the system are actually functioning to control the risk factors. Some of the control measures have to be embedded in the design and operational monitoring is a site inspection to verify that systems are constructed with the appropriate materials and designed to reduce stagnation. Other control measures are more prone to variation and need to be monitored regularly (ideally continuously) to determine that they are functioning properly:

- water temperature, at sentinel points and ideally continuously in warm water systems;
- disinfectant residual;
- turbidity;
- treated water quality, turbidity and nutrient content (biodegradable organic matter).

Monitoring - verification

Verification monitoring is used to verify, independent from the operational monitoring, that the control measures effectively control the risk of *Legionella pneumophila.* This is generally done by testing the water at the tap or point of aerosolization for the presence of *Legionella pneumophila,* using microbiological methods.

4 Microbiologically safe drinking-water in Member States/non-EU countries

Several Member States and non-EU countries as have recognized the shortcomings of the end-point testing approach for faecal indicator bacteria and HPC. They have been a driver for updating their drinking-water regulation or national recommendations with respect to WSPs and/or management strategies for specific (groups of) pathogens.

Several examples (not a complete listing) are given in Table 2. Generally speaking these regulations and recommendations are not simply an extension of the list of water quality standards and monitoring requirements, but much more a risk assessment/risk management evaluation of all the relevant elements of the water supply system and if they are capable of producing and delivering safe drinking-water.

In line with the Guidelines, the focus of water quality monitoring has also moved to operational monitoring, to determine whether the water supply system from catchment to tap is continuously providing safe drinking water. End-point water quality monitoring is used as a final check to verify that the control measures to produce and deliver safe drinking-water are effective.

Table 2. Examples of drinking-water regulations and recommendations with additional control strategies for microbiological safety

Country	Regulation	Additional requirements	Link
USA	Surface Water Treatment Rule, 1989	Bacteria, Giardia, viruses, Legionella Treatment performance	https://www.epa.gov/dwreginfo/surface-water-treatment-rule-documents
USA	(Long term 2) Enhanced Surface Water Treatment Rule, 2006	Cryptosporidium Treatment performance	https://www.gpo.gov/fdsys/pkg/FR-2006-01-05/pdf/06-4.pdf
Canada	Guidelines for Canadian Drinking Water Quality, 2012	Enteric viruses, enteric protozoa (Cryptosporidium, Giardia) Treatment performance	http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/sum_guide-res_recom/index-eng.php#t1
Australia	Australian Drinking Water Guidelines, 2011	Preventive risk management approach	https://www.nhmrc.gov.au/_files_nhmrc/file/publications/nhmrc_adwg_6_february_2016.pdf
England & Wales	The water supply regulation, 2001	Cryptosporidium risk assessment (no longer in force)	http://www.dwi.gov.uk/stakeholders/legislation/ws_wqregs2001.pdf
Scotland	Cryptosporidium directions, 2003	Cryptosporidium risk assessment	http://www.gov.scot/Resource/Doc/26487/0013541.pdf
England & Wales	Water supply (water quality) regulations, 2016	Risk assessment, Drinking Water Safety Plans	http://www.legislation.gov.uk/uksi/2016/614/contents/made
Germany	DVGW recommendations W 1000, 1001, 1002	Water Safety Plans	http://www.dvgw.de/wasser/organisation-management/sicherheit-in-der-wasserversorgung/

Country	Regulation	Additional requirements	Link
Germany	Recommendations by the Environment Ministry, 2014	Risk assessment of enteric viruses and protozoa	Bundesgesundheitsblatt - Gesundheitsforschung – Gesundheitsschutz October 2014, Volume 57, Issue 10, pp 1224-1230
Netherlands	Drinking Water Decree, 2011	Risk assessment of enteric bacteria, viruses, protozoa, Legionella	http://wetten.overheid.nl/BWBR0030111/2015-11-28
France	Guidelines for public warm water systems, 2010	Legionella	https://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000021795143&categorieLien=id
Germany	Trinkwasserverordnung, 2001	Legionella, risk assessment	https://www.gesetze-im-internet.de/bundesrecht/trinkwv_2001/gesamt.pdf

5 How to implement the risk-based approach to microbiological safety in the Directive?

As concluded in chapter 2, the requirements in the current Directive for microbial water quality testing for faecal indicator bacteria and colony counts at the point of compliance provide insufficient safeguards to ensure safe drinking water.

The Guidelines' approach towards assessing and managing microbial hazards in drinking-water addresses the gaps in the Directive. It ensures that drinking-water will also be safe with regards to enteric viruses and protozoa and towards opportunistic pathogens that may grow in distribution systems and plumbing systems in buildings.

The key elements in the Guidelines' approach (chapter 3) are system assessment and monitoring (both operational and for verification). Both system assessment and monitoring are already in the Directive; the recent revision of Annex II has more explicitly introduced system assessment in the Directive. The terminology used in the Directive for system assessment is "risk assessment", and this terminology will also be used in the rest of this paper.

Risk assessment

The objective of the risk assessment is to describe the water supply system from catchment to consumer and to identify hazards and risks to the safety of the drinking-water delivered through this system and to evaluate whether the control measures (from engineered barriers to hygiene protocols) are able to control these risks to such an extent that drinking-water can be regarded as safe. This element is so essential to the approach of microbiological safety, and the gaps towards microbiological safety in the Directive are so significant, that the risk assessment requires a central place in the requirements for microbiological safety in the Directive.

Monitoring

The Directive also identifies the objective of monitoring: "*to verify that the measures in place to control risks to human health throughout the water supply chain from the catchment area through abstraction, treatment and storage to distribution are working effectively and that water at the point of compliance is wholesome and clean*", a clear link to the risk-based approach in the Guidelines.

The overall approach for monitoring to verify microbiological risks are controlled and water at the point of compliance is wholesome and clean is the combination of operational monitoring and verification monitoring. This is particularly relevant for microbiological safety. Safeguarding drinking-water against the risks of micro-organisms, where a contamination event causes health risks acutely, requires continuous vigilance. Frequent to continuous monitoring of the efficacy of barriers against pathogen risks is key. The nature of the current methods for microbiological monitoring (lab-based, long time-to-result) makes them unsuitable for operational monitoring. Other parameters are much more suitable for operational monitoring and can be used to frequently or continuously monitor to verify that the control measures are working effectively. Examples are:

- the monitoring of disinfectant dose and residual in combination with contact time (flow) to monitor the efficacy of chemical disinfection;
- the monitoring of UV transmission, UV fluorescence and contact time (flow) to monitor the efficacy of UV disinfection (in combination with validation of UV reactor design and operation according to Ö-Norm 5873 or DVGW W294, for example);
- the continuous monitoring of turbidity in the effluent of (individual) filters to monitor removal efficiency of (coagulation and filtration processes.

The current Directive does indicate that "*… Member States shall take all measures necessary to ensure that, where disinfection forms part of the preparation or distribution of water intended for human consumption, the efficiency of the disinfection treatment applied is verified, …*" but does not incorporate this explicitly in the water quality parameters/values. Several Member States have embedded these operational monitoring requirements in technical specifications of good practice.

The following paragraphs describe the suggested approach for risk assessment and monitoring, for enteric pathogens and for *Legionella pneumophila.*

5.1 Enteric pathogens

Risk assessment

Effective management of drinking-water safety requires a comprehensive understanding of the system, of the range and magnitude of hazards and risk factors or hazardous events that may affect the safety of the drinking-water delivered through the system and the ability of the processes and procedures and infrastructure to manage these risks adequately. This is the purpose of the risk assessment. As described in chapter 3, risk assessment requires description, understanding and evaluation of:

Risk factors

- Contamination of source water with excreta from man, livestock, wildlife;
- Contamination of source water with (treated) domestic sewage, run off of manure, leaching of septic tanks and manure storage;
- Events leading to peak contamination of source waters, such as heavy rains, snowmelt and flooding;
- Insufficient protection of groundwater sources;
- Insufficient integrity of groundwater wells;
- Insufficient treatment, treatment failure or periods of suboptimal or poor treatment performance, allowing breakthrough of pathogens;
- Accumulation of pathogens in the treatment chain (such as via filter backwash water);
- Ingress of pathogens via open storage or openings, leaks etc. in the treatment plant;
- Ingress of pathogens in storage reservoirs or the piped network (leaks, low/no pressure events, repairs, cross-connections etc.).

Control measures

- Catchment protection measures (protected groundwater zones, safe setback zones, riparian buffer zones, sewer overflow diversion etc.);
- Source protection measures (intake stops/relocation, flow diversion, etc.);

- Treatment processes;
- Pressure and integrity of distribution network;
- Hygiene protocols for repair and maintenance works in treatment plants and distribution networks.

Together, evaluation of risk factors and control measures should result in the judgement of the ability of the water system to manage the risks adequately and deliver safe drinking-water.

This risk assessment is central to the safe water framework and water safety plans. Hence, implementing the risk-based approach in the Directive implies that a mandatory requirement for such a risk assessment is set.

Groundwater supplies

For water supply systems using groundwater as a source, the risk assessment could be implemented as a tiered assessment of the level of protection of the aquifer and abstraction infrastructure and processes.

1. *Evaluation of the level of protection of the aquifer*

Well-confined aquifers under intact, protective (clay) layers without influence from surface activities, mining or other underground activities are well protected and free of enteric pathogens.

(Semi) freatic karstic aquifers without sufficient protective, integer loam/clay layer or freatic sandy aquifers without such a protective layer or thick unsaturated zone are vulnerable to contamination with pathogens from leaking sewers, manure deposits etc.

Groundwater aquifers under the influence of surface water, including bank filtration and managed aquifer recharge are vulnerable to contamination with pathogens from surface water.

