EVALUATING HOUSEHOLD WATER TREATMENT OPTIONS:

Health-based targets and microbiological performance specifications

World Health Organization

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LIST OF ACRONYMS AND ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
ANSI	American National Standards Institute
DALY	disability-adjusted life year
DNA	deoxyribonucleic acid
EAWAG	Swiss Federal Institute of Aquatic Science and Technology
EPA	Environmental Protection Agency (USA)
ETV	environmental technology verification
GDWQ	*Guidelines for drinking-water quality*
HIV	human immunodeficiency syndrome
HWT	household water treatment
MPN	most probable number
NSF	NSF International
NTU	nephelometric turbidity unit
pI	isoelectric point
QMRA	quantitative microbial risk assessment
RNA	ribonucleic acid
SODIS	solar disinfection
TSC	tryptose-sulfite-cycloserine
USA	United States of America
USEPA	United States Environmental Protection Agency
UV	ultraviolet
WHO	World Health Organization
YLD	years of life lived with disability due to illness
YLL	years of life lost due to mortality

1. INTRODUCTION

Household water treatment (HWT) interventions may play an important role in protecting public health where existing water sources, including those delivered via a piped network or other improved sources, are untreated, are not treated properly or become contaminated during distribution or storage (UNICEF & WHO, 2009).

HWT applications are any of a range of technologies, devices or methods employed for the purposes of treating water at the household level or at the point of use in other settings, such as schools, health-care facilities and other community locations. Point-of-use water treatment is another term used for HWT. Proper household storage, including use of closed or narrow-necked containers to prevent contact with contaminated hands, is an essential component of household water management, but is not the focus of this document.

Properly formulated and locally relevant performance specifications are needed to protect users and inform decision-making regarding selection of technologies or approaches. This document provides a basis by which to evaluate the microbiological performance of HWT options by:

- establishing a series of health-based microbiological performance targets, ranging from an interim target to highly protective, to encourage incremental improvements in water safety (sections 2 and 3 and Appendix 1); and
- providing guidance to inform the development of new HWT testing protocols or supplement existing protocols (Appendix 2).

It also describes additional factors, including:

- those pertaining to national-level technology evaluation or verification programmes (Appendix 3); and
- justification for use of quantitative microbial risk assessment (QMRA) and performance targets for three classes of pathogens (Appendix 4).

These microbiological performance targets and testing protocols are intended to inform implementers, protect users and encourage technology development by providing a risk-based framework to assess the performance of HWT interventions. The document provides a basis to inform the development or revision of national or international technology performance evaluation programmes. It is underpinned by concepts established in the World Health Organization's (WHO) *Guidelines for drinking-water quality* (GDWQ), and the laboratory methods described are meant to be relevant in resource-limited settings. This document does not describe targets or protocols for chemical contaminants, although many of the same concepts regarding risk-based performance targets are relevant to this group of contaminants. The intended audiences are 1) national-level certification organizations, 2) regulatory authorities, 3) those involved in developing and evaluating technologies, including universities and researchers, and 4) manufacturers and implementers of HWT technologies.

The recommendations made in this document are advisory in nature and may be adapted to local contexts by regulatory agencies or national authorities, where applicable, including for product certification, pre-intervention performance evaluation or technology development and selection.

2. HEALTH-BASED PERFORMANCE TARGETS

The performance targets presented in this document were determined by applying the concept of tolerable disease burden (acceptable risk) as set forth in the fourth edition of the GDWQ (WHO, 2011). The GDWQ define the tolerable burden of disease as an upper limit of 10^{-6} disability-adjusted life year (DALY) per person per year (see Box 1).

Box 1. Disability-adjusted life year (DALY)

A common basis for comparing health outcomes is the disability-adjusted life year (DALY), now used extensively by WHO and others to estimate and compare burdens of disease and injuries (Havelaar & Melse, 2003). Health effects are weighted by severity from 0 (good health) to 1 (death). Effects are multiplied by the duration of the illness and the population affected to obtain a standardized estimate of total disease burden and premature death. DALYs thus represent the years of life lost due to mortality (years of life lost, YLL) and the years of life lived with disability (YLD) due to illness, standardized by outcome severity weights (Havelaar & Melse, 2003). The DALY metric is computed as: DALY = YLL + YLD. In mathematical terms, 10^{-6} DALY per person per year allows for the tolerable loss of 365 healthy days in a population of one million over the course of one year. This DALY limit is equivalent to one excess case of cancer per 100 000 people ingesting treated drinking-water over a 70-year period. The DALY metric is described further in the GDWQ (WHO, 2011).

Performance targets are values, expressed in terms of $\log_{10}$ reductions in microbe concentrations[1], that define treatment requirements in relation to source water quality. Ideally, these should be derived based on locally relevant data, but as such data are frequently not available, targets are commonly derived based on assumptions made in relation to three classes of pathogens present in drinking-water supplies.

Establishing a clear, causal link between pathogen levels in drinking-water and waterborne disease is problematic. QMRA provides a mechanism to make this link explicit based on current water quality data, exposure and dose–response models. The GDWQ recommend QMRA as an important option to assess risks and inform management decisions, particularly in situations where epidemiological data do not exist, until epidemiological data are obtained and/or where epidemiological studies may not be practical or appropriate. QMRA allows for an estimation of the health impacts of drinking-water quality control measures in a wide variety of settings (WHO, 2011). To the extent possible, QMRA takes into consideration and uses epidemiological data for both exposure assessment and health effects (dose–response) for risk characterization and estimation.

Use of QMRA is consistent with the three main WHO water-related guidelines (drinking-water, wastewater reuse and recreational water). Therefore, application of QMRA to HWT provides a harmonized framework for an integrated approach to estimating microbial risks in the water environment in general (Havelaar et al., 2001).

[1] Computed as $\log_{10}$ ($C_{\text{untreated water}}$ / $C_{\text{treated water}}$), where C = microbe concentration in water.

2.1 Target pathogens

It is neither feasible nor desirable to derive performance targets for all potentially waterborne pathogens given both the complexity of analyses required and the lack of sufficient data available. Thus, targets are derived for reference pathogens representing three classes of pathogens: bacteria, viruses and protozoa. These three classes are represented because each class is uniquely distinct in regard to the physicochemical and biological properties of the pathogens within the class and in terms of resistance to various treatment technologies. Given that all three classes occur widely in drinking-water supplies in low- and high-income countries and are associated with enteric disease in children in countries with a high burden of disease (Levin, 2009), they are all important.

The reference pathogens for bacteria (*Campylobacter jejuni*), viruses (rotavirus) and protozoan parasites (*Cryptosporidium*) were selected because they are relatively well characterized, of high public health importance and conservative with respect to dose–response and infectivity. In other words, if treatment options were in place to control these reference pathogens, there would be the expectation that other important pathogens within each class of pathogen would also be controlled.

2.2 Derivation of targets

Methods for deriving microbiological performance targets and default concentrations of pathogens are given in Appendix 1. Targets were derived using assumed levels of reference pathogens in untreated water, QMRA models described in the GDWQ and calculated $\log_{10}$ microbial reductions to meet health-based targets. To address the relatively common scenario where sufficient local data on relevant pathogens are not available, assumptions regarding background microbial water quality are used. Additional information on the analytical QMRA models can be found in chapter 7 of the GDWQ (WHO, 2011).

2.3 Tiered approach

Three recommended levels of performance for the reduction of bacteria, viruses and protozoa are illustrated in Figure 1. These range from a top-tier target equivalent to the WHO drinking-water guideline reference level of risk of 10^{-6} DALY per person per year to a bottom-tier, "interim" target relevant to the performance of currently available, low-cost technologies that have demonstrated health improvements. As described in the box above, DALYs are a common metric used to quantify and compare the burden of disease associated with different health hazards.

The top-tier standard of "highly protective" represents those technologies that, if used correctly and consistently over an entire year, will limit drinking-water disease burden to 10^{-6} DALY per person. This is an extremely conservative health-based target, and, from a health perspective, such technologies should be unequivocally recommended for use.

The second tier, "protective", has been established to allow for a less stringent level of tolerable disease excess, yet is still consistent with the goal of providing high-quality, safer water. The "protective" target defines pathogen removals that achieve a health-based target of 10^{-4} DALY per person per year. In areas with a suspected high burden of waterborne disease, technologies that meet the log removal standards in the second tier would still result in significant health benefits (see Box 2). Both the "highly protective" and "protective" targets are based on the removal of all three classes of pathogens, the justification for which is provided in Appendix 4.

Recognizing, however, that the "highly protective" and, to a lesser extent, "protective" targets are conservative and that achievement of these targets may not be the most cost-effective or achievable option in some situations, an "interim" target has been set. The "interim" target applies to those technologies that achieve "protective" removal targets for two classes of pathogens and have a proven impact on reducing diarrhoeal and waterborne infections. Achievement of this lower-tier target should be seen as an initial step in an effort to incrementally improve towards the ultimate target of "highly protective".

Figure 1. Flowchart for establishing health-based HWT performance targets

Are credible, local water quality data available?

Yes

Step 1 Derivation of targets

Taking into account **LOCAL** data, derive targets for relevant classes of pathogens.

No

Use **DEFAULT** assumptions for source water quality and QMRA calculations (see Appendix 1) to derive default performance targets.

Target	Log_{10} reduction required: **Bacteria**	Log_{10} reduction required: **Viruses**	Log_{10} reduction required: **Protozoa**
Highly protective	≥ **4**	≥ **5**	≥ **4**
Protective	≥ **2**	≥ **3**	≥ **2**
*Interim**	Achieves "protective" target for two classes of pathogens and results in health gains		

Summary of performance requirements for small-scale and household drinking-water treatment, based on reference pathogens *Campylobacter jejuni*, *Cryptosporidium* and rotavirus (see Appendix 1).

Step 2 Laboratory testing

Subject HWT technologies, including those used in combination approaches, to laboratory testing to assess their performance against derived or default log_{10} reduction targets according to protocols presented (Appendix 2) or other scientifically credible testing methods.

Step 3 National verification

Use resulting data in a national-level technology verification programme to protect public health. Product labelling, scope of testing/verification, testing methods, enforcement of standards and other programmatic considerations should be developed at the national level. WHO does not certify technologies (see Box 4 in section 3.3).

** Treatment options classified as "interim" should be recommended only when credible epidemiological evidence indicates that use of such devices results in reductions in waterborne disease.*

Box 2. The tolerable risk concept

The concept of tolerable, allowable or acceptable risk is the basis of the WHO approach to deriving water quality guidelines and encouraging incremental improvement. The "reference level of risk" due to exposure from drinking-water is 10^{-6} DALY per person per year (refer to chapter 3 of the GDWQ). Setting performance targets derived from a less stringent level of acceptable risk, such as 10^{-4} DALY per person per year, from waterborne exposure may be more achievable, yet still consistent with the goals of providing better quality, safer water. In the absence of local data (see section A1.5 in Appendix 1 for more information) and in applying a precautionary approach, the lowest level of performance in a given pathogen class from testing results should be used. For example, technologies resulting in a 5 $\log_{10}$ reduction in bacteria, a 5 $\log_{10}$ reduction in viruses and a 3 $\log_{10}$ reduction in protozoa would fail to meet the "highly protective" target, which requires a 4 $\log_{10}$ reduction level for protozoa and would therefore achieve the intermediate "protective" tier (Figure 1).

3. ESTABLISHING HEALTH-BASED PERFORMANCE TARGETS

3.1 General approach and principles

This document presents recommended microbiological performance targets for HWT technologies and supporting information that may be of use in implementing these at the national level. The following guiding principles have been used in the development of these recommendations:

- *Technologies should be as effective as possible against all classes of microbes*, with the goal of incremental improvements towards meeting the WHO recommended level of risk of 10^{-6} DALY per person per year attributable to drinking-water or relevant national health-based target.

- *Technologies that do not meet the recommended risk-based target of 10^{-6} DALY per person per year may contribute to a substantial reduction in waterborne disease risk*, particularly when the burden of disease is high. Whereas 10^{-6} DALY per person per year is most protective, setting multiple efficacy levels in a tiered approach is intended to stimulate innovation and incremental improvements while recognizing the potential beneficial impact of technologies whose performance is lower than the highest level. Therefore, an intermediate health-based target of 10^{-4} DALY per person per year is proposed.

- *Technologies that are effective against two but not all three classes of pathogens may be recommended for use if supported by epidemiological evidence of positive health impacts.* A bottom tier, "interim", includes technologies that meet two but not all three "protective" performance targets and demonstrate health gains based on epidemiological evidence. For example, free chlorine disinfection is effective against bacteria and viruses, but ineffective against *Cryptosporidium*, an important waterborne protozoan parasite.

- *Consistent and continuous use of HWT technologies is required for improvements in health associated with the consumption of drinking-water.* The goal of HWT is to make the water that users consume consistently safer. This means that technologies or methods must be continuously used by those whose existing water sources are unsafe. Factors related to HWT uptake and consistent, sustained use over the long term are critical for realizing health gains. The range of factors associated with consistent and sustained use and performance of HWT technologies are not within the scope of this document, but may be considered when setting technology verification guidelines at the local or national level (Appendix 3).

3.2 Default health-based microbiological performance targets

Performance targets were computed based on QMRA models. Further information on QMRA can be found in Appendix 4 and the GDWQ (WHO, 2011). The recommended levels of microbiological reduction calculated from QMRA models are presented in Figure 1 (above) and Table 1. Approaches for demonstrating performance are discussed further in Box 3.