2. *Evaluation of the level of protection of the abstraction system*

Intact wells or other abstraction systems with a protective clay, bentonite or other seal in the borehole will not allow run-off from the field (potentially containing excreta) to enter into the aquifer or abstracted groundwater through the abstraction system.

3. *Combination of 1 and 2*

If the result of the evaluation in 1 and 2 is that the aquifer is well-protected and abstraction means are secure, the water supply may be considered intrinsically safe. Regular re-evaluation of the protection level is sufficient. If the evaluation indicates vulnerability of the aquifer or abstraction means, go to step 4.

4. *Evaluation of pathogen sources in the 100d residence time zone surrounding the well or spring*

Evaluation of pathogen sources in the vicinity of the groundwater abstraction zone that could reach the aquifer or abstraction means, with special attention to potential peak events (during heavy rains, snowmelt, etc.).

If the result is that there are no pathogen sources in the vicinity that could contaminate the groundwater in the aquifer or during abstraction, the system may be considered vulnerable, but safe. Regular inspection of the absence of pathogen sources in the vicinity of the system is sufficient.

If there are pathogen sources in the vicinity, the system is vulnerable and prone to contamination and the abstracted groundwater should be monitored frequently for the presence of faecal contamination by testing for *E. coli* weekly. Since viruses are less effectively removed by soil passage than bacteria, also coliphages need to be monitored in the abstracted groundwater to determine the risk of virus contamination. These systems may require further treatment to remove/inactivate pathogens.

Surface water supplies and groundwater under the influence of surface water supplies

Surface water is generally contaminated with enteric pathogens, the level depending on the presence of sewage discharges, combined sewer overflows, septic tank seepage, input from shipping, manure discharges and run-off from agricultural lands and abundance of wildlife, particularly waterfowl.

The risk assessment is an evaluation of the presence, vicinity and abundance of the different contamination sources, as well as the vulnerability of the source water at the intake to peak contamination events, following heavy rainfall, snowmelt, flooding or other events. This is essentially a sanitary survey. The risk assessment should result in an understanding and characterization of the pathogen sources in the catchment and the pathways by which they can reach the intake (again particularly conditions that could lead to peak levels).

Treatment performance targets (see section 3.2) are set based on the concentration of reference pathogens in the source water. There are two approaches towards establishing the concentration of reference pathogens in source waters:

1. *A reference pathogen monitoring program*. This was part of the drinking-water legislation in the US (Information Collection Rule, Enhanced Surface Water Treatment Rule). It is currently part of the Dutch drinking-water legislation. Also the UK requires *Cryptosporidium* monitoring in source water of at-risk water supplies. The advantage of this approach is the site specific assessment of pathogen concentrations; the disadvantage is the high cost and limited availability of specialized pathogen testing laboratories throughout the EU.
2. *An estimation of reference pathogen concentrations*. The estimation can be based on a sanitary survey, potentially complemented with regular monitoring of *E. coli* and coliphages in source water. This does not inflict high costs and does not require specialized laboratories. The disadvantage is the uncertainty about the precision of the estimated pathogen concentration. The approach has been used in France for the (*Cryptosporidium*) risk assessment of 1700 water supplies and validated by subsequent *Cryptosporidium* monitoring, and allowed setting priorities for risk management (WHO, 2016).

The second approach is recommended, since this is generally applicable, is feasible throughout the EU for both large and small supplies and requires the least resources.

The concentration of reference pathogens in source water already contains control measures in the catchment area. This concentration is used to determine the performance target for the treatment (see section 3.2).

Once the treatment performance target for the three reference pathogens (Annex 2) is set, the ability of the train of treatment processes that is used to produce drinking-water to meet this performance target for each of the reference pathogens needs to be established. Also here, there are several approaches to establish the efficacy of the treatment processes.

1. *Pathogen monitoring before and after treatment processes*. For the same reasons as for pathogen monitoring in source water, pathogen monitoring is not recommended as the preferred approach to establish treatment performance. It can be used in pilot systems to understand the pathogen removal efficacy and the influence of process conditions thereon.
2. *Monitoring of surrogate parameters*. These could include *Escherichia coli* for removal/inactivation of enteric bacteria, coliphages for removal/inactivation of enteric viruses and spores of *Clostridium perfringens* for removal of enteric protozoan pathogens.
3. *Use generic information about the treatment process*. These could include type and operational monitoring data such as filter effluent turbidity or disinfectant residual concentration x contact time, as well as an evaluation of the level of compliance of the treatment process with state-of-the-art guidance on water treatment, and translate this into the $\log_{10}$-removal that can be assigned to the treatment process.

For the Directive, it is recommended to use approach 3 to determine treatment performance and to evaluate if the treatment is capable of meeting the treatment performance target. This approach is generally applicable, feasible throughout the EU for large and small water supplies.

Comparing required and achieved $\log_{10}$-removal gives indication whether the drinking-water supply system in its entirety – through resource protection measures and treatment – is capable to ensure microbial safety towards viruses, bacteria and protozoa.

A drawback of the recommended approaches is that it is uncertain whether the assignments of pathogen concentration in source water and of treatment performance accurately reflect the local situation. The uncertainties call for erring on the safe side, both in source water concentration and in treatment efficacy. On the other hand, if this is too conservative, this may lead to higher treatment performance demands than necessary, which may lead to costs that are higher than needed for adequate protection of microbial safety.

Member States could choose to validate their assessment with targeted monitoring of reference pathogens in source waters and/or with targeted monitoring and assessments of $\log_{10}$-removal of surrogate organisms such as *E. coli* as model for removal of bacterial pathogens, coliphages as model for removal of human pathogenic viruses and *Clostridium perfringens* spores as model for the removal of protozoan parasites.

Monitoring – operational

Operational monitoring is used to determine whether the control measures in the system are actually functioning to control the risk factors. Since delivery of safe drinking-water is a multiple barrier approach, operational monitoring of control measures in the catchment protection, source water, treatment and distribution need to be established.

Catchment protection

- For safe groundwater supplies: sanitary inspection of the integrity of the system and absence of contamination sources.
- For surface water supplies: river level, flow, turbidity to determine peak contamination events.

Treatment

Disinfectant residuals. Disinfection processes will be designed to achieve specified concentration x time (CT in mg-min/l) values for indicator organisms to achieve designated logs of reduction of pathogens. On-line and grab samples for disinfectant residuals provide almost instantaneous performance information regarding the in situ disinfectant concentration. The monitoring output allows for rapid adjustment of the disinfection dose, when needed. Free and combined chlorine residuals can indicate control of bacteria and viruses, but not some protozoa especially *Cryptosporidium*. Chlorine dioxide and ozone also provide better protozoan control barriers and they have quantified CT values that can be applied; however, turbidity measurements and goals (see below) are an essential part of demonstrating protozoan control.

Physical tests. Turbidity measurements can be made on line and rapidly by grab sampling. A turbidity goal of less than 0.3 NTU is a good performance indicator for filtration and removal of protozoans. Performance surrogates for high-pressure membranes (such as reverse osmosis or nanofiltration) include electrical conductivity (representing total dissolved solids rejection), which can be on line. Integrity of high and low-pressure membranes can be assessed by online turbidity measurements and periodic (daily) pressure decay tests (PDTs). UV performance can be monitored by fluence sensors, supplemented with flow and UV transmission monitoring.

Distribution

Inspection of integrity of network (with special attention to reservoirs), pressure monitoring, monitoring of residual disinfectant (where applied), inspection of strict adherence to hygiene protocols during construction, repair and maintenance works.

Monitoring – verification

Verification monitoring is used to verify, independent from the operational monitoring, that the control measures effectively control the risk of enteric pathogens. As indicated in section 3.2, *E. coli* and enterococci monitoring in treated water serves as independent verification that the treatment performance target is met for enteric bacteria. It is recommended to complement this with verification monitoring of coliphages to verify treatment performance against enteric viruses and spores of *Clostridium perfringens* to verify treatment performance against enteric protozoan pathogens. This is relevant for surface water supplies and for groundwater supplies that are vulnerable and prone to contamination.

5.2 Legionella pneumophila

Risk assessment

Risk assessment should focus on warm water systems in public buildings, and systems where aerosolization is possible. Different Member States have a different selection of public buildings that are specified under the Legionella legislation. It is recommended to evaluate the cost-benefit ratio of different selection criteria to make a selection for the EU Directive.

Factors that can lead to proliferation of, or exposure to, *Legionella pneumophila* in drinking-water distribution networks and in building plumbing systems include (adapted from WHO, 2007):

- poor treated water quality and treatment failures, particularly the removal of nutrients for growth of microbes

- distribution system problems, such as stagnation, dead zones and low flow rate
- construction materials that contribute to microbial growth and biofilm formation (particularly hemp (i.e. in shower hoses) and natural rubber (i.e. in O-rings))
- inefficient or ineffective disinfection (particularly when biofilms and amoeba are present to protect Legionella pneumophila from disinfectants)
- water temperature of 25–50 °C (even in small areas of the system)
- presence of biofilms (and amoeba)
- aerosol production (showerheads, nebulizers, toilet flushing etc.)

It is important to investigate whether the combination of factors present in the system is likely to lead to the proliferation of legionellae. These factors are strongly interrelated, with temperature being a key factor.

System assessment – control measures

The focus of attention control of *Legionella pneumophila* risks should be on the combination of risk factors, in line with the multiple barrier approach that forms the core of the WSP approach. Systems will need to be assessed individually, with particular attention to system sites and appendages where aerosol formation may occur.

- Sufficient removal of nutrients for microbial growth to produce water with no or very limited growth of microbes (biostability). Some source waters are naturally biostable. For source waters with high natural organic matter content, several (combinations of) treatment processes have been shown to produce biostable water
- Design and operate distribution networks and plumbing systems to limit stagnation, remove dead zones
- Use construction materials that do not promote microbial growth
- Use disinfecting agents that are more effective for control of Legionella pneumophila in biofilms, such as monochloramine
- Keep water temperature of cold water systems <25˚C and if possible <20˚C and of warm water systems >50 °C (even in small areas of the system). For circulating warm water systems, this implies that the temperature of the water leaving the heater should be 60˚C or higher. For non-circulating warm water systems, the piping to connect the heater to the tap should be as short as possible.
- Reduce presence of biofilms (and amoeba). The basic control measure is to remove nutrients (see above); regular cleaning and disinfection may also help to reduce biofilm presence

Monitoring – operational

Operational monitoring is used to determine whether the control measures in the system are actually functioning to control the risk factors. Some of the control measures have to be embedded in the design and operational monitoring is a site inspection to verify that systems are constructed with the appropriate materials and designed to reduce stagnation. Other control measures are more prone to variation and need to be monitored regularly (ideally continuously) to determine that they are functioning properly:

- water temperature, at sentinel points and ideally continuously in warm water systems
- disinfectant residual

- turbidity
- treated water quality, turbidity and nutrient content (biodegradable organic matter)

Monitoring – verification

Verification monitoring is used to verify, independent from the operational monitoring, that the control measures effectively control the risk of *Legionella pneumophila.* This would imply inclusion of *Legionella pneumophila* as parameter in Annex I of the Directive and setting a monitoring requirement. Monitoring should be aimed at warm water systems and the point of compliance, specifically at points of aerosolization, such as showerheads, for the presence of *Legionella pneumophila,* using standardized methods (ISO 11731, ISO 12869).

The German and Dutch legislation on *Legionella* in drinking-water and French guidelines require monitoring at public buildings (hospitals and other healthcare institutions, nursing homes, prisons, hotels etc.) with a frequency of once (Germany) or twice (Netherlands) per year. The water quality target is set to 100 / L (Netherlands) - 1000 / L (Germany, France). It is recommended to focus monitoring and target setting on *Legionella pneumophila* since this is the causative agent of legionellosis, and not on *Legionella* sp., since this genus contains many species that do not cause illness.

4 Implementing the risk-based approach for microbiological safety in the Directive – the place of current microbiological parameters in Annex I and II of the Directive

In this chapter, the microbiological parameters that are embedded in the Directive currently are placed within the safe water framework.

Currently, micro-organisms covered in the Directive are divided into two groups: those for which Member States must introduce into legislation and parametric values which are a maximum acceptable concentration (Directive Annex I part A) and those designated as indicator parameters for which there is greater flexibility in the introduction and in the parametric values and the way in which they are interpreted (Directive Annex I part C).

Table 3 describes the microbiological parameters in Annex I, part A and part C, the quality requirement in Annex I and the monitoring requirements in Annex II (version prior to October 6, 2015). The principle monitoring site is the point of compliance (Article 6).

Table 3. Microbiological parameters in DWD Annex I and Annex II (before October 6, 2015)

Parameter	Monitoring requirement	Quality requirement	Monitoring site
MICROBIOLOGICAL PARAMETERS (ANNEX I PART A)			
E. coli[1]	Yes, check frequency	Yes (0/100ml)	Point of compliance (consumer)
Enterococci	Yes, audit frequency	Yes (0/100ml)	Point of compliance (consumer)
INDICATOR PARAMETERS (ANNEX I PART C)			
Clostridium perfringens, including spores[1]	Yes, check frequency	Yes (0/100ml)	Point of compliance (consumer)
Coliform bacteria	Yes, check frequency	Yes (0/100ml)	Point of compliance (consumer)
Colony count 22˚C	Yes, check frequency	Yes, relative (no abnormal change)	Point of compliance (consumer)

[1] Only if surface water origin

With the revision of Annex II on 6 October 2015, changes were made to the requirements for monitoring. Annex II stipulates that the general objectives for monitoring programmes are to:

- Verify that the measures in place to control risks to human health throughout the water supply chain from the catchment area through abstraction, treatment and storage to distribution are working effectively and that water at the point of compliance is wholesome and clean;
- Provide information on the quality of the water supplied for human consumption to demonstrate that the obligations set out in Articles 4 and 5, and the parametric values laid down in Annex I, are being met;
- Identify the most appropriate means of mitigating the risk to human health.

Reviewing the microbiological parameters and the monitoring program for these parameters in the light of these new general objectives provides an important rationale for these parameters. Microbiological parameters can serve to:

- Verify that catchment protection measures are adequate. This requires monitoring of the source water with a monitoring program that is capable of detecting peak events (heavy rain, snowmelt, flooding, drought, heavy animal loads etc.) that may occur in the catchment and impact the presence and concentration of enteric pathogens in source water.
- Verify that the treatment processes are effectively removing pathogens. This requires frequent monitoring of treated water.
- Verify that the control measures of the distribution of drinking-water and in plumbing are adequately protecting against ingress of pathogens.
- Verify that the control measures to prevent/limit growth of micro-organisms in distribution networks and plumbing systems in buildings.
- Verify that the control measures to prevent/limit growth of *Legionella pneumophila* in distribution networks and plumbing systems in buildings.

Identification of the purpose of monitoring makes the role of the parameter in verifying effective control of microbiological hazards much clearer, not only to regulators from Member States but also politicians and the public. Such identification could also be beneficial for aiding in the assessment of hazards in small supplies for which there may only be limited resources and expertise available.

Table 4 identifies the role of the microbiological parameters that are currently in the Directive to verify that the measures in place to control microbiological risks to human health throughout the water supply chain from the catchment area through abstraction, treatment and storage to distribution are working effectively and that water at the point of compliance is wholesome and clean.

Table 4. Suggested implementation of the revised Annex II for the microbiological parameters in DWD Annex I with reference to their value in the risk-based approach

Parameter	Role in risk-based approach	Priority for inclusion	Monitoring requirement	Quality requirement	Monitoring site
MICROBIOLOGICAL PARAMETERS (ANNEX I PART A)					
E. coli[1]	Risk assessment: typing of source water contamination level, peak events	High	Yes	No	Source
E. coli[1]	Verification of treatment control for enteric bacterial pathogens[4]	High	Yes	Yes (0/100ml)	Post-treatment
E. coli[1]	Verification of distribution control against ingress of excreta	High	Yes	Yes (0/100ml)	Consumer

Parameter	Role in risk-based approach	Priority for inclusion	Monitoring requirement	Quality requirement	Monitoring site
Enterococci[1]	Risk assessment: typing of source water contamination level, peak events	Medium[2]	Yes	No	Source
Enterococci[1]	Verification of treatment control for enteric bacterial pathogens[4]	Medium[2]	Yes	Yes (0/100ml)	Post-treatment
Enterococci[1]	Verification of distribution control against ingress of excreta	Medium[2]	Yes	Yes (0/100ml)	Consumer
INDICATOR PARAMETERS (ANNEX I PART C)					
Clostridium perfringens spores[3]	Verification of treatment control for disinfection-resistant pathogens such as Cryptosporidium[4]	High	Yes	Yes	Post-treatment
Coliform bacteria	Verification of treatment control[4]	Low[5]	No	No	
Coliform bacteria	Verification of distribution control	Low[5]	No	No	
Colony count 22˚C	Verification of distribution/plumbing control against growth of micro-organisms, including opportunistic pathogens	High	Yes	Yes, relative (no abnormal change)	Consumer

[1] This is the (implicit) place of *E. coli* monitoring in the current Directive.

[2] Parameter is complementary to *E. coli.*

[3] *Clostridium perfringens* "including spores" should be changed to *Clostridium perfringens* spores

[4] Only if surface water (impacted) source

[5] Remove from Annex I part C. The added value compared to *E. coli* is limited and since coliforms may also multiply in water systems under certain conditions, the test results cannot be interpreted unequivocally.

The implications of the allocation of roles of the microbiological parameters in the verification of adequate control from catchment to consumer are:

- Some parameters can serve multiple roles. Testing of *E. coli* serves to verify controls in the catchment, treatment and distribution implies that the monitoring sites need to be in the source, after the treatment and at the consumers' tap and that the monitoring frequency needs to be geared towards the level required for adequate verification, given the potential risks. Also, the water quality requirement is valid for the water after treatment and in the distribution network up to the point of compliance, but not for the catchment or source water. There the monitoring serves to determine the required control by treatment.

- Some parameters serve a specific role. *Clostridium perfringens* spores serve to determine adequate control of pathogens that are resistant to disinfection in water supply systems that are using (or under the influence of) surface water as source. Monitoring sites are therefore the source and treated water, to establish the removal efficacy of the treatment processes. For this, only the spores of *Clostridium perfringens* have an indicator value, vegetative *Clostridium perfringens* have not. Therefore, the parameter should be *Clostridium perfringens* spores, and not *Clostridium perfringens*, including spores. Colony counts can serve to determine adequate control of growth of micro-organisms in the distribution network and plumbing systems. Their principle site for monitoring is at the point of compliance (at the consumer).
- Coliforms have little added value over *E. coli*. The ability of coliforms to grow in source waters, in water treatment and in the distribution network under some conditions make this parameter less suitable for verification monitoring (see Appendix 3 for more detailed evaluation of the limited value of this parameter). The proposal is to exclude this parameter from the Directive.
- Enterococci have little added value over *E. coli*, the information the enterococci provide is similar to *E. coli*.

In addition to the microbiological parameters, another parameter in the Directive has an important role in the risk-based approach: turbidity is a well-established parameter to monitor the performance of filtration processes. It can be monitored on-line in the effluent of (individual) filters and indicate whether the filtration process performs as expected. The more intense the operational monitoring (grab samples combined filter effluent, target <0.3 NTU versus on line sensors in individual filter effluents, target <0.1 NTU) the higher the level of removal that can be assigned to the filtration process. It is therefore recommended to include post-filtration turbidity monitoring as added role of the turbidity monitoring that is required under the Directive (Table 5).