Table 1. Performance requirements for HWT technologies and associated $\log_{10}$ reduction criteria for "interim", "protective" and "highly protective"

Reference microbe used in dose–response model	Assumed number of microbes per litre used in risk calculations[a]	Pathogen class	$\log_{10}$ reduction required[b]		
			Interim	Protective[c]	Highly[c] protective
			Requires correct, consistent and continuous use to meet performance levels		
Campylobacter jejuni	1	**Bacteria**	Achieves "protective" target for two classes of pathogens and results in health gains	≥ **2**	≥ **4**
Rotavirus[d]	1	**Viruses**		≥ **3**	≥ **5**
Cryptosporidium	0.1	**Protozoa**		≥ **2**	≥ **4**

[a] Assumptions for background water quality used to derive microbial reduction targets where local data are not available. These are based on an assumed wastewater content in untreated water of 0.01% by volume, using estimated background wastewater concentrations of reference microbes from Volume 2 of the WHO *Guidelines for the safe use of wastewater, excreta and greywater* (WHO, 2006). Local water quality data should be used if available and representative enough to inform QMRA using bacteria, viruses and protozoa. The use of alternative background water quality data will result in different $\log_{10}$ reductions required to achieve the relevant risk-based targets using the QMRA models as described in the GDWQ (WHO, 2011).

[b] Computed as $\log_{10}$ ($C_{\text{untreated water}}$ / $C_{\text{treated water}}$), where C = microbe concentration in water.

[c] Treated water achieving the $\log_{10}$ reduction required to meet the health-based target of 10^{-4} ("protective") and 10^{-6} ("highly protective") DALY per person per year, based on the given assumptions for background water quality and using the QMRA models as described in the GDWQ (WHO, 2011).

[d] The concentration of rotavirus is derived from high-income regions as presented in Table 7.4 of the GDWQ. Refer to the GDWQ for further explanation and validity for application in low-income regions (WHO, 2011).

Box 3. Demonstrating performance

The performance targets are intended to encourage testing of technologies using a standardized approach linking microbiological performance data with the defined health outcome targets. Scientifically credible and methodologically rigorous performance data meeting the standards of peer-reviewed research should be used in establishing performance. Existing international and national testing protocols for bacteria, viruses and protozoa (e.g. those published by the United States Environmental Protection Agency or NSF International/American National Standards Institute) or testing recommendations given in Appendix 2 should be used. Locally adapted targets and methodologies may also be developed and implemented at the national level by other stakeholders. Such certification or product testing programmes may establish data reporting requirements, incorporating peer review, to meet performance targets.

3.3 Testing protocols

The laboratory testing procedures are described in Appendix 2. These cover a range of HWT technologies, allow for substitution of non-pathogenic microbial surrogates

in challenge testing and are intended to be widely accessible and adaptable to local capabilities and conditions. Protocols describe general requirements for laboratory and testing facilities; appropriate procedures for experimental setup and test conditions; production and preparation of reference challenge bacteria, viruses and protozoa (and alternative non-pathogenic organisms as surrogates); and instructions for appropriately adding these microbes ("spiking") to test waters. Both general advice on production of scientifically credible testing data and technology- and microbe-specific details are given where appropriate. In addition, product certification and labelling are highlighted in Box 4 and discussed in greater depth in Appendix 2. The testing protocols are intended to be adaptable to local contexts and conditions while providing a common basis for technology comparison. Further references to inform creation and implementation of testing protocols are provided in Box 5.

Box 4. Product certification and product labelling

Requirements for labelling of products may be locally developed and should be approved by regulatory agencies at the national level. Labelling should supply enough information for consumers to make an informed choice regarding more and less effective technologies. Labels should be able to be easily compared and understandable. Other reportable quantities may also be locally required, such as flow rate or volume per day, expiry date or duration of use if applicable or indicator of expiry, and other supporting information regarding the performance against contaminants not described in this document (i.e. chemicals).

WHO does not endorse, certify or approve specific technologies for drinking-water treatment. Conforming to the recommended performance targets in this document is not grounds for any product labelling alluding to WHO approval, and under no circumstances should the WHO logo or name be used in advertising or labelling of products.

Box 5. Links to other WHO documents

The microbiological performance targets presented here have been derived using the risk-based approach articulated in the GDWQ based on assumed levels of pathogens in untreated water from the *Guidelines for the safe use of wastewater, excreta and greywater*. WHO recommends a water safety plan approach to drinking-water quality where possible. The following documents provide further information. All three can be found online at:

http://www.who.int/water_sanitation_health/publications/en/index.html

- WHO (2011). *Guidelines for drinking-water quality*, 4th ed. Geneva, World Health Organization.
- WHO (2006). *Guidelines for the safe use of wastewater, excreta and greywater.* Geneva, World Health Organization.
- WHO (2009). *Water safety plan manual: step-by-step risk management for drinking water suppliers.* Geneva, World Health Organization.

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APPENDIX 1. DERIVATION OF MICROBIOLOGICAL PERFORMANCE TARGETS

A1.1 Quantitative microbial risk assessment (QMRA)

QMRA draws upon advances in chemical risk assessment, dose–response relationships for microbial pathogens and the epidemiology of infectious disease to predict health risks associated with exposure to microbial pathogens. This appendix provides general background information on the methods used to identify and quantify health risks resulting from exposure to pathogens in drinking-water and the development and identification of health-based targets for water treatment technologies. It is important to note that QMRA is an evolving methodology that relies on assumptions in the absence of relevant data, such as background levels of pathogens in untreated water. The dose–response data used to derive the constants applied in the QMRA calculations are based on highly controlled studies in which healthy subjects were given a known concentration of a specific pathogen and the subsequent effects on health were carefully assessed.

A1.2 Reference pathogens

The bacterium *Campylobacter jejuni*, rotavirus and the protozoan parasite *Cryptosporidium* are key waterborne reference pathogens cited in the GDWQ (WHO, 2011) and are used here to derive performance targets. Reference target pathogens were chosen to represent classes of pathogens in water (bacteria, viruses, protozoa) with respect to occurrence, concentration and health impact. These are microbes that are widely present in human populations and in faecally contaminated water worldwide and whose dose–response relationships and occurrence in water are relatively well characterized, thus enabling their use as targets for estimating health risks associated with the presence of bacteria, viruses and protozoa in water.

These microbes can be used in QMRA models and analyses to estimate the potential health effects resulting from ingestion of a certain number of these microbes of each class in water over time. HWT technology challenge testing with these or with surrogate microbes can be used to calculate $\log_{10}$ reduction values for use in obtaining waterborne exposures for the risk assessment model. More information on each reference pathogen is available in the microbial fact sheets found in chapter 11 of the GDWQ (WHO, 2011).

A1.2.1 *Campylobacter jejuni*

Campylobacter spp. are among the most common bacterial etiological agents of acute gastroenteritis worldwide (Acheson & Allos, 2001). They are shed in high concentrations in faeces by infected humans and animals, are infectious for humans in relatively low numbers and sometimes cause a severe neurological disorder after enteric infection (Guillain-Barré syndrome). In many parts of the world, *Campylobacter* spp. are highly prevalent in livestock animals, including poultry, cattle, swine and sheep. *Campylobacter jejuni* is the most commonly isolated species of *Campylobacter* associated with infection in humans, and waterborne transmission has been documented.

A1.2.2 Rotavirus

Rotaviruses are considered to be among the most common causes of infant gastroenteritis and resulting mortality in the world, causing up to 527 000 deaths (or 29% of all deaths due to diarrhoea) annually (Parashar et al., 2009). They are shed in high concentrations in the faeces of infected persons, and they are infectious for humans at relatively low doses. Also, there are many different human rotaviruses, and repeated infections are possible because of lack of cross-protection among strains or subtypes and only short-lived immunity. Most human rotaviruses are transmitted person to person or in aerosols, but infection via faecally contaminated water is a possible transmission route. Like other viruses, rotaviruses are relatively resistant to or unaffected by some water treatment processes.

A1.2.3 *Cryptosporidium*

Cryptosporidium is a pathogen of concern worldwide. It is an etiological agent of childhood diarrhoeal disease in both Africa and Asia (Levin, 2009) and was the organism responsible for the major outbreak of enteric disease in the United States of America (USA) in 1993 (MacKenzie et al., 1994). In addition, it has also been shown to be one of the main causes of infection and disease in individuals living with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) (Ghimire, Sapkota & Manandhar, 2004; Mor & Tzipori, 2008) in high disease burden settings. *Cryptosporidium* spp. infect a wide range of animals as well as humans. Of the *Cryptosporidium* species infecting humans, *C. hominis* is prevalent. Faecally shed oocysts of *Cryptosporidium* spp. are relatively stable and persistent in the environment, and they are commonly present in faecally contaminated water worldwide. Oocysts of *Cryptosporidium* are also relatively resistant to chemical disinfectants such as chlorine, are infectious at relatively low doses and cause serious and persistent infection in immunocompromised individuals. *Cryptosporidium parvum* and *C. hominis* are among the most important of waterborne pathogens (Steiner et al., 1997; Chappell et al., 2006; Tzipori & Widmer, 2008).

A1.3 Estimating default pathogen concentrations

Microbiological performance targets are based on the reduction of microbe levels in drinking-water to an acceptable risk level and therefore are based on the assumed background concentration of particular pathogens (by class) in water.

A conservative estimate of potential pathogen concentrations in untreated water may be calculated based on estimates of pathogen concentrations in wastewater, for which pathogen occurrence and distribution have been relatively better characterized compared with environmental water sources. This is partly based on the fact that pathogens are relatively more difficult to detect, quantify and identify in typical drinking-water sources, where they may exist in dilute concentrations. Specific pathogens vary spatially and temporally in human and animal populations. In a given population and geographic area, some pathogens may rarely, if ever, be present, and others may be present all of the time, but at variable levels, based on the proportion of the population infected. Tables A1.1 and A1.2 provide estimates of concentrations of the reference pathogens in faeces and in raw domestic or municipal sewage.

Table A1.1. Estimates of the occurrence of reference pathogens in wastewater

Reference pathogen	Number per gram of faeces[a]	Total number excreted daily per infected person[b]	% of population shedding[c]	Estimated number per litre of wastewater[d]	Other reported values in wastewater (numbers per litre)	References
Campylobacter jejuni	1×10^6	1×10^8	10	10 000	32 000–500 000	Stelzer (1988); Jones, Betaieb & Telford (1990); Stampi et al. (1992); Koenraad et al. (1994)
Rotavirus	1×10^9	1×10^{11}	1–10	100–100 000[e]	1000–90 700	Gerba et al. (1996); AWWA (1999)
Cryptosporidium	1×10^7	1×10^9	1	1000	Up to 10 000	Feachem et al. (1983); Metcalf & Eddy, Inc. (2003); Bitton (2005)

[a] As reported in the literature or estimated given the best available data.
[b] Using Feachem et al.'s (1983) assumptions that persons over 15 years of age excrete 150 g of faeces daily, that persons under 15 years of age excrete 75 g of faeces per day and that two thirds of the infected people are under 15 years of age; this yields a mean faecal weight of 100 g per infected person per day.
[c] As estimated by Feachem et al. (1983) in their hypothetical "tropical community of 50,000 in a developing country". This would not represent an outbreak situation, where a much higher proportion of the population would be shedding the microbe.
[d] Using the following assumptions: 100 litres of sewage per person per day, 90% inactivation of microbes within a short time.
[e] Limited data available. Reported arithmetic mean concentrations in raw sewage vary from less than 100 to 90 700 (Gerba et al., 1996). The risk assessment model assumes 1000 rotaviruses per litre of wastewater.

Table A1.2. Example occurrence of selected indicators and pathogens in faeces, wastewater and raw water[a]

Microbe	Number per gram of faeces	Number per litre of untreated wastewater	Number per litre of raw water
Faecal coliforms (*Escherichia coli* and *Klebsiella*)	10^7 (mostly non-pathogenic)	10^6–10^{10}	100–100 000
Campylobacter spp.	10^6	100–10^6	100–10 000
Vibrio cholerae[b]	10^6	100–10^6	100–10^8
Enteroviruses	10^6	1–1000	0.01–10
Rotaviruses	10^9	50–5000	0.01–100
Cryptosporidium	10^7	1–10 000	0–1000
Giardia intestinalis	10^7	1–10 000	0–1000

[a] Local data will vary.
[b] *Vibrio* can grow in the aquatic environment.
Source: Extracted from WHO (2011), which cites the following sources of information: Feachem et al. (1983); Stelzer (1988); Jones, Betaieb & Telford (1990); Stampi et al. (1992); Koenraad et al. (1994); Gerba et al. (1996); AWWA (1999); Maier, Pepper & Gerba (2000); Metcalf & Eddy, Inc. (2003); Bitton (2005); Lodder & de Roda Husman (2005); Schijven & de Roda Husman (2006); Masini et al. (2007); Rutjes et al. (2009); Lodder et al. (2010)

The numeric values of estimated pathogen concentrations in these tables should be taken as indicative and not exact. Many factors contribute to the variability of concentrations of both faecal indicator bacteria and pathogens in faeces, wastewater and environmental waters. Values can vary depending on per capita daily water use, diet and other factors influencing per capita faecal excretion and seasonal factors, such as wet or dry weather, that can influence the extent of disease (and shedding) and concentration of faecal matter in wastewater.