Table 5. Suggested implementation of the revised Annex II for the non-microbiological parameters in DWD Annex I with reference to their value in the risk-based approach

Parameter	Role in risk-based approach	Priority for inclusion	Monitoring requirement	Quality requirement	Monitoring site
INDICATOR PARAMETERS (ANNEX I PART C)					
Turbidity	Operational monitoring of efficacy of physical removal by filtration processes[1]	High	Yes	Yes	Post-filtration

[1] Where filtration is barrier against enteric pathogens.

The added role of the turbidity parameter is part of the operational monitoring that is key to the risk-based approach. It is recommended to strengthen the operational monitoring requirements in the Directive and translate the general requirement in Article 7.1 "*… Member States shall take all measures necessary to ensure that, where disinfection forms part of the preparation or distribution of water intended for human consumption, the efficiency of the disinfection treatment applied is verified, …*" to more explicit requirements for operational monitoring:

- *Chemical disinfection*: monitor disinfectant dose, disinfectant residual, temperature, pH and flow;
- *UV irradiation*: monitor (validated) UV fluence in reactor, UV transmission of water and flow;
- *Membrane filtration processes*: monitor particle removal;
- *Warm water systems*: temperature, residual disinfectant and turbidity;

Several Member States have documents of best practice that contain such requirements.

5. Implementing the risk-based approach for microbiological safety in the Directive – suggested parameters for inclusion

In this chapter, new parameters are suggested for inclusion in the Directive to complement the existing parameters to implement the safe water framework in the Directive. The safe water framework also involves risk assessment, suggestions for implementing this element are also included.

Complete implementation of the risk-based approach requires the inclusion of several new microbiological parameters in the Directive (Table 6).

1. **Selected reference pathogens**
   - *Campylobacter*, enterovirus and *Cryptosporidium* as reference enteric pathogens (Appendix 2).
   - *Legionella pneumophila* as reference pathogen for opportunistic pathogens that can grow in distribution networks and plumbing systems.

It is recommended to include these reference pathogens as parameters in the list of health-related microbiological parameters (Annex IA), with reference to their role in the risk assessment, to evaluate the ability of the system to deliver safe drinking water. It is not recommended to include a monitoring requirement for these reference pathogens (see Chapter 5) or a direct water quality requirement. Instead, the risk assessment should demonstrate the ability of the system to manage the risks of each individual reference pathogen.

2. **New parameters for verification monitoring**
   - *Legionella pneumophila* to verify the level of control of the risk of *Legionella pneumophila.*
   - *Coliphages* to verify the level of control of the risks of enteric viruses.

For these parameters, both a monitoring requirement and water quality target should be set.

Table 6. Suggested new microbiological parameters in DWD Annex I with reference to their value in the risk-based approach

Parameter	Role in risk-based approach	Priority for inclusion	Monitoring requirement	Quality requirement
MICROBIOLOGICAL PARAMETERS (ANNEX I PART A)				
Campylobacter	Risk assessment: reference pathogen for enteric bacterial pathogens in EU. Raw water (surface water) characterization, basis for treatment performance target[1]	High	No (optional)	No. Indirect, via treatment performance target

Parameter	Role in risk-based approach	Priority for inclusion	Monitoring requirement	Quality requirement
Enterovirus	Risk assessment: reference pathogen for enteric virus pathogens in EU. Raw water (surface water) characterization, basis for treatment performance target[1]	High	No (optional)	No. Indirect, via treatment performance target
Cryptosporidium	Risk assessment: reference pathogen for enteric protozoan pathogens in EU. Raw water (surface water) characterization, basis for treatment performance target[1]	High	No (optional)	No. Indirect, via treatment performance target
Legionella pneumophila	Risk assessment: reference pathogen for pathogens that are able to grow in water distribution networks or plumbing systems in EU.[2]	High	No	No
Legionella pneumophila	Verification of distribution/plumbing control.[2]	High	Yes[2]	Yes
INDICATOR PARAMETERS (ANNEX I PART C)				
Coliphages	Risk assessment: typing of source water contamination level, peak events[3]	High	Yes[3]	No
Coliphages	Verification of treatment control for enteric viruses.[1]	High	Yes[1]	Yes

[1] If surface water (impacted) source, owner needs to conduct a risk assessment for this pathogen to demonstrate that the water supply system is capable of producing and delivering safe drinking water.

[2] For warm water systems in public buildings.

[3] For groundwater sources.

6. Consideration of other microbial hazards

This chapter evaluates whether microbiological hazards that are not explicitly embedded in the current or suggested parameters in the Directive are sufficiently controlled by the level of water safety control that is instigated by the suggested amendments of the Directive (see previous chapters).

The Guidelines (and the current preparation of the first addendum) contain an overview of micro-organisms for which transmission through drinking-water has been proven. The information in these tables has been adapted in the tables below to reflect the EU context (Table 7). Several of the pathogens (particularly helminths) are less prevalent in the EU and/or pathogens are primarily transmitted in endemic areas via consumption of untreated or poorly treated surface water that is contaminated with these pathogens. For others, waterborne outbreaks have been reported in other parts of the world, but not in the EU. For each pathogen (group), the basis for adequate control in the Directive is given. In many cases, the rationale is that the risk of drinking-waterborne transmission of this pathogen is adequately controlled by the control measures needed to control the respective reference pathogen.

Table 7. Pathogens transmitted through drinking-water[a], not to be explicitely included in the DWD

Pathogen	Health significance[b]	Persistence in water supplies[c]	Resistance to chlorine[d]	Relative infectivity[e]	Important animal source	Basis for control in the Directive
Bacteria						
Escherichia coli – diarrhoeagenic[f]	High	Moderate	Low	Low	Yes	Controlled via Campylobacter control
Escherichia coli – enterohaemorrhagic	High	Moderate	Low	High	Yes	Controlled via Campylobacter control
Mycobacteria (non-tuberculous)	Low	May multiply	High	Low	No	Partially controlled via Legionella control
Salmonella typhi	Low	Moderate	Low	Low	No	If present, controlled via Campylobacter control
Other salmonellae	High	May multiply	Low	Low	Yes	Controlled via Campylobacter control
Shigella	Medium	Short	Low	High	No	Controlled via Campylobacter control
Vibrio cholera O1 O139	Low	Short to long[g]	Low	Low	No	If present, controlled via Campylobacter control
Viruses						
Adenoviruses	Moderate	Long	Moderate	High	No	Medium, more resistant to UV than Enteroviruses
Astroviruses	Moderate	Long	Moderate	High	No	Low, controlled via Enterovirus control
Noroviruses, Sapoviruses	High	Long	Moderate	High	Potentially	Medium, due to peak contaminations in source water, controlled via Enterovirus control
Hepatitis E virus	High	Long	Moderate	High	Potentially	Low, controlled via Enterovirus control
Hepatitis A virus, Rotaviruses	Moderate	Long	Moderate	High	No	Low, controlled via Enterovirus control
Reoviruses	Moderate	Long	Moderate	High	Potentially	Medium, controlled via Enterovirus control

Pathogen	Health significance[b]	Persistence in water supplies[c]	Resistance to chlorine[d]	Relative infectivity[e]	Important animal source	Basis for control in the Directive
Protozoa						
Acanthamoeba	Medium (eye infection in contact lens wearers)	May multiply	Low	High	No	Low, partial control via Legionella control
Cryptosporidium	High	Long	High	High	Yes	High, reference pathogen
Cyclospora cayetanensis	Low	Long	High	High	No	Low, controlled via Cryptosporidium control
Entamoeba histolytica	Low	Moderate	High	High	No	Low, controlled via Cryptosporidium control
Giardia intestinalis	High	Moderate	High	High	Yes	Medium, controlled via Cryptosporidium control
Naegleria fowleri	Low	May multiply[h]	Low	Moderate	No	Low, partial control via Legionella control
Helminths						
Dracunculus medinensis	Low	Moderate	Moderate	High	No	Low, controlled via Campylobacter, Enterovirus, Cryptosporidium control

[a] This table contains pathogens for which there is some evidence of health significance related to their occurrence in drinking-water supplies in the EU region.

[b] Health significance relates to the incidence and severity of disease, including association with outbreaks in the EU region.

[c] Detection period for infective stage in water at 20 °C: short, up to 1 week; moderate, 1 week to 1 month; long, over 1 month.

[d] When the infective stage is freely suspended in water treated at conventional doses and contact times and pH between 7 and 8. Low means 99% inactivation at 20 °C generally in < 1 min, moderate 1–30 min and high > 30 min. It should be noted that organisms that survive and grow in biofilms, such as *Legionella* and mycobacteria, will be protected from chlorination.

[e] From experiments with human volunteers, from epidemiological evidence and from experimental animal studies. High means infective doses can be 1–10^2 organisms or particles, moderate 10^2–10^4 and low > 10^4.

[f] Includes enteropathogenic, enterotoxigenic, enteroinvasive, diffusely adherent and enteroaggregative.

[g] *Vibrio cholerae* may persist for long periods in association with copepods and other aquatic organisms.

[h] In warm water.

Antibiotic resistance

The discharge of antibiotic resistant bacteria (ARB) and the ability of resistance genes (ARG) to transfer to other bacteria in the environment is increasingly recognized as important public health issue. In general, the barriers against enteric bacteria (with *Campylobacter* as reference pathogen) will result is very high removal and inactivation rates of bacteria in general, including ARB. The control of enteric bacteria therefore also means that drinking-water is controlled against ARB.

The risk of transfer of antibiotic resistance genes in water treatment or distribution networks might be possible, but whether this occurs in practice and under what conditions this will or will not occur is insufficiently well researched and understood to currently recommend for specific control measures for ARG.

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Appendix 1

Health-based target setting

Setting performance target for water treatment is based on the safe level of pathogens in treated water. The Guidelines define “safe” drinking-water. The general requirement “not represent a significant risk” is translated into a health target: a DALY of $<10^{-6}$ per person per year. DALY (Disability Adjusted Life Years) is a metric for the burden of disease in a population (i.e. based on the years of life lost by disease and the years of life lived with a disability, weighed for the severity of the disability). DALYs are used extensively in setting priorities in public health policy at WHO or national level. The safe level of 10^{-6} DALY is approximately equivalent to the tolerable lifetime cancer (mortality) risk of 10^{-5} that is used in the Guidelines as the tolerable risk level to determine guideline values for the maximum concentration of genotoxic carcinogens in drinking-water. A more detailed explanation of DALY’s and health-based target setting can be found in the Guidelines (chapter 3) and supporting documents.

DALYs are not a practical metric in water supply, so the Guidelines translate the DALY-target to performance targets for the water supply system. The 10^{-6} DALY per person per year corresponds to a certain (very low) concentration of pathogens in drinking-water. The water supply system should be able to produce water with these very low pathogen concentrations. The difference between these very low concentrations in drinking-water and the concentration of pathogens in source water sets the performance target for the water treatment.

Appendix 2

Reference pathogens

The Guidelines state that it is not practical, and there are insufficient data, to set performance targets for all potentially waterborne pathogens, including bacteria, viruses, protozoa and helminths. A more practical approach is to set performance targets for reference pathogens, which represent groups of pathogens, taking into account variations in characteristics, behaviours and susceptibilities of each group to different treatment processes. Typically, different reference pathogens will be identified to represent bacteria, viruses, protozoa and helmints. The Guidelines present criteria for the selection of appropriate reference pathogens, including the conclusiveness of the evidence of waterborne transmission, sensitivity to removal or inactivation by water treatment processes, infectivity, incidence and severity of disease. Some of the criteria, such as environmental persistence and sensitivity to treatment processes, relate to the specific characteristics of the reference pathogens (such as the high chlorine-resistance and zoonotic transmission of *Cryptosporidium*). Other criteria are subject to regional circumstances and conditions. These include waterborne disease burden, which can be influenced by health, immunity and nutrition status, the occurrence of a pathogen in the human and animal population and in source waters and general availability of information about these pathogens. These criteria may lead to different pathogens for different regions. Here, the criteria are used to select reference pathogens for the EU region.

Only enteric pathogens for which waterborne transmission is conclusively established are taken into account. It is very likely that pathogens from the same group for which current knowledge about waterborne transmission is inconclusive are sufficiently under control when the reference pathogens are used to target sufficient control measures in catchment protection and water treatment.

Enteric protozoa

Enteric protozoa for which waterborne transmission is conclusively established are *Cryptosporidium, Giardia intestinalis, Entamoeba histolytica and Cyclospora cayetanensis.* These protozoa are evaluated against the criteria (Table A2.1).

Table A2.1. Evaluation of waterborne parasitic protozoa for reference pathogen in the EU. More dots mean higher significance.

Pathogen	Sufficient information in EU	Occurrence in EU domestic wastewater, livestock	Persistence in water/soil	Removal by treatment	Infectivity, incidence, burden of disease in EU
Cryptosporidium	●●	●●	●●●	●●●	●●
Giardia intestinalis	●●	●●●	●●	●●	●●
Entamoeba histolytica		●●	●●	●●	●
Cyclospora cayetanensis		●	●●	●●	●

Sufficient information is available in the EU about *Cryptosporidium* and *Giardia intestinalis*. *Cryptosporidium* is more challenging to water treatment systems and based on this, *Cryptosporidium* is selected as reference pathogen for enteric protozoa. Other enteric protozoa for which waterborne transmission is established are *Cyclospora cayetanensis* and *Entamoeba histolytica,* but for these there is limited information about occurrence, fate and removal. The disease incidence in the EU is also higher for *Cryptosporidium* and *Giardia.*

Helminths

No reference pathogen from the group of helminths is selected, since it is very likely that adequate control of the reference pathogens for the other pathogen groups will also protect against health risks from helminths that are more readily removed by water treatment and soil passage than the other groups of pathogens.

Viruses

There is a range of enteric viruses for which waterborne transmission is established (Table A2.2). Presence of sufficient information in the EU context is focused on information on information on occurrence of *infectious* viruses in water (using cell culture methods). Many recent studies on virus occurrence use molecular detection methods, that detect both infectious and non-infectious virus particles. Only a small fraction of virus particles in water is infectious.

Table A2.2. Evaluation of waterborne enteric viruses for reference pathogen in the EU. More dots mean higher significance.

Pathogen	Sufficient information	Occurrence in EU domestic wastewater	Persistence in water/soil	Removal by treatment	Infectivity, incidence, burden of disease
Enteroviruses	●●	●●	●●	●●	●●●
Adenoviruses	●	●●●[3]	●●	●●(●)[4]	●●
Noroviruses		●●●[1]	●●	●●	●●●
Astroviruses		●[1]	●●	●●	●●●
Hepatitis A virus		●[1]	●●	●●	●●●
Hepatitis E virus		●[1]	●●	●●	●●●
Rotaviruses	●	●●[1]	●●	●●	●●
Sapoviruses		?	●●	●●	●●

Based on this, culturable enteroviruses are selected as reference pathogens for the enteric viruses.

Bacteria

Table A2.3. Evaluation of waterborne enteric bacteria for reference pathogen in the EU. More dots mean higher significance.

Pathogen	Sufficient information	Occurrence in EU domestic wastewater	Persistence in water/soil	Removal by treatment	Infectivity, incidence, burden of disease
Campylobacter	●●	●●●	●	●	●●●
Enteropathogenic E. coli	●	●	●	●	●●
Enterohemorrhagic E. coli	●	●	●	●	●●●
Salmonella	●	●	●	●	●●●
Salmonella typhi			●	●	●
Shigella	●	●	●	●	●●
Vibrio cholerae			●●	●	●

Based on this, Campylobacter is selected as reference pathogen for bacteria.

[3] Mainly based on molecular methods
[4] Adenovirus is very resistant to UV

Appendix 3

Technical overview of microbiological parameters included in DWD Annex I Part A and C

The text of these fact sheets was largely based on the Microbial fact sheets in the WHO GDWQ (2016) and the WHO/OECD background document Assessing Microbial Safety of Drinking Water (2003), complemented with information from the scientific literature.

Clostridium perfringens spores

General description

Clostridium spp. are Gram-positive, anaerobic, sulfite-reducing bacilli. They produce spores that are exceptionally resistant to unfavourable conditions in water environments, including UV irradiation, temperature and pH extremes, and disinfection processes, such as chlorination. The characteristic species of the genus, C. perfringens, is a member of the normal intestinal flora of 13–35% of humans and other warm- blooded animals. Other species are not exclusively of faecal origin. Like E. coli, C. perfringens does not multiply in most water environments and is a highly specific indicator of faecal pollution.

Indicator value

In view of the exceptional resistance of C. perfringens spores to disinfection processes and other unfavourable environmental conditions, C. perfringens has been proposed as an indicator of protozoa in treated drinking-water supplies. In addition, C. perfringens can serve as an indicator of faecal pollution that took place previously and hence can indicate sources liable to intermittent contamination. The evidence that Clostridium is a reliable indicator for enteric viruses is limited and inconsistent, largely based on one study of reductions by drinking-water treatment. Results should be treated with some caution, as the exceptionally long survival times of its spores are likely to far exceed those of enteric pathogens. Clostridium perfringens spores are smaller than protozoan (oo)cysts and may be useful indicators of the effectiveness of filtration processes.

Source and occurrence

Clostridium perfringens and its spores are virtually always present in sewage. The organism does not multiply in water environments. Clostridium perfringens is present more often and in higher numbers in the faeces of some animals, such as dogs, than in the faeces of humans and less often in the faeces of many other warm-blooded animals. The numbers excreted in faeces are normally substantially lower than those of E. coli.

Application in practice

Vegetative cells and spores of C. perfringens are usually detected by membrane filtration techniques in which membranes are incubated on selective media under strict anaerobic conditions. These detection techniques are not as simple and inexpensive as those for other indicators, such as E. coli and intestinal enterococci.

Significance in drinking-water

The presence of C. perfringens in drinking-water can be an indicator of intermittent faecal contamination. Potential sources of contamination should be investigated. Filtration processes designed to remove enteric viruses or protozoa should also remove C. perfringens. Detection in water immediately after treatment should lead to investigation of filtration plant performance.

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Colony counts or Heterotrophic plate counts at 22˚C

General description

Colony counts or Heterotrophic Plate Counts (HPC) measurement detects a wide spectrum of heterotrophic microorganisms, including bacteria and fungi, based on the ability of the organisms to grow on rich growth media, without inhibitory or selective agents, over a specified incubation period and at a defined temperature. The spectrum of organisms detected by HPC testing includes organisms sensitive to disinfection processes, such as coliform bacteria; organisms resistant to disinfection, such as spore formers; and organisms that rapidly proliferate in treated water in the absence of residual disinfectants. The tests detect only a small proportion of the microorganisms that are present in water. The population recovered will differ according to the method and conditions applied. Although standard methods have been developed, there is no single universal HPC measurement. A range of media is available, incubation temperatures used vary from 20 °C to 37 °C and incubation periods range from a few hours to 7 days or more.

Indicator value

The test has little value as an indicator of pathogen presence but can be useful in operational monitoring as a treatment and disinfectant indicator, where the objective is to keep numbers as low as possible. In addition, HPC measurement can be used in assessing the cleanliness and integrity of distribution systems and the presence of biofilms.