Using Table A1.1 and the assumption that untreated, uncharacterized water is 0.01% wastewater, background levels of reference pathogens have been estimated in order to calculate $\log_{10}$ reductions.

Table A1.3 presents the calculation of required $\log_{10}$ reductions to meet the WHO reference level of risk, 10^{-6} DALY per person per year ("highly protective"). Table A1.4 provides the calculation of the $\log_{10}$ reduction requirements consistent with "protective" performance, which is based on the reference level of risk of 10^{-4} DALY per person per year.

Table A1.3. Example calculation of required $\log_{10}$ reduction of microbes by treatment technology to achieve the "highly protective" WHO reference risk level of 1×10^{-6} DALY per person per year

	Units	*Cryptosporidium*	*Campylobacter jejuni*	Rotavirus
Raw water quality (C_R), assumed	Organisms per litre	**0.1**	**1**	**1**
Treatment efficacy required to reach tolerable risk (PT)	$\log_{10}$ reduction required	**3.88**	**3.98**	**4.96**
Drinking-water quality (C_D)	Organisms per litre	1.32×10^{-5}	1.05×10^{-4}	1.10×10^{-5}
Consumption of drinking-water (V)	Litres per person per day	1	1	1
Exposure by drinking-water (E)	Organisms per day ingested	1.34×10^{-5}	1.04×10^{-4}	1.10×10^{-5}
Dose–response (r)	Probability of infection per organism	0.20	0.019	0.59
Risk of infection ($P_{inf,d}$)	Per day	2.67×10^{-6}	1.99×10^{-6}	6.53×10^{-6}
Risk of infection ($P_{inf,y}$)	Per year	9.74×10^{-4}	7.25×10^{-4}	2.38×10^{-3}
Risk of diarrhoeal illness given infection ($P_{ill\|inf}$)		0.7	0.3	0.5
Risk of diarrhoeal illness (P_{ill})	Per year	6.82×10^{-4}	2.18×10^{-4}	1.19×10^{-3}
Disease burden (db)	DALYs per case	1.47×10^{-3}	4.60×10^{-3}	1.40×10^{-2}
Susceptible fraction (f_s)	Percentage of population	100%	100%	6%
Disease burden (DB)	DALYs per year	1×10^{-6}	1×10^{-6}	1×10^{-6}
Formulae	$C_D = C_R \div 10^{PT}$ $E = C_D \times V$ $P_{inf,d} = E \times r$		$P_{ill} = P_{inf,y} \times P_{ill\|inf}$ $DB = P_{ill} \times db \times f_s \div 100$	

Source: Adapted from WHO (2011). The format and calculations contained in this table follow the same approach as described in the GDWQ.

Table A1.4. Example calculation of required log_{10} reduction of microbes by treatment technology to achieve the "protective" WHO reference risk level of 1×10^{-4} DALY per person per year

	Units	*Cryptosporidium*	*Campylobacter jejuni*	Rotavirus
Raw water quality (C_R), assumed	Organisms per litre	**0.1**	**1**	**1**
Treatment efficacy required to reach tolerable risk (PT)	Log_{10} reduction required	**1.85**	**1.97**	**2.90**
Drinking-water quality (C_D)	Organisms per litre	1.40×10^{-3}	1.08×10^{-2}	1.26×10^{-3}
Consumption of drinking-water (V)	Litres per day	1	1	1
Exposure by drinking-water (E)	Organisms per day ingested	1.40×10^{-3}	1.08×10^{-2}	1.26×10^{-3}
Dose–response (r)	Probability of infection per organism	0.20	0.019	0.59
Risk of infection ($P_{inf,d}$)	Per day	2.80×10^{-4}	2.07×10^{-4}	7.49×10^{-4}
Risk of infection ($P_{inf,y}$)	Per year	0.097	0.073	0.24
Risk of diarrhoeal illness given infection ($P_{ill\|inf}$)		0.7	0.3	0.5
Risk of diarrhoeal illness (P_{ill})	Per year	0.068	0.022	0.12
Disease burden (db)	DALYs per case	1.47×10^{-3}	4.60×10^{-3}	1.40×10^{-2}
Susceptible fraction (f_s)	Percentage of population	100%	100%	6%
Disease burden (DB)	DALYs per year	1×10^{-4}	1×10^{-4}	1×10^{-4}
Formulae	$C_D = C_R \div 10^{PT}$ $E = C_D \times V$ $P_{inf,d} = E \times r$		$P_{ill} = P_{inf,y} \times P_{ill\|inf}$ $DB = P_{ill} \times db \times f_s \div 100$	

Source: Adapted from WHO (2011). The format and calculations contained in this table follow the same approach as described in the GDWQ.

A1.4 Interim performance targets

The "interim" target is directed towards countries with a high disease burden where drinking-water quality is poor and incremental improvements in pathogen reduction would be expected to result in significant health gains. Historically, such gains have been demonstrated through modest improvements in drinking-water quality as indicated by reductions of bacterial indicator species in the range of 90–99% (Frankland, 1885; Hazen, 1900; Baker, 1948). For example, field epidemiological trials indicate that HWT technologies that meet the "interim" performance target may be associated with measurable reductions of diarrhoeal diseases in users compared with non-users

(Brown, Sobsey & Loomis, 2008). Furthermore, in certain cases, such as free chlorine disinfection (which is not effective against *Cryptosporidium*), the existing evidence based on numerous field trials indicates a protective effect against diarrhoeal disease (Arnold & Colford, 2007). Among all technologies, observed reductions of diarrhoeal disease risk in shorter-term field epidemiological HWT intervention studies are often in the range of 15–50% (Clasen et al., 2007; Waddington et al., 2009), with the lower end representing situations where studies incorporated more rigorous methods to limit bias or the existing drinking-water was of fairly good quality. This suggests that these technologies may reduce disease burdens and serve as a temporary disease prevention measure before more efficacious household or community water treatment technologies can be put in place.

Technologies in the "interim" target may underperform for one class of pathogens, but must still provide reductions that meet the health-based target of at least 10^{-4} DALY for two of the three classes of pathogens. In addition, to achieve the "interim" level, technologies must demonstrate through epidemiological evidence significant diarrhoeal disease reductions. Criteria for what constitutes credible epidemiological evidence are outside the scope of this document. It is important to note, however, from a global normative perspective (i.e. which needs to account for varying local conditions), that efficacy against all three pathogen classes is preferred. The multi-barrier approach, discussed in further detail in Appendix 2, should be considered to address underperformance for one pathogen class.

A1.5 Using local data to calculate microbiological performance targets

Local data may be used to establish $\log_{10}$ reduction criteria for meeting health-based targets by employing the framework articulated in chapter 7 of the GDWQ. If such data are to be used in setting risk-based performance criteria for localized areas, data should capture seasonal and geographical variability with respect to bacteria, viruses and protozoa, ideally for reference microbes (*Campylobacter jejuni*, rotavirus and *Cryptosporidium*). Where data are absent or are insufficiently detailed, default levels specified in this document may be used (see Table A1.1 above). Data on specific target pathogens should be used, if possible, when the HWT intervention is intended to prevent exposure to certain individual pathogens rather than all pathogens that may be present (e.g. cholera outbreaks). Background data should be sufficient to reliably estimate the mean or median microbial levels as well as the magnitude of variability of microbial levels (expressed as standard deviations and 95% confidence intervals) and also extreme values. These data should include sampling during periods of greater vulnerability to contamination and likely higher risk (e.g. wet weather, impacts from known faecal contamination sources such as periodic sewage discharges and other site-specific factors that increase faecal contamination). National certification or technology testing programmes may set local requirements based on QMRA principles articulated here and local microbial data. National certification programmes may also choose to set background assumptions for water quality (microbial counts for the three classes of pathogens in untreated water) that are based on representative monitoring data from local, regional or national surveys.

APPENDIX 2. TECHNOLOGY-SPECIFIC TESTING PROTOCOLS FOR EVALUATION OF HOUSEHOLD WATER TREATMENT PERFORMANCE

A2.1 Foundation for development and implementation of technology-specific testing

Before embarking on laboratory testing, clear technology evaluation programmes must exist, accompanied by a regulatory and enforcement framework that is adapted to the local institutional landscape. Rigorous protocols and standard metrics are needed, which can be effectively communicated to all stakeholders.

Whereas the technology performance risk-based targets are standard measures that may be adopted internationally, testing programmes and specific requirements for individual technologies may vary according to local needs and local resources. This document is intended to provide a basis for the creation of such guidelines and protocols.

Verification programmes need to be placed within a carefully considered institutional structure. National verification programmes may need to consider:

- acceptability and applicability of existing microbiological performance or epidemiological data to local approval of technologies for use;
- scope and content of the testing protocols;
- approval of technology-specific testing protocols;
- regulatory authority;
- enforcement of standards and labelling;
- labelling of products;
- whether the programme is to be voluntary or compulsory;
- reporting rules and data quality control;
- certification of testing laboratories and criteria for independent verification;
- recertification of products;
- publishing and disseminating results to maintain transparency;
- costs of testing and responsible party for paying costs;
- reciprocity rules recognizing other testing programmes.

While these and other factors are outside the scope of this document, they are critical to implementing a successful national HWT technology performance evaluation, also referred to in some countries as a technology verification programme. The experience of the Bangladesh Environmental Technology Verification – Support to Arsenic Mitigation (BETV-SAM) Project (http://www.betv-sam.org/) has suggested that a national water treatment technology performance evaluation and verification programme can be developed through the cooperation of sector-wide stakeholders with support from the private sector. Although the programme is not without limitations, including the costs and ongoing challenge of facilitating the update of verifications after improvements are made to devices, it may provide "lessons learnt" to other environmental technology verification programmes at the national level.

A2.2 Guiding principles and factors to consider when developing HWT testing protocols

The general approach for developing and implementing technology-specific testing can be summarized as identification of candidate technologies, laboratory testing against contaminants of interest and other testing and considerations. These are described in further detail below:

1) *Identification of candidate technologies*. A wide range of HWT devices and methods are now available throughout the world. These technologies vary by cost, availability, effectiveness and many other factors. Selection of candidate technologies for corporate, government, trade group, nongovernmental organization or multilateral verification programmes may be based on local or international evidence, availability of technologies locally or individual technologies of interest. Preliminary screening of technologies using simple, locally relevant metrics may be helpful in determining which ones to subject to further testing.

2) *Laboratory testing to determine efficacy against contaminants of interest*. Because HWT is specifically intended to reduce pathogens in water, microbiological efficacy testing is essential to protect end users of technologies. Recommended levels of pathogen removal and a generalized approach to technology verification are described here. Protocols for laboratory testing, data quality control and reporting should be developed locally and may be technology specific.

3) *Other testing as necessary, relevant to national conditions*. Some of the additional considerations that may be of use in technology verification locally are described in Appendix 3. Because the utility of HWT in protecting public health depends on coverage, continued and correct use, and effectiveness under a range of conditions over the long term, other factors that may be relevant to HWT sustainability may be included in local technology verification programmes. Metrics and protocols should be locally relevant and developed as appropriate by stakeholders.

In considering the specific task of developing HWT testing protocols, several important principles should be considered. These are highlighted below:

- *Protocols should result in data that demonstrate the effectiveness of HWT technologies against bacteria, viruses and protozoa*. HWT technologies ideally should demonstrate efficacy in reducing all classes of waterborne microbial pathogens. If only two classes of pathogens are removed effectively, the technology must demonstrate health benefits in order to achieve the "interim" target. For further explanation, refer to section 2.3 of the main text.

- *Where possible, existing testing protocols should be used or adapted*. A number of protocols for microbiological testing of water treatment technologies exist and may be locally applicable. Protocols published by the United States Environmental Protection Agency (USEPA) and NSF International (NSF)/American National

Standards Institute (ANSI) (USEPA, 1987; NSF, 2003) are methodologically rigorous and, if properly applied, can result in scientifically credible microbiological performance data.

- *Different technologies may require different approaches to demonstrating performance*. The wide range of HWT options now in use may limit the utility of a single standardized protocol.

- *Protocols should be rigorous but flexible*. Options should exist to enable protocols to be adapted to new technologies, alternative test microbes and different contexts, as long as scientifically credible evidence is the result.

- *Laboratory testing should closely model actual field use*. Performance testing under simulated field use conditions may yield data that more closely estimate long-term effectiveness in actual household use.

- *Protocols may be locally developed or adapted*. Laboratory and human capacities vary. Protocols are not useful if they are not possible given local resources and constraints. Suggested protocols in this document are sensitive to this and can be accomplished with basic microbiological laboratory capacity. Locally developed protocols to demonstrate performance may be more suited to local conditions. Standard operating procedures for laboratory work and reporting should be developed locally.

- *Protocols should be accompanied by an appropriate institutional framework*. Technology performance testing data need to be locally interpreted and acted upon so that users can benefit from the information generated in testing programmes. The process of designing and implementing technology verification programmes is complex and benefits from the input of a wide range of stakeholders.

Testing procedures to characterize or verify technology performance need to include a range of key operational parameters that are known to influence microbial reduction efficiency. Such testing should present a reasonable challenge to the effectiveness of the technology against microbial pathogens in water. Some of the key known parameters affecting HWT technology performance are given in Table A2.1. These factors should be considered in developing or implementing technology-specific testing protocols.