Source and occurrence

Heterotrophic microorganisms include both members of the natural (typically non-hazardous) microbial flora of water environments and organisms present in a range of pollution sources. They occur in large numbers in raw water sources. The actual organisms detected by HPC tests vary widely between locations and between consecutive samples. Some drinking-water treatment processes, such as coagulation and sedimentation, reduce the number of HPC organisms in water. However, the organisms proliferate in other treatment processes, such as biologically active carbon and sand filtration. Numbers of HPC organisms are reduced significantly by disinfection practices, such as chlorination, ozonation and UV light irradiation. However, in practice, none of the disinfection processes sterilizes water; under suitable conditions, such as the absence of disinfectant residuals, HPC organisms can grow rapidly. HPC organisms can grow in water and on surfaces in contact with water as biofilms. The principal determinants of growth or “regrowth” are temperature, availability of nutrients, including assimilable organic carbon, lack of disinfectant residual and stagnation.

Application in practice

No sophisticated laboratory facilities or highly trained staff are required. Results on simple aerobically incubated agar plates are available within hours to days, depending on the characteristics of the procedure used.

Significance in drinking-water

After disinfection, numbers would be expected to be low; for most uses of HPC test results, however, actual numbers are of less value than changes in numbers at particular locations. In distribution systems, increasing numbers can indicate a deterioration in cleanliness, possibly

stagnation and the potential development of biofilms. HPC can include potentially “opportunistic” pathogens such as Acinetobacter, Aeromonas, Flavobacterium, Klebsiella, Moraxella, Serratia, Pseudomonas and Xanthomonas. However, there is no evidence of an association of any of these organisms with gastrointestinal infection through ingestion of drinking-water in the general population.

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Enterococci

General description

Intestinal enterococci are a subgroup of the larger group of organisms defined as faecal streptococci, comprising species of the genus Streptococcus. These bacteria are Gram-positive and relatively tolerant of sodium chloride and alkaline pH levels. They are facultatively anaerobic and occur singly, in pairs or as short chains. Faecal streptococci including intestinal enterococci all give a positive reaction with Lancefield's Group D antisera and have been isolated from the faeces of warm-blooded animals. The subgroup intestinal enterococci consists of the species Enterococcus faecalis, E. faecium, E. durans and E. hirae. This group was separated from the rest of the faecal streptococci because they are relatively specific for faecal pollution. However, some intestinal enterococci isolated from water may occasionally also originate from other habitats, including soil, in the absence of faecal pollution.

Indicator value

The intestinal enterococci group can be used as an indicator of faecal pollution. Most species do not multiply in water environments. The numbers of intestinal enterococci in human faeces are generally about an order of magnitude lower than those of E. coli. Important advantages of this group are that they tend to survive longer in water environments than E. coli (or thermotolerant coliforms), are more resistant to drying and are more resistant to chlorination. Intestinal enterococci have been used in testing of raw water as an indicator of faecal pathogens that survive longer than E. coli and in drinking-water to augment testing for E. coli. In addition, they have been used to test water quality after repairs to distribution systems or after new mains have been laid.

Source and occurrence

Intestinal enterococci are typically excreted in the faeces of humans and other warm-blooded animals. Some members of the group have also been detected in soil in the absence of faecal contamination. Intestinal enterococci are present in large numbers in sewage and water environments polluted by sewage or wastes from humans and animals.

Application in practice

Enterococci are detectable by simple, inexpensive cultural methods that require basic bacteriology laboratory facilities. Commonly used methods include membrane filtration with incubation of membranes on selective media and counting of colonies after incubation at 35–37 °C for 48 hours. Other methods include a most probable number technique using microtitre plates where detection is based on the ability of intestinal enterococci to hydrolyse 4-methyl-umbelliferyl-β-D-glucoside in the presence of thallium acetate and nalidixic acid within 36 hours at 41 °C.

Significance in drinking-water

The presence of intestinal enterococci provides evidence of recent faecal contamination, and detection should lead to consideration of further action, which could include further sampling and investigation of potential sources such as inadequate treatment or breaches in distribution system integrity.

Selected bibliography

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Escherichia coli

General description

Escherichia coli is the most widely used indicator of faecal pollution and is in use already since the late 19th century. It is a bacterium that is present in very high numbers (up to 10^9 per gram) in human and (warm-blooded) animal faeces. *E. coli* is a natural and essential part of the bacterial flora in the gut of humans and animals. Most *E. coli* strains are nonpathogenic and reside harmlessly in the colon. However, certain serotypes (such as O157:H7) do play a role in intestinal and extra-intestinal diseases, such as urinary tract infections, and have been involved in drinking-waterborne outbreaks. It is found in sewage, treated effluents, and all natural waters and soils subject to recent faecal contamination, whether from humans, wild (warm-blooded) animals, or livestock activity. Because animals can excrete pathogens that are infective in humans, the presence of *E. coli* from (particularly farm) animal faeces is also indicative of a potential human health risk. *E. coli* is rarely found in water in the absence of faecal pollution, although there is some evidence for growth in some environments, such as tropical soils. Water temperatures and nutrient conditions present in drinking-water distribution systems are highly unlikely to support the growth of these organisms.

Indicator value

Escherichia coli is considered the most suitable indicator of faecal contamination. In most circumstances, populations of thermotolerant coliforms are composed pre-dominantly of E. coli; as a result, this group is regarded as a less reliable but acceptable indicator of faecal pollution. Escherichia coli (or, alternatively, thermotolerant coliforms) is the first organism of choice in monitoring programs for verification, including surveillance of drinking-water quality. These organisms are also used as disinfection indicators, but testing is far slower and less reliable than direct measurement of disinfectant residual. In addition, E. coli is far more sensitive to disinfection than are enteric viruses and protozoa.

Methods

Escherichia coli are measured using culture methods. The principle of these methods has been in use for water quality monitoring for over 100 years. The methods are relatively simple and cheap and well-established throughout the EU. The standard methods EN ISO 9308-1 and 9308-2 are the culture methods specified in the EU DWD. These methods require at least 18 h to produce a result. Others culture methods have been approved or are in the process of approval as alternative method. The use of molecular methods, such as RT-PCR, allows for a very rapid and specific detection of *E. coli*. A short time-to-result is very beneficial for adequate protection of the health of the drinking water consumers.

Significance in drinking-water

The presence of E. coli (or, alternatively, thermotolerant coliforms) provides evidence of recent faecal contamination, and detection should lead to consideration of further action, which could include further sampling and investigation of potential sources such as inadequate treatment or breaches in distribution system integrity.

Guideline value derivation

The guideline value is absence in 100 ml of drinking water. This guideline value has been unchanged since the first edition of the EU DWD and WHO GDWQ, and actually since the beginning of bacteriological water quality examinations. The origin of the guideline value is not completely clear. The early water microbiologists (at the end of the 19th century) were already analyzing drinking water sources for the presence of bacteria, including for the presence of *Bacterium coli* (later renamed as *Escherichia coli*) as an indicator of contamination of the water with human fecal material, and hence the potential presence of pathogens (at that time the bacterial pathogens that cause typhoid or cholera).

The guideline value of 0/100 ml seems to be based on practical considerations (100 ml was a convenient volume to use in bacterial water testing), and on the observation that water derived from deep wells and springs, which was protected from contamination by excreta, consistently contained 0/100 ml, while shallow well water and surface water contained more than 0/100 ml (Savage, 1906). It is intriguing to consider that the main water quality parameter to protect the health of the EU citizens has no direct health basis. A few studies in the EU (France, UK) have looked at the health risk associated with non-compliance with the EU DWD for *E. coli* (and enterococci) (refs). These studies focused on small water supplies, since these are notorious for non-compliance and serve around 10% of the EU population (Hulsmann,). The studies indicate that people (particularly children) that consume non-compliant water have a higher (up to 6 times) rate of diarrhoeal illness than people that consume compliant water. This association was particularly observed when water was non-compliant for both *E. coli* and enterococci.

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Total coliform bacteria

General description

Total coliform bacteria include a wide range of aerobic and facultatively anaerobic, Gram-negative, non-spore-forming bacilli capable of growing in the presence of relatively high concentrations of bile salts with the fermentation of lactose and production of acid or aldehyde within 24 hours at 35–37 °C. Escherichia coli and thermotolerant coliforms are a subset of the total coliform group that can ferment lactose at higher temperatures (see below). As part of lactose fermentation, total coliforms produce the enzyme f3-galactosidase. Traditionally, coliform bacteria were regarded as belonging to the genera Escherichia, Citrobacter, Klebsiella and Enterobacter, but the group is more heterogeneous and includes a wider range of genera, such as Serratia and Hafnia. The total coliform group includes both faecal and environmental species.

Indicator value

Total coliforms include organisms that can survive and grow in water. Hence, they are not useful as an indicator of faecal pathogens, but they can be used to assess the cleanliness and integrity of distribution systems and the potential presence of biofilms. However, there are better indicators for these purposes. It has been proposed that total coliforms could be used as a disinfection indicator. However, the test for total coliforms is far slower and less reliable than direct measurement of disinfectant residual. In addition, total coliforms are far more sensitive to disinfection than are enteric viruses and protozoa. HPC measurements detect a wider range of microorganisms and are generally considered a better indicator of distribution system integrity and cleanliness.

Source and occurrence

Total coliform bacteria (excluding E. coli) occur in both sewage and natural waters. Some of these bacteria are excreted in the faeces of humans and animals, but many coliforms are heterotrophic and able to multiply in water and soil environments. Total coliforms can also survive and grow in water distribution systems, particularly in the presence of biofilms.

Application in practice

Total coliforms are generally measured in 100 ml samples of water. A variety of relatively simple procedures are available based on the production of acid from lactose or the production of the enzyme f3-galactosidase. The procedures include membrane filtration followed by incubation of the membranes on selective media at 35–37 °C and counting of colonies after 24 hours. Alternative methods include most probable number procedures using tubes or microtitre plates and presence/absence tests. Field test kits are available.