A2.3 Choice of candidate technologies

Reviews of HWT and safe storage technologies have advanced the current knowledge about practical aspects of these interventions and their applications (Sobsey, 2002; IRC, 2005; Hygiene Improvement Project, 2006; Lantagne, Quick & Mintz, 2006). Physical methods for small-scale water treatment include boiling, heating (using fuel and solar), filtering, settling and ultraviolet (UV) radiation (solar or UV lamps). Chemical methods include coagulation–flocculation and precipitation, ion exchange, chemical disinfection with germicidal agents (primarily chlorine) and adsorption. Combinations of these methods simultaneously or sequentially (e.g. coagulation combined with disinfection) often yield more effective results as "multi-barrier" technologies (Souter et al., 2003). Other combinations

Table A2.1. Technology-specific parameters, variables or conditions that may affect performance

Technology	Testing parameters, variables or conditions potentially affecting performance
Chemical disinfection	Concentration and type of disinfectant, type of treatment reactor, reaction (contact) time, pH, temperature, dissolved solids (organic and inorganic) and suspended constituents (e.g. turbidity or suspended particles) that can interfere with microbial inactivation by disinfectant consumption or physical protection of the target microbes
Membrane, porous ceramic or composite filters	Turbidity or suspended matter, dissolved solids (organic or inorganic), temperature, pH, contact time or flow rate, filter surface chemistry, filter media pore size distribution, filter geometry; operational parameters include flow rate, flux, intermittent or continuous flow, length of filter run, factors influencing fouling or clogging, filter media cleaning procedures and cycles, and vulnerability to bypassing filter medium (faulty filter element seals and other failures of filter element integrity)
Granular media filters	Turbidity, temperature, pH, contact time, filter surface chemistry, dissolved and colloidal constituents, filter bed geometry, hydraulic residence time and flow profile (e.g. extent of plug flow or short-circuiting), and extent of biological activity on filter media particles or on filter bed surface; operational parameters include flow rate, flux, intermittent or continuous flow, length of filter run, filter media cleaning procedures and cycles
Solar disinfection	Incident solar radiation, aids to solar energy capture (e.g. solar reflectors), temperature, time, dissolved oxygen in water, turbidity or suspended matter; UV-absorbing dissolved constituents in water and UV penetrability of container walls, soluble constituents subject to sunlight-induced chemical changes that modulate antimicrobial activity (e.g. photo-Fenton reactions) and metallic oxide or other particulate additives or coatings intended to increase disinfection efficiency
UV light (lamp/ light-emitting diode) technologies	Intensity of incident radiation (mW/cm^2) and delivered UV fluence or dose ($mW{\cdot}s/cm^2$), UV wavelengths in the germicidal range, exposure time, dissolved oxygen, turbidity or suspended matter (measured as transmittance or absorbance), dissolved constituents or solutes (that absorb UV energy or alter its reactivity with target microbes)
Thermal technologies	Temperature, exposure time, dissolved or suspended constituents that protect or physically stabilize or chemically protect microbes (e.g. clays and proteins)
Coagulation, precipitation and/or sedimentation	Type (chemical properties) of coagulant or precipitant, chemical dose, contact time, pH, mixing (conditions for coagulation–flocculation or precipitation), settling conditions for sedimentation (static; no mixing), turbidity or suspended matter, dissolved solutes (organic and inorganic), particle sizes and vessel geometry
Combination (multi-barrier) approaches or other emerging technologies	Combinations of the above variables and conditions, depending on which chemical and physical treatment methods are used together or in series

UV, ultraviolet

or multiple barriers are media filtration followed by chemical disinfection, media filtration followed by membrane filtration or composite filtration combined with chemical disinfection. The above-mentioned reviews as well as other reviews of technologies have suggested that the success of interventions is highly context specific, with no one technology or method representing a universal best solution (Clasen et al., 2007). The availability of materials, the quality of raw water available, cultural factors and user preferences or cost may determine which technology is most suited to HWT applications in resource-limited settings, such as technologically less developed countries.

A2.4 Experimental setup and test conditions

Microbiological testing protocols described below are intended to be adaptable to local contexts while providing a common basis for technology performance evaluation in the absence of locally developed technology testing or verification programmes. The scope of these protocols is restricted to microbiological performance and provides general guidance and recommendations for scientifically credible technology-specific testing of HWT. These protocols represent one, but not the only, method for demonstrating microbiological performance.

To the extent possible, experimental setups for these HWT technologies should model actual use conditions for the target context. For example, media or membrane filters should be tested over time, using intermittent flow, for the typical length of filter runs or use cycles, including periodic cleaning, and with water qualities representative of or worse than those of the water to be treated (i.e. "worst case" water, such as that given as "test water 2" in Table A2.2). Solar or chemical disinfection should be tested as batch processes, if this is how they are to be used in practice, and with water that has a physicochemical quality similar to or worse than that of the water to be treated, providing conditions that will yield a conservative estimate of the technology's performance in the field. Testing over the expected or claimed duration of use of the treatment technology, such as the total volume of water treatable before replacement of a functional component (e.g. a filter element, disinfectant delivery module or UV lamp), should be done to document the ability to achieve effective performance over this duration of use.

Table A2.2. Two recommended challenge waters for use in laboratory verification of all technologies, intended to model a range of possible untreated water sources

	Test water 1	Test water 2
Description	High-quality groundwater, surface water, caught (newly harvested) rainwater or other water free of disinfectant residual	High-quality groundwater, surface water, rainwater or other water free of disinfectant residual with 20% by volume primary wastewater effluent or 1% by volume untreated raw sewage, sterilized or pasteurized
Turbidity	< 5 NTU	> 30 NTU
pH	7.0–9.0	6.0–10.0
Temperature	20 °C ± 5 °C	4 °C ± 1 °C

NTU, nephelometric turbidity unit

Specific parameters for use in technology performance evaluation and validation studies are given below as general guidelines. The actual experimental setup for each candidate technology, however, should, to the extent possible, *exactly reflect or model* the manufacturer's or implementer's recommendations for daily household use. This may encompass test water volumes treated, processing times (e.g. contact or exposure times or flow rates), temperature or other relevant physical conditions, and representativeness of raw water quality (e.g. maximum turbidity and pH range). Where testing is to be done over time (e.g. with granular media, porous or membrane filters), at least 20 litres per day is indicated as the appropriate minimum volume to be used in laboratory verification. For those technologies that treat volumes smaller than 20 litres, manufacturers' recommendations should be followed. However, daily water produced should still be 20 litres per day during testing, as an estimated minimum drinking-water volume for a household for one day. This has implications for batch treatment systems in which production of a total of 20 litres per day may actually require treatment of several batches of the smaller water volumes specified by manufacturers.

Separate individual treatment units should be used for effectiveness testing against each separate microbe (e.g. *Escherichia coli*, bacteriophages, *Clostridium perfringens* spores) to prevent any interaction between these microbes that could potentially influence the validity of the treatment performance and test microbe assays. For example, *E. coli* B could be infected by indigenous bacteriophages present in the challenge water, resulting in unanticipated increased bacteriophage production in the test water and decreased numbers of *E. coli* B bacteria due to lysis of the *E. coli* cells by the bacteriophages. If the simultaneous use of multiple test microbes is considered, preliminary experiments should be done in the test water to be used to show that the concentrations of the added test microbes in the challenge test water do not change appreciably for a time period corresponding to the treatment time.

A2.4.1 Volume and physicochemical characteristics of water to be tested

For each water quality condition, at least 20 litres of each challenge water (Table A2.2) spiked with specified amounts of test microbes per batch or run or per day should be tested. Technology challenge tests should be performed over appropriate and representative time intervals specified or recommended for using media filters, UV technologies or membrane/porous filters. Suggested non-microbial challenge water quality parameters (pH, turbidity and temperature) are given in Table A2.2. However, the tested water quality parameters should also be representative of those in the waters where the technology is to be used, and local adaptation of these waters may be required. For example, if technologies are being considered for use in tropical climates, challenge studies for test water 2 can be done at a temperature of 20 °C or the lowest water temperature expected in the location of proposed use. If technologies are intended to treat water volumes less than 20 litres (e.g. 1 litre or 10 litres) per batch or per run, then multiple challenge tests with these specified smaller water volumes are recommended to document the ability to treat a total of 20 litres of water per day.

A2.4.2 Recommended materials for adjusting test water characteristics

The following materials are recommended for adjusting the turbidity and pH of the test water:

Turbidity

- AC Fine test dust, manufactured by AC Spark Plug Division of General Motors, which is commonly used in the USA and is specified for the addition of turbidity in the protocol by the USEPA (1987). This material may be difficult to obtain in countries outside North America.
- Finely ground dry clay. This could be any clay representative of the type of clay found in soils and therefore in waters where the technologies will be used.
- Naturally occurring turbidity in test water. If local waters where the technology is to be used are available, these waters should be tested with their naturally occurring turbidity. Waters with different levels of turbidity can be obtained by collecting samples at different times when differing conditions (e.g. rainfall events) have created different turbidity levels. Water collected at different times and having different turbidities can be blended to create test waters with specific target turbidities. If necessary, the turbidity of these collected waters can be adjusted to target levels. The turbidity of test waters can be reduced by settling (plain sedimentation) and collecting (decanting) the resulting supernatant water, which has reduced turbidity. The turbidity of test waters can be increased by settling, removing (decanting) the resulting supernatant and retaining the resulting bottom water, which has increased turbidity.

pH

- Inorganic acids or bases (e.g. hydrochloric acid, sodium hydroxide).

Consideration must be given to the order of adjusting turbidity and pH when using certain clays. This is because the clay as turbidity material could change the pH, and changing the pH could also cause previously added turbidity material to precipitate or coagulate.

A2.5 Technology-specific testing protocols

The following sections describe common HWT technologies and provide recommended technology-specific testing procedures. These may be adapted for use as needed, consistent with the guiding principles for performance testing articulated previously. These recommendations are not intended to be comprehensive but are a guide to developing detailed testing protocols that will, if executed competently, result in scientifically credible testing data. We recommend reviewing existing studies from the peer-reviewed scientific literature for additional information to aid in designing and conducting appropriate studies to establish the microbiological performance of specific HWT technologies.

A2.5.1 Chemical disinfection

Chemical disinfection of drinking-water includes any chlorine- or iodine-based technologies, including chlorine dioxide, as well as bromine, ozone, other oxidants, strong acids and bases, ferrates and some antimicrobial metals (e.g. silver and copper). Chemical disinfection is most widely done with technologies using free chlorine (hypochlorous acid) and, to lesser

extents, dicyanurates and tricyanurates of free chlorine, chloramines, chlorine dioxide or other forms of chlorine oxidants. The chlorine, bromine and iodine technologies as well as ozone and other oxidants all share similar mechanistic features. Disinfection with metals has been done with soluble, colloidal and larger solid (metallic) forms added to water. Disinfection of household drinking-water in developing countries is done primarily with free chlorine, however. This is because it is quite effective, widely available, easy to dose properly in principle and inexpensive. Disinfection of drinking-water with iodine, which is also a strong oxidant, is generally not recommended for extended use. This is because there are concerns about its adverse biological (toxic) effects on certain metabolic functions and particularly effects on the thyroid gland. Furthermore, elemental iodine is difficult to prepare, handle and deliver to water as a solution. Iodine can be used in emergency or other short-term interventions where other options are not indicated. Iodine can be delivered to water through several means, including aqueous solutions, tablets or iodinated synthetic polymer resins that slowly release active iodine. Tetraglycine hydroperiodide tablets that liberate free iodine in water have been widely used in field military and recreational settings. Ozone is not recommended for household water treatment. This is because it is difficult and expensive to generate at controlled doses in drinking-water and it requires a reliable source of energy to power the ozone generator. Strong acids or bases are not recommended as chemical disinfectants for drinking-water, as they are hazardous chemicals that can alter the pH of the water to dangerously low or high levels. However, as an emergency or short-term intervention, the juices of some citrus fruits, such as limes and lemons, can be added to water to inactivate *Vibrio cholerae* bacteria, if enough is added to sufficiently lower the pH of the water (probably to pH less than 4.5) (Sobsey, 2002). The advisability of using silver and copper as drinking-water disinfectants remains uncertain due to the lack of evidence of efficacy supported by performance technology evaluations as proposed in this document.

Chlorination is the chemical treatment method that had such a dramatic effect on public health in the more technologically developed countries over the last century. It has proven successful in HWT applications as well, although not in all cases. Among the most successful intervention models is the United States Centers for Disease Control and Prevention/WHO Safe Water system developed in the 1990s (Mintz, Reiff & Tauxe, 1995). A systematic review of 21 point-of-use chlorination studies yielded a pooled risk ratio of 0.71 (95% confidence interval = 0.58–0.87) for diarrhoeal disease in children who used the intervention, although the authors noted that the median study length (30 weeks) was short (Arnold & Colford, 2007).

Laboratory verification of chemical disinfectant technologies should follow the manufacturer's or implementer's recommendations for daily HWT use in terms of dose and contact time as well as quality and quantity of the water to be treated. Important considerations are how the chemical is dosed into the water, mixed and allowed to react over time. For some well-established chemical disinfectants, such as free chlorine (hypochlorous acid/hypochlorite), the parameters for dosing and contact time are well established. However, adaptations to household use may require some departures from the use practices typically employed in community water supply systems. Typically, hypochlorous acid for HWT use is added to water as a concentrated solution (typically as 0.5–6% hypochlorous acid) or as a tablet to provide a dose of about 3 mg/l (range 1–5 mg/l). As with its use in community water supplies, it is desirable to maintain a measurable free chlorine residual in the water throughout the period of water use. In addition to determining chemical disinfectant dose, contact time and maintenance of a

residual, testing procedures also need to specify or at least measure temperature and other key water quality parameters that can influence disinfection efficacy, such as pH, turbidity and chlorine-demanding solutes such as dissolved organic matter and ammonia. At a minimum, test waters 1 and 2 (Table A2.2) should be used to establish effectiveness over a range of water quality characteristics. In testing microbial inactivation efficacy, it is necessary to measure the initial concentration of the target microbes in the water as well as the microbe concentration remaining after one or more periods of exposure (contact times). Further details are given below. When samples of chemically disinfected water are taken for microbe analysis after an exposure period (a specified contact time), the disinfectant chemical in the water should be immediately chemically neutralized (i.e. converted to a form lacking antimicrobial activity) to end further activity against the microbes in the test water sample. In the case of free chlorine, for example, such chemical neutralization is done by adding a reducing agent such as sodium thiosulfate or sodium bisulfite to the test water sample following exposure.

A2.5.1.1 Experimental time period and sampling schedule

Batches (specified volumes) of challenge waters of defined quality are spiked (experimentally contaminated with target microbes) and then treated or processed by the chemical disinfection technology according to the instructions given for household use. Relevant parameters to be controlled and monitored include water quality, chemical dose, mixing, contact time, temperature and storage conditions of the disinfected water. Samples of untreated (raw challenge) water and treated water are taken for analysis according to microbiological methods outlined below. As noted above, it is essential to chemically neutralize chlorine in samples to be analysed for microbes at the time they are taken to stop further microbiocidal action beyond the sampling time. Failure to do this allows chlorine or other chemical disinfectants to continue to exert antimicrobial activity beyond the sampling time, resulting in an overestimation of the extent of microbial inactivation. A minimum of three batch processes for each microbial challenge is recommended.

A2.5.1.2 Special considerations

The choice of target microbes is an important consideration in technology verification studies for chemical disinfection of household water by chlorine or other oxidants. It is preferable to do such studies with the microbes that are known to be present in the source water and pose the highest waterborne disease burden. If the important waterborne pathogens are not known or studies with the known, relevant pathogens are not possible, it is recommended that test challenge waters be spiked with sufficient concentrations of indicator bacteria, viruses and protozoan parasite surrogates to follow the extent and possibly the kinetics of inactivation over time. Recommended indicator bacteria, viruses and surrogates for protozoan parasites are, respectively, *Escherichia coli*, bacteriophages of *E. coli* (coliphages) and spores of either *Clostridium perfringens* or *Bacillus* spp. to document log reductions by chlorine or other chemical disinfection treatment. Care must be taken to prepare microbial stocks for spiking that do not add excessive chlorine demand to the test water. Microbial stocks may need to be purified to reduce their chlorine (or other test disinfectant) demand prior to use in spiking studies. Preliminary studies may be needed to ensure the absence of excessive chlorine demand in spiked test waters. Doing challenge studies with a given test water and specified conditions of temperature, pH and other key variables in triplicate at a minimum and performing microbial assays in triplicate are recommended. It is recommended that a minimum 20-litre volume be subjected to treatment to account for daily water use in the home. However, larger or smaller volumes can be

tested if a pre-made disinfectant dose unit (such as a tablet) is intended for a specified volume other than 20 litres. Multiple test units in parallel may be used if the unit volume to be treated is less than 20 litres in order to quantify the time burden and other uses of resources by household members in treating their water.

Measuring the pH of test waters as well as the concentrations of certain solutes in the test waters is of particular importance, because some chemical disinfectants, such as free chlorine and chlorine dioxide, differ in microbiocidal efficacy at low and high pH. Free chlorine is more effective as a microbiocide as hypochlorous acid, which predominates at low pH (pH 6 or lower), than as hypochlorite ion, which predominates at higher pH (pH 9 or higher). In contrast, chlorine dioxide is more viricidal at high pH than at low pH. Furthermore, solutes that react with free chlorine, such as ammonia and organic compounds, can result in the loss of free chlorine residual and lower microbiocidal activity. Chloramines, which are formed by the reaction of free chlorine with ammonia, are only weakly microbiocidal compared with free chlorine, and chlorinated organic compounds resulting from the reaction of chlorine with natural organic matter are not microbiocidal at all.

A2.5.2 Membrane or structured porous media (ceramic, porous carbon block, etc.) filters

Point-of-use water filtration technologies include cloth or fibre filters, membrane filters, porous ceramic filters, carbon block filters, composite filters or similar technologies. These filters reduce microbes by a combination of physical and chemical (and, in some cases, biological) processes, including physical straining, sedimentation and adsorption. Filtration technologies are finding increasing application in developing countries where chemical disinfection or boiling may not always be practical or effective (Colwell et al., 2003).

Traditional membrane technology is generally expensive and therefore less known for effectiveness when applied to small-scale drinking-water treatment in developing countries. However, reverse osmosis, nanofilters and other membrane technologies are common in developed countries (Hörman et al., 2004), may be used by travellers to developing countries (Backer, 2002) and are now being evaluated and field implemented in developing countries (Boisson et al., 2010). These advanced filters may include composite filters that employ several methods for reduction of microbes in water. Some low-cost applications of these types of filters have been developed and may have an increasing role to play in the future of HWT in developing countries.

Cloth filters, such as those of sari cloth, have been recommended for reducing *Vibrio cholerae* in water when these pathogens are associated with copepods or other eukaryotes in water (Huo et al., 1996; Colwell et al., 2003). These cloths will not significantly retain dispersed bacteria not associated with copepods, other crustaceans, suspended sediment or large eukaryotes because the pores of the cloth fabric (> 20 μm) are not sufficiently small to exclude bacteria. However, where appropriate, these filters can have significant health impacts. Colwell et al. (2003) reported a 48% reduction in cholera associated with use of the filters over a 35-month trial that included 65 villages in rural Bangladesh and approximately 133 000 participants. Cloth filters have also been critical interventions in guinea worm (dracunculiasis) eradication programmes (Olsen, Magnussen & Anemana, 1997; Aikhomu, Brieger & Kale, 2000).

Filtration through porous ceramic material is also used to reduce microbes in water. Ceramic technologies exist in many forms, the most prevalent being the ceramic "candle" filters (Clasen et al., 2004; Clasen, Brown & Collin, 2006) or

the ceramic "pot" filters of the type promoted by the nongovernmental organization *Potters for Peace* (e.g. Brown, Sobsey & Proum, 2007). Filters are generally gravity-driven and are often used in a nested bucket system to safely store treated water. Field trials of commercially produced ceramic "candle" filtration devices have suggested that they do provide an effective barrier against microbial pathogen indicators in water and that interventions are associated with significant health gains in users compared with non-users of the technologies. Studies of locally produced, low-cost ceramic pot filters in Cambodia have suggested that these interventions are also effective. Diarrhoeal reductions associated with filter use were approximately 50% in two field trials in rural Cambodia, with filters providing a mean 99% reduction of *E. coli* in household drinking-water and a mean 90–99% reduction in viruses in laboratory testing.

Filters having a structured porous barrier to retain microbes and other contaminants should be tested according to the implementer's or manufacturer's recommendations for use in the target setting. A flow rate, average volume treated per day (minimum 20 litres) and other operational parameters that closely mimic actual household use conditions should be used. As with chemical disinfection, volumes of test waters of defined quality should be spiked with known concentrations of target microbes and treated by the filtration process, and both the initial (spiked) feed water and the treated filtrate water should be assayed to determine the microbial concentrations and extent of microbial reduction. At a minimum, test waters 1 and 2 (Table A2.2) should be used to establish effectiveness over a range of water quality characteristics.

A2.5.2.1 Experimental time period and sampling schedule

Because the effectiveness of filtration technologies is known to vary over time, a minimum time corresponding to an anticipated use cycle before routine maintenance, cleaning or replacement should be used for verification testing. If manufacturers or implementers do not specify typical filter use cycles between cleaning or other maintenance cycles, a minimum 14-day test period is recommended. Samples of spiked challenge water and filtrate should be taken for microbial assay at least on days 0, 1, 3, 7 and 14 of the challenge test. If the filtration technology has a specified lifetime or if it requires periodic cleaning, challenge testing with spiked water should take place at intervals of 0%, 25%, 50%, 75% and 100% of the life cycle or the cleaning cycle of the filter and should include challenge testing with spiked water into the next cycle of use after cleaning to document continued performance. At each sampling time, other relevant water quality parameters, such as turbidity, should also be measured in challenge water and filtrate, as well as key operational variables, such as flow rate. A minimum of two filtration units should be tested in parallel using the same challenge water and test microbes to document performance reproducibility and detect variability. If the duration of use of the filter between cleaning or use cycles is longer than 14 days, the length of the total test period should be extended according to the manufacturer's or implementer's recommendations, and microbial challenge tests should be performed at intervals corresponding to 0%, 25%, 50%, 75% and 100% of the overall use or life cycle period.

A2.5.2.2 Special considerations

Ceramic and some other structured porous media filters are regularly cleaned during use in the household. In challenge tests, filters should be cleaned according to the implementer's or manufacturer's exact recommendations, including frequency and method of cleaning. However, in evaluating these filter technologies, no disinfectants or other antimicrobial agents should be used on the filters during cleaning. If these disinfectant agents are recommended for regular use with the filter, they should be included in a technology evaluation performance test under the "multi-barrier" category.

A2.5.3 Granular media filters

Granular media filters include filters containing sand, diatomaceous earth or other particulate media in packed beds, layers or surfaces over or through which water is passed. These filters retain microbes by a combination of physical and chemical processes, including physical straining, sedimentation and adsorption. Some may also employ chemically active antimicrobial or bacteriostatic surfaces or other chemical modifications. Other granular media filters are biologically active because they develop layers of microbes and their associated exopolymers on the surface of or within the granular medium matrix. This biologically active layer, called the *schmutzdecke* in conventional slow sand filters, retains microbes and often leads to their inactivation and biodegradation. A household-scale filter with a biologically active surface layer that can be dosed intermittently with water has been developed called the BioSand filter, which is an intermittently operated slow sand filter (Stauber et al., 2006). The BioSand system has been the subject of several studies (Kaiser et al., 2002; Duke et al., 2006; Stauber et al., 2009) suggesting that the filters can be effective in reducing waterborne microbes and improving health among users.

Filters using porous granular media to retain microbes and other contaminants should be tested according to the implementer's or manufacturer's recommendations for use in the target setting. A flow rate, average volume treated per day (minimum 20 litres) and other operational parameters that closely represent actual household use conditions should be used. At a minimum, test waters 1 and 2 (Table A2.2) should be used to establish effectiveness over a range of water quality characteristics.

A2.5.3.1 Experimental time period and sampling schedule

Because the effectiveness of these technologies is known to vary over time, challenge tests should be performed using a minimum time period of operation corresponding to the filter use cycle recommended by the manufacturer or implementer, including the filter cleaning step. If no time period is specified by the manufacturer or implementer, a minimum time period of 14 days should be used for verification testing. Samples of spiked challenge water and filtrate should be taken for microbe testing at least on days 0, 1, 3, 5 and 14, and any cleaning or maintenance operations should be included. If the duration of use of the filter between cleaning or use cycles is longer than 14 days, the length of the total test period should be extended according to the manufacturer's or implementer's recommendations, and microbial challenge tests should be performed at intervals corresponding to 0%, 25%, 50%, 75% and 100% of the overall use or life cycle period. Testing replicate filtration units in parallel using the same challenge water and test microbes to document performance is recommended, with two parallel filters at a minimum.

A2.5.3.2 Special considerations

Most granular media filters require periodic filter backwashing or other cleaning, although the frequency of this may depend on raw water characteristics. Backwashing or cleaning should be conducted during the testing period exactly in accordance with the manufacturer's or implementer's recommendations. It is recommended that technology evaluation challenge tests be performed over at least one cycle of filter use, including a cleaning step, such as backwashing or sand surface harrowing and decanting, and the subsequent period of filter operation until the end of the filter run or use cycle before the next round of filter cleaning or maintenance.

Granular media filters that are biologically active, such as intermittently operated slow sand filters, may "mature" or "ripen" over time, and reductions of some microbes by such treatment may not reach maximum or optimum performance until the filter

has biologically matured or ripened (Stauber et al., 2006). In challenge tests of such filters, the microbial reduction may increase well after a minimum 14-day testing period, because the filter was still maturing and had not reached its maximum state of performance. For such filters, technology performance data should be collected from periodic challenge tests over an extended time interval of use to better represent the microbial reduction capability of the filter in the target setting and where long periods of use (e.g. several months per filter run cycle) are ordinarily indicated or expected.

A2.5.4 Solar disinfection

There are a number of technologies using solar irradiation to disinfect water, and mechanisms for reduction of microbes and technologies have been well studied (e.g. Acra et al., 1980; Acra, Raffoul & Karahagopian, 1984; Joyce et al., 1996; Kehoe et al., 2004; Lonnen et al., 2005; Méndez-Hermida et al., 2005; Berney et al., 2006a,b). Some use solar radiation to inactivate microbes in either dark or opaque containers by relying on heat from sunlight energy. Others, such as the SODIS system developed at the Swiss Federal Agency for Environmental Science and Technology (EAWAG), use clear plastic containers penetrated by UV radiation from sunlight and rely on the combined action of the UV radiation, oxidative activity associated with dissolved oxygen and heat. Other physical forms of solar radiation exposure systems also employ combinations of these solar radiation effects in other types of containers, such as UV-penetrable bags and panels, to improve the microbial quality of water. A number of field trials have been conducted to evaluate the health impacts and field effectiveness of the technology (Conroy et al., 1996, 1999, 2001; Rainey & Harding, 2005).