Significance in drinking-water

Total coliforms are less suitable as indicator organisms. They have been regarded as indicator for faecal contamination, but because they can survive and multiply in plant material, soil, sediments in water reservoirs etc, they are not a conclusive indication of faecal contamination. They have been used as model organisms for treatment efficacy, but also here this is hampered by their potential to grow under certain conditions in the water systems. E. coli and enterococci serve as more conclusive indicators for faecal contamination and also to assess treatment efficacy, so there

is no need for coliforms as parameter. The presence of total coliforms in distribution systems and stored water supplies can reveal growth and possible biofilm formation or contamination through ingress of foreign material, including soil or plants. Growth and biofilm formation are monitored more adequately with colony counts and ingress of foreign material, without the presence of E. coli or enterococci is unlikely to be associated with a health risk, so also here, the role of the coliforms as parameter is of little added value.

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Appendix 4

Technical overview of microbiological parameters suggested for inclusion in DWD Annex I Part A and C

The text of these fact sheets was largely based on the Microbial fact sheets in the WHO GDWQ (2016) and the WHO/OECD background document Assessing Microbial Safety of Drinking Water (2003), complemented with information from the scientific literature.

Campylobacter

General description

Campylobacter spp. are microaerophilic (require decreased oxygen) and capnophilic (require increased carbon dioxide), Gram-negative, curved spiral rods with a single unsheathed polar flagellum. Campylobacter spp. are one of the most important causes of acute gastroenteritis worldwide. Campylobacter jejuni is the most frequently isolated species from patients with acute diarrhoeal disease, whereas C. coli, C. laridis and C. fetus have also been isolated in a small proportion of cases. Two closely related genera, Helicobacter and Arcobacter, include species previously classified as Campylobacter spp.

Human health effects

An important feature of C. jejuni is relatively high infectivity compared with other bacterial pathogens. As few as 1000 organisms can cause infection. Most symptomatic infections occur in infancy and early childhood. The incubation period is usually 2–4 days. Clinical symptoms of C. jejuni infection are characterized by abdominal pain, diarrhoea (with or without blood or faecal leukocytes), vomiting, chills and fever. The infection is self-limited and resolves in 3–7 days. Relapses may occur in 5–10% of untreated patients. Other clinical manifestations of C. jejuni infections in humans include reactive arthritis and meningitis. Several reports have associated C. jejuni infection with Guillain-Barré syndrome, an acute demyelinating disease of the peripheral nerves.

Source and occurrence

Campylobacter spp. occur in a variety of environments. Wild and domestic animals, especially poultry, wild birds and cattle, are important reservoirs. Pets and other animals may also be reservoirs. Food, including meat and unpasteurized milk, are important sources of Campylobacter infections. Water is also a significant source. The occurrence of the organisms in surface waters has proved to be strongly dependent on rainfall, water temperature and the presence of waterfowl.

Routes of exposure

Most Campylobacter infections are reported as sporadic in nature, with food considered a common source of infection. Transmission to humans typically occurs by the consumption of animal products. Meat, particularly poultry products, and un-pasteurized milk are important sources of infection. Contaminated drinking-water supplies have been identified as a source of outbreaks. The number of cases in these outbreaks ranged from a few to several thousand, with sources including unchlorinated or inadequately chlorinated surface water supplies and faecal contamination of water storage reservoirs by wild birds.

Significance in drinking-water

Contaminated drinking-water supplies have been identified as a significant source of outbreaks of campylobacteriosis. The detection of waterborne outbreaks and cases appears to be increasing. Waterborne transmission has been confirmed by the isolation of the same strains from patients and drinking-water they had consumed. Within a water safety plan, control measures that can be applied to manage potential risk from Campylobacter spp. include protection of raw water supplies from waste from humans and animals, adequate treatment and protection of water during distribution. Storages of treated and disinfected water should be protected from bird faeces. Campylobacter spp. are faecally borne pathogens and are not particularly resistant to disinfection. Hence, E. coli (or thermotolerant coliforms) is an appropriate indicator for the presence/absence of Campylobacter spp. in drinking-water supplies.

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Coliphages

General description

Bacteriophages (phages) are viruses that use only bacteria as hosts for replication. Coliphages use E. coli and closely related species as hosts and hence can be released by these bacterial hosts into the faeces of humans and other warm-blooded animals. Coliphages used in water quality assessment are divided into the major groups of somatic coliphages and F-RNA coliphages. Differences between the two groups include the route of infection.

Somatic coliphages initiate infection by attaching to receptors permanently located on the cell wall of hosts. They replicate more frequently in the gastrointestinal tract of warm-blooded animals but can also replicate in water environments. Somatic coliphages consist of a wide range of phages (members of the phage families Myoviridae, Siphoviridae, Podoviridae and Microviridae) with a spectrum of morphological types.

F-RNA coliphages initiate infection by attaching to fertility (F-, sex) fimbriae on E. coli hosts. These F-fimbriae are produced only by bacteria carrying the fertility (F-) plasmid. As F-fimbriae are produced only in the logarithmic growth phase at temperatures above 30 °C, F-RNA phages are not likely to replicate in environments other than the gastrointestinal tract of warm-blooded animals. F-RNA coliphages comprise a restricted group of closely related phages, which belong to the family Leviviridae, and consist of a single-stranded RNA genome and an icosahedral capsid that is morphologically similar to that of picornaviruses. F-RNA coliphages have been divided into serological types I–IV, which can be identified as genotypes by molecular techniques such as gene probe hybridization. Members of groups I and IV have to date been found exclusively in (non-human) animal faeces, and group III in human faeces. Group II phages have been detected in human faeces and no animal faeces other than about 28% of porcine faeces. This specificity, which is not fully understood, offers a potential tool to distinguish between faecal pollution of human and animal origin under certain conditions and limitations.

Indicator value

Phages share many properties with human viruses, notably composition, morphology, structure and mode of replication. As a result, coliphages are useful models or surrogates to assess the behaviour of enteric viruses in water environments and the sensitivity to treatment and disinfection processes. In this regard, they are superior to faecal bacteria and could be considered for inclusion in verification and surveillance monitoring where source waters are known to be affected by human faecal waste. However, there is no direct correlation between numbers of coliphages and numbers of enteric viruses. In addition, coliphages cannot be absolutely relied upon as an indicator for enteric viruses. This has been confirmed by the isolation of enteric viruses from treated and disinfected drinking-water supplies that yielded negative results in conventional tests for coliphages.

F-RNA coliphages provide a more specific indicator of faecal pollution than somatic phages. In addition, F-RNA coliphages are better indicators of the behaviour of enteric viruses in water environments and their response to treatment and disinfection processes than are somatic coliphages. This has been confirmed by studies in which the behaviour and survival of F-RNA coliphages, somatic phages, faecal bacteria and enteric viruses have been compared. Available

data indicate that the specificity of F-RNA serogroups (genotypes) for human and animal excreta may prove useful in the distinction between faecal pollution of human and animal origin. However, there are shortcomings and conflicting data that need to be resolved, and the extent to which this tool can be applied in practice remains to be elucidated. Owing to the limitations of coliphages, they are best used in laboratory investigations, pilot trials and possibly validation testing. They are not suitable for operational or verification (including surveillance) monitoring.

Source and occurrence

Coliphages are excreted by humans and animals in relatively low numbers. As a re-sult of their respective modes of replication and host specificity, somatic coliphages are generally excreted by most humans and animals, whereas F-RNA coliphages are excreted by a variable and generally lower percentage of humans and animals. Available data indicate that in some communities, F-RNA phages are detectable in 10% of human, 45% of bovine, 60% of porcine and 70% of poultry faecal specimens. Somatic coliphages have been found to generally outnumber F-RNA phages in water environments by a factor of about 5 and cytopathogenic human viruses by a factor of about 500, although these ratios vary considerably. Sewage contains somatic coliphages in numbers of the order of 106–108 per litre; in one study, slaughterhouse wastewater was found to contain somatic coliphages in numbers up to 1010 per litre. There are indications that they may multiply in sewage, and somatic coliphages may multiply in natural water environments using saprophytic hosts. Somatic phages and F-RNA phages have been detected in numbers up to 105 per litre in lake and river water.

Application in practice

Somatic coliphages are detectable by relatively simple and inexpensive plaque assays, which yield results within 24 hours. Plaque assays for F-RNA coliphages are not quite as simple, because the culture of host bacteria has to be in the logarithmic growth phase at a temperature above 30 °C to ensure that F-fimbriae are present. Plaque assays using large petri dishes have been designed for the quantitative enumeration of plaques in 100 ml samples, and presence/absence tests have been developed for volumes of water of 500 ml or more.

Significance in drinking-water

As coliphages typically replicate in the gastrointestinal tract of humans and warm-blooded animals, their presence in drinking-water provides an indicator of faecal pollution and hence the potential presence of enteric viruses and possibly also other pathogens. The presence of coliphages in drinking-water also indicates shortcomings in treatment and disinfection processes designed to remove enteric viruses. F-RNA coliphages provide a more specific indicator for faecal pollution. The absence of coli-phages from treated drinking-water supplies does not confirm the absence of patho¬gens such as enteric viruses and protozoan parasites.

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Cryptosporidium

General description

Cryptosporidium is an obligate, intracellular, coccidian parasite with a complex life cycle including sexual and asexual replication. Thick-walled oocysts with a diameter of 4–6 μm are shed in faeces. The genus Cryptosporidium has about 13 species, with human infections predominantly caused by C. hominis and the cattle genotype of C. parvum. Other Cryptosporidium species have been reported to cause infrequent infections. Cryptosporidium was discovered to infect humans in 1976, and waterborne transmission was confirmed for the first time in 1984.