Solar disinfection technologies should be tested in accordance with the implementer's or manufacturer's recommendations for use in the target setting. Incident solar radiation that controls UV intensity and thermal flux depends on the latitude, altitude, weather, season, exposure orientation and specific design features of the water vessel, and water quality. Therefore, either technology performance evaluation or verification should take place in an area that is representative of the target context and conditions in terms of incident solar radiation (measured in W/m^2) and other relevant conditions, or these conditions may be simulated in the laboratory and field, for example, according to methods recommended by Oates et al. (2003) or other scientifically credible methods.

Only those results obtained under conditions of testing that are representative of those equivalent to the specific setting in which the technology is to be used (e.g. incident radiation, temperature, water turbidity, length of time exposed to sunlight) should be considered valid for expected performance in the field. If key operating and environmental conditions vary seasonally in the regions where the technology is to be used, it is recommended that performance evaluation testing be done to include representative average (central tendency) and boundary (maximum and minimum) conditions potentially influencing performance. At a minimum, test waters 1 and 2 (Table A2.2) should be used to establish effectiveness over a range of water quality characteristics.

A2.5.4.1 Experimental time period and sampling schedule

The recommended volume of each quality of challenge water to be tested is 20 litres, which represents the estimated minimum daily need of a household. Normally, this volume will need to be distributed among individual polyethylene terephthalate bottles if using SODIS or similar solar disinfection systems that employ small volumes. Testing of the length of time

of sunlight exposure, taking into consideration the intensity of the sunlight (sunny day versus cloudy day), should be according to the specifications of the manufacturer, implementer or other testing sponsor. Treated water from individual bottles can be aseptically combined as a composite sample from which an aliquot can be taken for microbial analysis. A recommended minimum of three such challenge tests should be performed, each with triplicate microbial analysis.

A2.5.4.2 Special considerations

Dissolved oxygen in water should be measured both before and after treatment, if possible, as this has been shown to influence the microbial effectiveness of solar disinfection technologies performed in clear (UV-penetrable) plastic bottles. For such technologies, solar radiation, especially in the UV wavelength range, should also be measured, and UV fluence (dose) should be calculated, as this is a key contributor to microbial inactivation. The temperature of the water during exposure should also be measured, as elevated temperature may also contribute to microbial inactivation. For technologies that use opaque containers and rely primarily on elevated temperature to inactivate microbes by pasteurization, temperature should be monitored over time. If there is a particular target temperature specified by the technology provider or implementer, the time to reach this target temperature and the duration of time that water was kept at this target temperature before ending the treatment period should be recorded. The turbidity of water to be treated should also be measured, and the extent to which this turbidity either stays suspended in water or settles in the bottle during sunlight exposure should be considered, as this could influence the kinetics and extent of microbial reduction by these solar processes.

A2.5.5 UV light technologies using lamps, including UV light-emitting diodes

UV radiation has been used in drinking-water treatment for over 100 years (Ward, 1893; Baker, 1948), and its mechanisms for inactivating microbes have now been well characterized (Sobsey, 1989; Blatchley & Peel, 2001). The technology's increasing use is due in part to its proven effectiveness against chlorine-resistant protozoan pathogens, such as *Cryptosporidium* and *Giardia*. A number of drinking-water treatment technologies employ UV light radiation from UV lamps to inactivate microbes. For household or small-scale water treatment, most employ low-pressure mercury arc lamps producing monochromatic UV radiation at a germicidal wavelength of 254 nm. Typically, these technologies allow water in a vessel or in flow-through reactors to be exposed to the UV radiation from the UV lamps at sufficient dose (fluence) to inactivate waterborne pathogens.

Technologies using UV lamps must be tested in accordance with the manufacturer's or implementer's recommendations for use, including specific properties of the lamps, power input, water treatment vessel, treatment reactor or orientation of lamp relative to the water to be treated, incident UV intensity (in mW/cm^2 or other standard units), estimated UV dose delivered (fluence, based on intensity and exposure time), reported in standard units (e.g. mJ/cm^2), and flow rate. At a minimum, test waters 1 and 2 (Table A2.2) should be used to establish effectiveness over a range of water quality characteristics.

A2.5.5.1 Experimental time period and sampling schedule

Spiked challenge waters should be treated according to the manufacturer's or implementer's instructions as a batch process. Samples of untreated (raw challenge) and treated water should be taken for analysis according to the microbiological methods outlined below. A minimum of three batch processes for each microbial challenge is recommended.

A2.5.5.2 Special considerations

Adenoviruses are more resistant to UV disinfection than any known non-pathogenic surrogate virus. However, the virus surrogate coliphage MS2 is relatively resistant to UV radiation and can be used to evaluate the performance of UV disinfection HWT technologies (Thurston-Enriquez et al., 2003). If the UV treatment process is operated as a flow-though or continuous flow reactor and flow rate can vary within a specified range, triplicate spiked water challenge tests should be performed at the average, maximum and minimum flow rates to document the range of microbiocidal effectiveness across the flow rate range.

A2.5.6 Thermal (heat-based) technologies

Thermal technologies are those whose primary mechanism for the destruction of microbes in water is heat produced by burning fuel. This includes boiling and heating to pasteurization temperatures (typically > 63 °C for 30 minutes). For example, pasteurization (Iijima et al., 2001) was found to improve household drinking-water quality in a trial in Kenya. Another field trial from Bangladesh demonstrated inactivation of thermotolerant coliforms using a pasteurization process (Islam & Johnston, 2006). Relatively low heat (55 °C) for several hours may inactivate key protozoan pathogens in water, such as *Cryptosporidium parvum*, *Giardia intestinalis* and *Entamoeba histolytica* (Feachem et al., 1983; Sobsey & Leland, 2001; Sobsey, 2002; Spinks et al., 2006). Boiling remains the most common form of household-scale water treatment worldwide, having been used to treat drinking-water since antiquity.

Because boiling of drinking-water is the most widespread practice for treating drinking-water in the world and, in theory, the most effective for reducing pathogens, it should, like other existing methods of water treatment, not be discouraged when alternative technologies are not as effective or are less likely to be used correctly, consistently and continuously. In practice, however, boiling may not be as effective as other strategies, for various reasons. Disadvantages to boiling include the following: boiling does not reduce sediment or turbidity; boiling may negatively affect taste; boiling heats up water so that it cannot be drunk immediately; the temperature achieved may not be easily measured; and the method may use large amounts of fuel. Boiling may not be a cost-effective or practical option in many places. Boiled water still must be safely stored to avoid contamination in the household, as well.

Technologies that use thermal energy for heat inactivation as the main mechanism for microbial reductions in water should be tested according to the manufacturer's or implementer's recommendations for use. The specifics of the temperature required and the length of time at this temperature that must be maintained for proper treatment should be included in the testing conditions. At a minimum, test waters 1 and 2 (Table A2.2) should be used to establish effectiveness over a range of water quality characteristics.

A2.5.6.1 Experimental time period and sampling schedule

Spiked challenge waters should be treated according to the manufacturer's or implementer's instructions as a batch process. If the process is a flow-through reactor, the manufacturer's instructions for operating conditions in challenge tests should be followed. Samples of untreated (raw challenge) and treated water should be taken for analysis according to the microbiological methods outlined below. A minimum of three such challenge tests should be performed, each with triplicate microbiological analysis.

A2.5.6.2 Special considerations

In thermal treatment processes, it takes time for the water being treated to reach the target temperature and then for the water to cool before use. Therefore, the changes in temperature of the water being treated should be measured, and these temperatures should be compared with those specified as acceptable by the manufacturer. The rate and extent of microbial inactivation are dependent on time–temperature conditions, so documenting these conditions is critical in evaluating performance for microbial reductions. Time–temperature conditions that are considered acceptable according to the manufacturer's or implementer's specifications and that are also representative of conditions where the technology will be used should be used in challenge tests.

A2.5.7 Coagulation–flocculation and/or sedimentation

Coagulation or precipitation is any device or method employing a natural or manufactured coagulant or precipitant to coagulate and/or precipitate suspended particles, including microbes, to enhance their sedimentation. Sedimentation is any method for water treatment using the settling of suspended particles, including microbes, to remove them from the water. These methods may be used along with cloth or fibre media for a straining step to remove the flocculated particles ("floc") that have formed. This category includes simple sedimentation, or that achieved without the use of a chemical coagulant. Coagulant–flocculant products have been tested in the laboratory and field (e.g. Rangel et al., 2003; Reller et al., 2003; Souter et al., 2003; Crump et al., 2004a; Chiller et al., 2006). Promising results have been achieved with low-cost, locally available coagulants for use in simple coagulation/filtration systems (Babu & Chaudhuri, 2005).

Some combination systems are commercial products in the form of granules, powders or tablets containing a chemical coagulant such as an iron or aluminium salt and a disinfectant such as chlorine. When added to water, these chemicals coagulate and flocculate impurities to promote their rapid and efficient sedimentation and also deliver the chemical disinfectant (e.g. chlorine) to inactivate microbes. To use these combined coagulant–flocculant–disinfectant products, they are added to specified volumes of water, allowed to react for floc formation, usually with brief mixing to promote coagulation–flocculation, then allowed to remain unmixed for the floc to settle; the clarified supernatant water is then decanted off, usually through a cloth or other fine mesh medium to strain out remaining particles. The recovered supernatant is then stored for a period of time to allow for additional chemical reactions and disinfection to occur before the water is consumed.

Technology evaluation or verification challenge tests of coagulation–flocculation and sedimentation for the removal of microbes should be performed according to the manufacturer's or implementer's recommendations for normal household use in the target context. Specific representative conditions of the volume of water to be treated (minimum of 20 litres), coagulant dose (if applicable), mixing conditions (e.g. stirring method) and the specified or recommended method for removing floc from the treated water (physical straining, settling, decanting, etc.) should be included. At a minimum, test waters 1 and 2 (Table A2.2) should be used to establish effectiveness over a range of water quality characteristics.

A2.5.7.1 Experimental time period and sampling schedule

Spiked challenge waters should be treated according to the manufacturer's or implementer's instructions as a batch process. Samples of untreated (raw challenge) and treated water (e.g. settled supernatant water after coagulation–flocculation and settling) should be taken

for analysis according to recommended microbiological methods. A minimum of three such challenge tests should be performed, each with triplicate microbiological analysis per sample.

A2.5.7.2 Special considerations

Challenge tests should be performed on volumes of treated water specified by the manufacturer or implementer. These volumes may be based on the unit in which the treatment chemical is provided (e.g. tablet or sachet). Multiple test volumes of challenge water should be treated if the recommended unit volume to be treated is less than the recommended 20 litres per day per household. In this case, samples of pretreatment and post-treatment water to be assayed for microbial and other parameters can be combined from multiple units or batches tested in parallel.

Raw water quality is a critical factor in coagulation–flocculation and precipitation. The efficacy of these physicochemical processes is often highly dependent on such water quality parameters as pH, dissolved solids, alkalinity, hardness, turbidity and the concentrations and types of dissolved and colloidal matter. Therefore, the conditions of these parameters in challenge waters to be tested should be representative of those where the technology will be used, and they should be within the acceptable range at which the treatment chemicals can be effectively used.

For coagulation–flocculation processes, mixing conditions (e.g. speed and duration) can be critical to effective performance, as can subsequent setting times. Therefore, challenge tests should be performed under conditions for these parameters specified by the manufacturer or implementer.

A2.5.8 Combination (multi-barrier) approaches

Multi-barrier approaches are any combination of the above technologies used together, either simultaneously or sequentially, for water treatment. Examples include such combinations as coagulation/disinfection, media filtration/disinfection and media filtration/membrane filtration. Some combination systems are commercial products in the form of granules, powders or tablets containing a chemical coagulant such as an iron or aluminium salt and a disinfectant such as chlorine. When added to water, these chemicals coagulate and flocculate impurities to promote their rapid and efficient sedimentation and also deliver the chemical disinfectant (e.g. chlorine) to inactivate microbes. To use these combined coagulant–flocculant–disinfectant products, they are added to specified volumes of water, allowed to react for floc formation, usually with brief mixing to promote coagulation–flocculation, then allowed to remain unmixed for the floc to settle; the clarified supernatant water is then decanted off, usually through a cloth or other fine mesh medium to strain out remaining particles. The recovered supernatant is then stored for a period of time to allow for additional chemical reactions and disinfection to occur before the water is consumed.

Multi-barrier treatment technologies should be tested according to the manufacturer's or implementer's recommendations for normal household use in the target context. Challenge tests should be performed at representative and acceptable conditions for the water volume to be treated (but also meeting the minimum 20 litres for daily household use), water quality, flow rate (if a continuous flow or flow-through process), dose (if applicable; measured in the appropriate units) and duration of treatment (treatment process time or cycle). At a minimum, test waters 1 and 2 (Table A2.2) should be used to establish effectiveness over a range of water quality characteristics.

A2.5.8.1 Experimental time period and sampling schedule

Where one component of the treatment system is a media or membrane filtration process, a minimum treatment time corresponding to that recommended by the manufacturer or implementer should be used. If no such minimum treatment time is recommended, time periods that are realistic for the type of process and representative of the conditions where the technology will be used should be used. For filters that are used over an extended period of time, a recommended minimum test period of 14 days should be used for each challenge test. If appropriate for the technology, a batch test may be used. A minimum of three such challenge tests should be performed, each with triplicate microbiological analysis per sample.

A2.5.8.2 Special considerations

Challenge tests of technology performance should be performed using water quality conditions representative of those where the technology will be used and within the acceptable range of water quality conditions specified by the manufacturer or implementer. At a minimum, three conditions of water quality, representing maximum, minimum and average conditions in waters from the locations where the technology will be used, should be tested.

For combination treatments that include a chemical disinfectant, the guidance provided above in section A2.5.1 should be followed. In particular, disinfectant dose, disinfectant residual in treated water and contact time should be measured, and the residual disinfectant present in the water upon sampling should be chemically neutralized before performing microbiological analyses.

A2.6 Microbiological testing protocols and laboratory requirements

A2.6.1 International testing protocols

Laboratory demonstration of the effectiveness of HWT technologies against microbes in water can be accomplished through several available standard and consensus protocols. Internationally recognized standards and guidelines for performance or effectiveness testing exist, such as the USEPA's *Guide standard and protocol for testing microbiological water purifiers* (USEPA, 1987) or the NSF's *Protocol P231: Microbiological water purifiers* (NSF, 2003). Although existing protocols are rigorous and detailed, they often require specialized facilities and expertise that may not be widely available in some countries and other resource-limited settings. Methods presented here are alternatives to existing protocol standards and guidelines that can be applied to performance testing of HWT technologies in resource-limited settings.

A2.6.2 Testing facilities and laboratory requirements for HWT technology performance evaluation

Microbiological challenge testing to meet risk-based performance guidelines or relevant standards should be performed in adequate facilities. It is recommended that evaluation of the performance of HWT technologies be done in appropriate and preferably certified laboratories by personnel trained and experienced in microbiology and water quality sciences and their laboratory analytical methods. For technology verification studies with human pathogens, such testing should be done in laboratories certified as biosafety level II (WHO, 2004). For technology studies with non-pathogenic microbes, such as non-pathogenic strains of *Escherichia coli*, non-pathogenic bacterial spores and bacterial

viruses (e.g. coliphages), it is recommended that such testing should be done in laboratories certified as biosafety level I (WHO, 2004). If such testing is done in countries where such biosafety certification is not available, laboratories are encouraged to meet and follow the specifications described in the WHO *Laboratory biosafety manual* (WHO, 2004).

Microbiological performance evaluation for HWT processes is best done by entities that are already experienced in such technical work and that have developed or can develop detailed protocols and specific test plans for such studies. They should also be knowledgeable of quality assurance and either have developed or be able to develop a quality assurance project plan for the technology performance evaluation. Adequate equipment and facilities, trained and experienced staff, protocols, test plans, standard operating procedures and bench sheets, data management systems and quality assurance project plans are critical elements for ensuring reliability and quality in collection, analysis and reporting of data from HWT technology performance evaluation or validation studies.

A2.7 Challenge microbes: Selection and preparation

A2.7.1 Challenge microbe levels in test waters

Sufficient test microbes need to be present in or added to test waters to be able to quantify sufficient reductions by treatment to meet the specified health-based target. For example, consider the case where achieving the WHO reference level of risk of 10^{-6} DALY per person per year requires a 4 $\log_{10}$ reduction of the test microbe by the HWT technology and one analyses only 100 ml of the treated water for remaining levels of these microbes after treatment. Analysing a 100 ml volume of water provides a lower detection limit of 1 microbe per 100 ml. In this case, there needs to be at least 10 000 (10^4) of these microbes per 100 ml of initially seeded water, such that a 4 $\log_{10}$ or greater reduction would result in 1 or 0 microbes per 100 ml of treated water. That is, the microbes would be reduced from an initial 10 000 (4 $\log_{10}$) per 100 ml to 1 microbe (0 $\log_{10}$) or less per 100 ml of treated water (initial 4 $\log_{10}$ – final 0 $\log_{10}$ = a 4 $\log_{10}$ reduction).

A2.7.2 Microbe choice

The choice of test microbes is critical. The choice is best made from reliable local or regional data on which pathogens are the most important ones contributing to the population burden of waterborne disease. The pathogen of greatest risk in causing waterborne disease or contributing the most to waterborne disease burden is the one to target for control by HWT or other control measures. Knowing which pathogen poses the greatest waterborne disease risk makes it possible to choose, verify and implement the technology that will best reduce concentrations of this pathogen to an acceptable level of risk (e.g. the WHO reference risk level of 10^{-6} DALY per person per year). Also, identifying key etiological agents of waterborne disease risk informs the choice of the candidate technologies to effectively treat the water and efficiently reduce this pathogen and its attendant disease risk.

For example, if the pathogen of greatest waterborne disease risk and health burden is *Vibrio cholerae* (the cause of cholera) in a particular community and it is known that this pathogen can be extensively reduced by a novel ceramic filter, solar disinfection or chlorine to achieve the recommended 10^{-6} DALY per person per year reference level of risk, then any of these technologies can be subjected to testing to verify their effectiveness in achieving the required reduction. Alternatively, if facilities and resources do not exist for using the bacterium *V. cholerae* in challenge testing, a laboratory strain of *E. coli* might be used as an acceptable surrogate test microbe, as

it is a bacterium with similar properties and may be more easily grown and handled in the laboratory. It is a surrogate for the pathogen of concern and is meant to represent the effectiveness of the technology against all members of its class (bacteria).

Recommended test pathogens and the suggested alternative pathogen or indicator microbes to use as substitutes for them in technology performance evaluation or validation studies are shown in Table A2.3. These microbes are not the only ones to consider, as there may be other pathogens that are responsible for the greatest waterborne disease burden in a particular community or country and its water sources, and therefore more appropriate microbial indicators might be needed for them. If so, another target pathogen of interest or an indicator for it would be a more appropriate choice for technology performance evaluation or validation. Appropriate choices for pathogen surrogates in testing should be determined by parallel testing of the pathogen and the proposed surrogate with the treatment process or technology in question, a task that has been performed for some treatment processes, with results in peer-reviewed studies. Above all, the surrogate microbe should conservatively estimate the extent of reduction efficiency of the target pathogen.

Table A2.3. Key test pathogens and alternative indicator microbes for use in the laboratory verification of HWT technology

Target pathogen	Recommended alternatives	Comments/special considerations
Campylobacter jejuni	*E. coli* spp., *Enterococcus* spp. (e.g. *E. faecalis* and *E. faecium*), *Salmonella* spp., *V. cholerae*	*C. jejuni* is associated with a relatively high DALY; *Salmonella* spp. and *C. jejuni* are common enteric pathogens. *E. coli* resembles them (Gram-negative, rod-shaped) and has non-pathogenic strains. *Enterococcus* spp., especially *E. faecium* and *E. faecalis*, are abundant in faeces, prevalent and persistent in faecally contaminated water and utilized as faecal indicators of recreational water quality.
Rotavirus	Echovirus 12, MS2, φX-174, other bacteriophages	Rotavirus is highly infectious and causes high disease burdens in children; echovirus 12, a human picornavirus, resembles other enteroviruses, has low pathogenicity and is superficially similar to hepatitis A and E viruses, noroviruses and astroviruses. MS2 and φX-174 are coliphages superficially resembling human enteric viruses, and they respond similarly to them in many water treatment processes.
Cryptosporidium or *Giardia*	*Clostridium perfringens* spores, other spore-forming bacteria (e.g. naturally occurring aerobic spores in natural waters or added as *Bacillus* spp. spores), inert particles, *Entamoeba histolytica or Entamoeba* spp.	*Cryptosporidium* and *Giardia* are prevalent waterborne protozoa causing major disease burdens. As there are no established non-pathogenic protozoa resembling them, *C. perfringens* (or sulfite-reducing clostridia) spores, *Bacillus* spp. spores or naturally occurring aerobic spores in natural waters are suggested as surrogates or indicators. Because chlorine-based technologies are not effective against *Cryptosporidium*, *C. perfringens* (sulfite-reducing clostridia) spores would not be an adequate indicator organism because they are inactivated by chlorine. *E. histolytica* or other human *Entamoeba* species (e.g. *E. dispar* or *Entamoeba coli*) are acceptable for use in challenge tests as well. For technologies relying on physical straining only, inert particles 4–6 μm in diameter may be used. Manufactured fluorescent microspheres have been successfully used for this purpose.

Local laboratory capacity may limit the extent of microbiological testing available, especially in early product screening. *Escherichia coli* or other bacterial indicator species, which may be assayed using kits or other simple, low-cost methods, may be the only available choice in many contexts. Detecting specific bacteria as surrogates or indicators is now facilitated by chromogenic bacteriological media. Such media contain a specific substrate that facilitates detection of the target surrogate bacteria in water containing high background concentrations of other bacteria by a specific colour change (in the bacterial colony or in the broth culture medium) indicating the presence of the target bacteria. Systematic testing of untreated and treated water spiked with *E. coli* or another bacterial indicator can provide useful information about technology effectiveness and can be used to indicate whether a technology or method reaches the recommended levels of effectiveness *for bacteria only*. Such testing is encouraged and can be useful in determining whether a water treatment method merits further testing. Bacterial tests cannot, however, be used to indicate or infer levels of effectiveness against other classes of microbes, such as viruses or protozoan parasites.

A2.7.3 Microbe preparation and state

The methods of preparation and the state of the test microbes to be spiked into test waters in technology performance evaluation or verification challenge studies are critically important. It is recommended that test microbes be prepared by methods resulting in reproducible microbial stocks of appropriate and consistent physical, chemical and biological quality. For most studies, it is recommended that the test microbes be sufficiently purified and dispersed so as to be discrete particles (as opposed to aggregates or being attached to or embedded in other particles) and in suspensions free of excessive solutes and non-microbial particles that could interfere with disinfection treatment processes.

It is important to determine the extent to which microbial aggregation, particle (solids) association and the presence of interfering particles and solutes in water influence the performance of an HWT technology. These conditions of the physical state of the microorganisms and the composition and concentrations of other constituents in water tend to alter microbe response to treatment and treatment effectiveness, and they occur widely in environmental waters used for drinking-water supply. However, determining the effects of these interfering conditions on technology performance in reducing microbes is best done using test microbes and waters in which these factors are controlled and well characterized.

A2.7.4 Bacteria

Historically, standards and guidelines for microbiological performance testing of water treatment technology and resulting treated water quality requirements have been based upon the reduction of members of the coliform group of bacteria. Now, specifically *E. coli* is the WHO-recommended member of the group most representative of faecal contamination of drinking-water. *Escherichia coli* and other coliforms are mostly non-pathogenic, plentiful in the enteric tract of humans and animals (about a billion cells per gram of faeces) and easily cultured in the laboratory. Some coliforms other than *E. coli* are also commonly present in the environment. The use of non-pathogenic laboratory strains of *E. coli* is recommended for bacterial challenge testing of HWT technology when pathogen testing is not possible. *Escherichia coli* B (ATCC 11303) is a widely available strain and therefore an appropriate choice for this purpose, although

others are possible as well. *Campylobacter jejuni* or less pathogenic *Campylobacter* species, such as *Campylobacter coli*, and less pathogenic species of *Salmonella* can be used for technology performance evaluation or validation if the laboratory meets biosafety level II requirements. *Enterococcus* species, especially *E. faecium* and *E. faecalis*, which are abundant in human faeces and are prevalent and persistent in faecally contaminated ambient waters, are also suitable candidate surrogates for pathogenic bacteria in technology performance evaluations. Laboratories performing microbiological performance testing will need to have experience and capacities to handle the appropriate bacteria. There are several standardized methods for the enumeration of *E. coli* and other bacteria in water (see below).

A2.7.4.1 Method of production and handling procedures

For test bacteria, it is recommended that they be grown overnight (or possibly longer for some that grow slowly) as pure cultures in non-selective broth media. The use of selective growth media is generally not recommended, because the bacteria may become injured or physiologically altered when grown in them. Injured bacteria respond differently to disinfection processes and other environmental stresses. Furthermore, bacteria often tend to become injured and physiologically altered when in water and other environmental media. Starting with bacteria cultures that are already injured or physiologically altered from inhibitors in their growth medium can further compromise the physiological state of the cells in unpredictable ways that are not typical for pathogenic bacteria present in water from human or animal waste and other sources.

For many bacteria of interest in water as pathogens or indicators of pathogens, broth cultures prepared for water technology testing can be used immediately, or they can be stored refrigerated for use within a given work week. New cultures should be made weekly. The cells of these broth cultures can be used directly for challenge studies of some treatment technologies if the cells are at high concentrations (allowing for considerable dilution into test waters), relatively dispersed and free of excessive amounts of extraneous solids and dissolved solutes that will interfere with the mode of action of the treatment technology. However, for many HWT technologies, the bacteria cells may need to be further purified to reduce inhibitory materials that would compromise the testing conditions. Such purification is usually done by centrifuging the cells out of the culture medium at several thousand times gravity for several tens of minutes and resuspending them either in a medium compatible with the test water or in the test water itself.