Human health effects

Cryptosporidium generally causes self-limiting diarrhoea, sometimes including nausea, vomiting and fever, which usually resolves within a week in normally healthy people, but can last for a month or more. Severity of cryptosporidiosis varies according to age and immune status, and infections in severely immunocompromised people can be life-threatening. The impact of cryptosporidiosis outbreaks is relatively high due to the large numbers of people that may be involved and the associated socioeconomic implications. The total cost of illness associated with the 1993 outbreak in Milwaukee, USA, has been estimated at US$ 96.2 million.

Source and occurrence

A large range of animals are reservoirs of C. hominis/parvum, but humans and live-stock, particularly young animals, are the most significant source of human infectious organisms. Calves can excrete 1010 oocysts per day. Concentrations of oocysts as high as 14 000 per litre for raw sewage and 5800 per litre for surface water have been reported. Oocysts can survive for weeks to months in fresh water. Cryptosporidium oocysts have been detected in many drinking-water supplies. However, in most cases, there is little information about whether human infectious species were present. The currently available standard analytical techniques provide an indirect measure of viability and no indication of human infectivity. Oocysts also occur in recreational waters.

Routes of exposure

Cryptosporidium is transmitted by the faecal–oral route. The major route of infection is person-to-person contact. Other sources of infection include the consumption of contaminated food and water and direct contact with infected farm animals and possibly domestic pets. Contaminated drinking-water, recreational water and, to a lesser extent, food have been associated with outbreaks. In 1993, Cryptosporidium caused the largest waterborne outbreak of disease on record, when more than 400 000 people were infected by the drinking-water supply of Milwaukee, USA. The infectivity of Cryptosporidium oocysts is relatively high. Studies on healthy human volunteers revealed that ingestion of fewer than 10 oocysts can lead to infection.

Significance in drinking-water

The role of drinking-water in the transmission of Cryptosporidium, including in large outbreaks, is well established. Attention to these organisms is therefore important. The oocysts are extremely resistant to oxidizing disinfectants such as chlorine, but investigations based on assays for infectivity have shown that UV light irradiation inactivates oocysts. Within a water safety plan,

control measures to reduce potential risk from Cryptosporidium should focus on prevention of source water contamination by human and livestock waste, adequate treatment and protection of water during distribution. Because of their relatively small size, the oocysts represent a challenge for removal by conventional granular media–based filtration processes. Acceptable removal requires well-designed and well-operated systems. Membrane filtration pro¬cesses that provide a direct physical barrier may represent a viable alternative for the effective removal of Cryptosporidium oocysts. Owing to the exceptional resistance of the oocysts to disinfectants, E. coli (or, alternatively, thermotolerant coliforms) cannot be relied upon as an indicator for the presence/absence of Cryptosporidium oocysts in drinking-water supplies.

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Enteroviruses

General description

The genus Enterovirus is a member of the family Picornaviridae. This genus consists of 69 serotypes (species) that infect humans: poliovirus types 1–3, coxsackievirus types A1–A24, coxsackievirus types B1–B6, echovirus types 1–33 and the numbered entero-virus types EV68–EV73. Members of the genus are collectively referred to as entero-viruses. Other species of the genus infect animals other than humans—for instance, the bovine group of enteroviruses. Enteroviruses are among the smallest known viruses and consist of a single-stranded RNA genome in a non-enveloped icosahedral capsid with a diameter of 20–30 nm. Some members of the genus, notably poliovirus, coxsackievirus B, echovirus and enterovirus, are readily isolated by cytopathogenic effect in cell cultures.

Human health effects

Enteroviruses are one of the most common causes of human infections. They have been estimated to cause about 30 million infections in the USA each year. The spectrum of diseases caused by enteroviruses is broad and ranges from a mild febrile illness to myocarditis, meningoencephalitis, poliomyelitis, herpangina, hand-foot-and-mouth disease and neonatal multi-organ failure. The persistence of the viruses in chronic conditions such as polymyositis, dilated cardiomyopathy and chronic fatigue syndrome has been described. Most infections, particularly in children, are asymptomatic, but still lead to the excretion of large numbers of the viruses, which may cause clinical disease in other individuals.

Source and occurrence

Enteroviruses are excreted in the faeces of infected individuals. Among the types of viruses detectable by conventional cell culture isolation, enteroviruses are generally the most numerous in sewage, water resources and treated drinking-water supplies. The viruses are also readily detected in many foods.

Routes of exposure

Person-to-person contact and inhalation of airborne viruses or viruses in respiratory droplets are considered to be the predominant routes of transmission of enteroviruses in communities. Transmission from drinking-water could also be important, but this has not yet been confirmed. Waterborne transmission of enteroviruses (coxsackie-virus A16 and B5) has been epidemiologically confirmed for only two outbreaks, and these were associated with children bathing in lake water in the 1970s.

Significance in drinking-water

Enteroviruses have been shown to occur in substantial numbers in raw water sources and treated drinking-water supplies. In view of their prevalence, drinking-water represents a likely, although unconfirmed, source of enterovirus infection. The limited knowledge on the role of waterborne transmission could be related to a number of factors, including the wide range of clinical symptoms, frequent asymptomatic infection, the diversity of serotypes and the dominance of person-to-person spread. Enteroviruses have been detected in drinking-water supplies that met accepted specifications for treatment, disinfection and conventional indicator organisms. Within a

water safety plan, control measures to reduce potential risk from enteroviruses should focus on prevention of source water contamination by human waste, followed by adequate treatment and disinfection. The effectiveness of treatment processes used to remove enteroviruses will require validation. Drinking-water supplies should also be protected from contamination during distribution. Owing to the higher resistance of the viruses to disinfection, E. coli (or, alternatively, thermotolerant coliforms) is not a reliable indicator of the presence/absence of enteroviruses in drinking-water supplies.

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Legionella

General description

The genus Legionella, a member of the family Legionellaceae, has at least 50 species comprising 70 distinct serogroups. Legionellae are Gram-negative, rod-shaped, non-spore-forming bacteria that require L-cysteine for growth and primary isolation. Legionella spp. are heterotrophic bacteria found in a wide range of water environments and can proliferate at temperatures above 25 °C.

Human health effects

Although all Legionella spp. are considered potentially pathogenic for humans, L. pneumophila is the major waterborne pathogen responsible for legionellosis, of which two clinical forms are known: Legionnaires' disease and Pontiac fever. The former is a pneumonic illness with an incubation period of 3–6 days. Host factors influence the likelihood of illness: males are more frequently affected than females, and most cases occur in the 40- to 70-year age group. Risk factors include smoking, alcohol abuse, cancer, diabetes, chronic respiratory or kidney disease and immunosuppression, as in transplant recipients. Pontiac fever is a milder, self-limiting disease with a high attack rate and an onset (5 hours to 3 days) and symptoms similar to those of influenza: fever, headache, nausea, vomiting, aching muscles and coughing. Studies of seroprevalence of antibodies indicate that many infections are asymptomatic.

Source and occurrence

Legionella spp. are members of the natural flora of many freshwater environments, such as rivers, streams and impoundments, where they occur in relatively low numbers. However, they thrive in certain human-made water environments, such as water cooling devices (cooling towers and evaporative condensers) associated with air-conditioning systems, hot water distribution systems and spas, which provide suitable temperatures (25–50 °C) and conditions for their multiplication. Devices that support multiplication of Legionella have been associated with outbreaks of Legionnaires' disease. Legionella survive and grow in biofilms and sediments and are more easily detected from swab samples than from flowing water. Legionellae can be ingested by trophozoites of certain amoebae such as Acanthamoeba, Hartmanella and Naegleria, which play an important role in their persistence in water environments.

Routes of exposure

The most common route of infection is the inhalation of aerosols containing the bacteria. Such aerosols can be generated by contaminated cooling towers, warm water showers, humidifiers and spas. Aspiration has also been identified as a route of infection in some cases associated with contaminated water, food and ice. There is no evidence of person-to-person transmission.

Significance in drinking-water

Legionella spp. are common waterborne organisms, and devices such as cooling towers, hot water systems and spas that utilize mains water have been associated with outbreaks of infection. Owing

to the prevalence of Legionella, the potential for ingress into drinking-water systems should be considered as a possibility, and control measures should be employed to reduce the likelihood of survival and multiplication. Disinfection strategies designed to minimize biofilm growth and temperature control can minimize the potential risk from Legionella spp. The organisms are sensitive to disinfection. Monochloramine has been shown to be particularly effective, probably due to its stability and greater effectiveness against biofilms. Water temperature is an important element of control strategies. Wherever possible, water temperatures should be kept outside the range of 25–50 °C and preferably 20–50 °C to prevent the growth of the organism. In hot water systems, temperatures leaving heaters should be above 60 °C, and temperatures above 50°C should be maintained throughout associated pipework. However, maintaining temperatures of hot water above 50 °C may represent a scalding risk in young children, the elderly and other vulnerable groups. Where temperatures in hot or cold water distribution systems cannot be maintained outside the range of 25–50 °C, greater attention to disinfection and strategies aimed at limiting development of biofilms are required. Accumulation of sludge, scale, rust, algae or slime deposits in water distribution systems supports the growth of Legionella spp., as does stagnant water. Systems that are kept clean and flowing are less likely to support excess growth of Legionella spp. Care should also be taken to select plumbing materials that do not support microbial growth and the development of biofilms.

Legionella spp. represent a particular concern in devices such as cooling towers and hot water systems in large buildings. As discussed in chapter 6, specific water safety plans incorporating control measures for Legionella spp. should be developed for these buildings. Legionella are not detected by HPC techniques, and E. coli (or, alternatively, thermotolerant coliforms) is not a suitable indicator for the presence/ absence of this organism.

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