For physical water treatment processes based on exposure to high temperatures, such as boiling, thermal pasteurization (e.g. solar disinfection in opaque containers) or exposure to sunlight (solar disinfection) or UV radiation from lamps, bacteria cultures can be used directly by adding the cells in their culture medium directly to test waters. The thermal and UV radiation effects causing bacterial inactivation are likely to be about the same for cells added to test waters either directly as culture medium or after further purification by washing procedures, as long as there is sufficient dilution of the culture in the test water to prevent constituents from causing interferences (e.g. absorbing or blocking UV disinfection treatment). However, for performance testing of most other HWT technologies, further purification of bacteria cells prior to spiking them into test waters is recommended. This is because undesirable physical states of the bacteria and impurities in the culture medium can interfere in unpredictable ways with the evaluation of the HWT technology.

For example, in challenge tests of chlorine or other chemical oxidant disinfection technologies, washing the test bacteria by centrifuging them out of the culture medium and resuspending them in either test water or other water having low chlorine demand is necessary to free the cells from the high chlorine-demanding solutes of the bacterial culture. Adding bacteria still in their culture medium to test water can introduce high concentrations of chlorine-demanding solutes that cause the dosed chlorine to be quickly consumed, resulting in no chlorine residual remaining during the intended contact time.

As another example, chemical disinfection, filtration and sedimentation studies are best done using dispersed cells. Some bacteria can be grown in broth culture media and remain mostly dispersed (as individual discrete cells) without further treatment. For such bacteria, further treatment to disperse the cells may not be necessary. However, if the test bacteria have a tendency to mostly aggregate or clump, it may be necessary to disperse the cells by physical or chemical treatment. In the case of testing filtration technologies, the use of dispersed cells is important in order to document that the pore size distribution of the filter is small enough to exclude individual cells. If the cells are aggregated as large clumps, the effective size of the particles is much larger than the size of the individual (discrete) bacteria cells and therefore not adequately representative of the performance of the filter in removing individual (discrete) cells. Treatments to disperse cells include physical treatments, such as sonication (e.g. in an ultrasonic bath) or pre-filtration through a membrane filter that removes bacterial aggregates but allows passage of non-aggregated (individual or discrete) cells, and chemical treatments, such as the addition of low concentrations of a surfactant (e.g. a non-ionic detergent) to the cultured bacteria to disperse the cells.

A2.7.4.2 Methods for enumerating bacteria in spiked samples

Bacteria are usually assayed by either quantal (presence/absence) or enumerative methods. Quantal assays involve making serial dilutions of a sample, inoculating multiple cultures for each sample dilution, scoring each of the inoculated cultures as positive or negative for characteristic bacterial growth and then using the data for positive and negative cultures of key sample dilutions to estimate bacterial density as a most probable number (MPN) per unit sample volume. Enumerative culture methods usually are based on counting bacteria colonies on a solid medium (typically an agar medium) or a membrane filter that has received a unit volume of sample. Enumerative (colony count) methods employing agar media include pour, spread and spot (drop) plates. A unit volume of the water sample is inoculated either undiluted or after dilution onto a plate of solidified agar medium (by spreading over the entire surface of the agar or by applying individual small volumes to the agar surface as discrete drops or spots), or it is mixed with molten agar medium and poured into a culture dish to then solidify. In the membrane filter method, a volume of the undiluted or diluted sample is filtered through a microporous membrane filter that retains bacteria. The membrane is then placed onto the surface of a plate with growth medium. After incubation for bacteria to grow and form colonies on agar plates or on membrane filters of culture medium plates, the colonies are counted to determine the bacteria concentration as colony-forming units per unit volume of water.

A wide range of techniques and methodologies exist for the enumeration of *E. coli* in water samples (NRC, 2004), including methods published in *Standard methods for the examination of water and wastewater* (Eaton et al., 2005) and methods by the USEPA (e.g. USEPA, 2002a,b), ASTM International (e.g. ASTM, 2006) and the

International Organization for Standardization (e.g. ISO, 2000), among others. EPA Methods 1603 and 1604 (USEPA, 2002a,b) and Standard Method 9222 (Eaton et al., 2005) describe basic membrane filtration techniques for the enumeration of *E. coli* in water samples. These methods are recommended for use in quantifying *E. coli* samples in spiked untreated and treated water samples.

A2.7.5 Viruses

Viruses are obligate intracellular parasites that must be grown and assayed in living host cells. Viruses used to evaluate drinking-water treatment technologies can be target human viral pathogens, viruses of *E. coli* bacteria (coliphages) or other bacteriophages that serve as indicator or surrogate viruses. Some taxonomic groups of coliphages and other bacteriophages resemble human viral pathogens in general size, shape, composition, environmental persistence and response to water treatment processes. However, compared with human viral pathogens, they are much easier and safer to propagate, assay and handle for technology performance evaluation or verification studies. The target human viral pathogens of greatest risk in drinking-water include hepatitis A and E viruses (causes of infectious hepatitis), rotaviruses and noroviruses (Norwalk virus and related human caliciviruses or noroviruses). These viruses must be used in laboratories certified as biosafety level II. Hence, technology performance validation testing of HWT processes with these viruses is limited to the few laboratories regionally that are both certified as biosafety level II and also knowledgeable about and proficient in water treatment technology verification procedures. In these laboratories, hepatitis A virus, rotaviruses and caliciviruses can be grown and assayed in mammalian cell cultures. The viruses can be purified and seeded into test waters for treatment technology verification studies. Human noroviruses have not been grown in mammalian cell cultures in the laboratory and therefore are not practical or convenient for use in water treatment technology verification studies. Some non-human caliciviruses or noroviruses, such as feline calicivirus and murine norovirus, are surrogates for human noroviruses that can be used for HWT technology evaluation and verification studies. However, using these viruses also requires a biosafety level II laboratory and appropriately trained and experienced personnel.

A2.7.5.1 Coliphages as surrogates for human viruses in laboratory testing

Where possible, human viruses can be used in virus challenge testing of HWT technology. However, these viruses are human pathogens, they are classified as biosafety level II agents (or higher) and as such they require specialized equipment, facilities and training. Therefore, the use of bacteriophages (viruses of bacteria) as surrogates for human viruses is an acceptable and even preferred alternative, especially where biosafety level II laboratories and laboratory analysts trained in biosafety level II procedures are not available. Bacteriophages are widely accepted and used for water treatment technology validation, including for UV radiation, filtration and chemical disinfection technologies. Several different viruses of *E. coli* (coliphages) and other enteric bacteria are effective, convenient and widely used for water treatment technology testing. These include the F+RNA coliphages of the Leviviridae family, such as MS2 and Q-Beta, small deoxyribonucleic acid (DNA)-containing coliphages belonging to the Microviridae family, such as φX-174, and larger DNA-containing bacteriophages of the Tectiviridae family, such as PRD-1. Critical to the use of these bacteriophages is the availability of appropriate strains of

E. coli or other host bacteria. These bacterial hosts must be routinely maintained and tested for their ability to efficiently grow and assay the bacteriophages for which they are a sensitive and specific host cell. Preferred host cells include *E. coli* strains F-amp and K12 for MS2 and Q-Beta, *E. coli* C for φX-174, and *Salmonella typhimurium* LT2 (a pathogenic host bacterium) for PRD-1. MS2 and φX-174 are recommended for use and are further addressed here.

MS2 is a male-specific (F+), icosahedral, non-enveloped coliphage with an isoelectric point (pI) of 3.9. It is often used in modelling enteric viruses, due to its similarity to poliovirus and hepatitis in size (diameter = 24–25 nm), shape (icosahedral) and nucleic acid (single-stranded ribonucleic acid [RNA]). It belongs to the environmentally stable genotype I of the F+RNA coliphages, and its presence in water has been shown to be strongly associated with enteric viruses in environmental samples. It is also useful in laboratory applications due to its ease of recovery and enumeration, non-pathogenic nature and ease of attaining high titres. Human enteric viruses and F+ coliphages probably do not correlate well in relative numbers at any given time in the environment due to the varying presence of human enteric virus pathogens in communities. Coliphages are normally present in faecally contaminated water, but enteric viruses may be present only periodically, such as during an outbreak, when the pathogen is being shed by infected people (Grabow, 2001). φX-174 is a somatic, small (25 nm diameter), spherical virus (pI = 6.6) with DNA as the nucleic acid. It is also useful as an indicator for enteric viruses in water, owing to its easy detection and correlation with enteric viruses in water and wastewater (Grabow, 2001). The electrostatic properties of bacteriophages might differ from those of the enteric viruses. Therefore, they may not behave identically in terms of adsorption or association with other particles in water, and inactivation of these bacteriophages may not be representative of all other viruses. Sobsey et al. (1995) found, however, that F-specific (male) coliphages behaved comparably to hepatitis A virus and the simian rotavirus SA-11 in the bench-scale modelling of drinking-water treatment processes such as flocculation, coagulation and sedimentation; rapid sand filtration; and chlorine disinfection. There is also some evidence that MS2 can be a conservative estimator of other viruses, including poliovirus type 1, in slow sand filtration (Schijven et al., 2003). MS2 coliphage is a conservative indicator for viral pathogens in water subjected to UV irradiation treatment, requiring a higher UV dose to be inactivated relative to other pathogens, including rotavirus, poliovirus and hepatitis A virus (Jevons, 1982; Wolfe, 1990; Wilson, 1992).

A2.7.5.2 Preparation and purification of bacteriophage stocks

Bacteriophages can be grown in their cultured host cells and then recovered and purified for use in HWT technology performance evaluation or validation studies. Bacteriophages can be grown (propagated) by allowing them to infect and replicate in their host cells in liquid broth enrichment cultures using methods similar to those for their quantal enrichment assay. Usually, the host bacteria are first propagated in liquid broth culture medium to the log phase of growth. Then, the bacteriophages are added to the bacteria culture at a ratio of about 1 bacteriophage per 10–1000 cells. The culture is reincubated with constant mixing to allow for several cycles of bacteriophage infection, replication and host cell lysis (typically 2–5 hours). The resulting culture is then centrifuged at 1000–5000 times gravity for 15–30 minutes to sediment the lysed host cell debris, and the supernatant is recovered as crude bacteriophage stock. Bacteriophages can also be propagated by recovering them

from the agar medium of plaque assay plates having confluent (100%) lysis of host cells. This can be done by scraping the virus-containing agar and lysed host bacteria material into a small volume of buffered water, mixing this suspension to release the viruses from the recovered agar medium and lysed host cells, centrifuging at moderate speed (1000–5000 times gravity for 10–30 minutes) to sediment the agar and host cell debris, and then recovering the resulting supernatant as crude bacteriophage stock. Crude bacteriophage stock can be used for technology verification of thermal treatment processes such as boiling or solar disinfection with heat only.

For use in water treatment technology performance evaluation or validation studies on technologies such as chemical disinfection, UV disinfection and other chemical treatments (e.g. coagulation and precipitation), it is recommended that viruses be further purified by chloroform or other organic solvent (e.g. fluorocarbon) extraction. The recovered supernatant virus stocks from either plaque assay or enrichment culture propagation can be solvent-extracted by adding the solvent (e.g. chloroform or fluorocarbon) to the supernatant virus stock at a ratio of 1 part solvent to 2–10 parts virus stock. The mixture is mixed vigorously by hand or with a vortex mixer to create an emulsion. The emulsion is then centrifuged at 3000–5000 times gravity for 30 minutes to separate the aqueous virus material from the organic solvent. The resulting aqueous supernatant containing the virus is recovered by aspirating or decanting, leaving the organic solvent and any sedimented debris behind.

For use in evaluating certain filtration technologies for water treatment, it may be advisable to further purify the virus suspension by removing large aggregates of virus particles by pre-filtration filtration and recovering the filtrate as relatively dispersed virus stock. Pre-filtration is usually through low protein binding membrane filters, such as those made of polycarbonate or specially treated cellulose esters. Chloroform-extracted virus can be filtered successively through 1 µm (or 0.45 µm) and then 0.2 µm pore size filters, and the filtrate can be recovered as dispersed virus stock.

The various methods for growing and purifying bacteriophage stocks are summarized in Carlson (2004).

A2.7.5.3 Methods for enumerating bacteriophages in spiked samples

Coliphages are easily grown and assayed in bacterial cultures by standard techniques that are widely used in general, medical, food and environmental microbiology. Standard coliphage growth and assay procedures have been developed, evaluated and certified by national and international entities (Mooijman et al., 2001, 2005; USEPA, 2001a,b; Sobsey et al., 2004). These include both quantal and enumerative assays in which the concentrations of coliphages in water samples are quantified by their ability to infect and lyse their host bacteria cells. In the enumerative assays, the viruses form clear zones of lysis (plaques) in lawns of host bacteria in agar medium plates. In this method, a volume of water sample is combined with host bacteria and then combined with molten agar medium. The mixture is poured into a culture plate to harden and then incubated to allow the bacteria to infect and lyse the host cells in the agar medium; these zones of lysis, called plaques, are then counted. Bacteriophage concentration is expressed as plaque-forming units per unit volume of water sample. This agar layer plaque assay method for bacteriophage enumeration is analogous to the agar medium colony count methods for enumeration of bacteria.

In the quantal assay methods, multiple sample volumes are inoculated into separate liquid cultures of the host bacteria in liquid broth culture medium and incubated to allow the bacteriophage to infect and lyse the host cells. Enrichment cultures positive

for coliphages are detected by removing a small volume of each culture, placing it as a spot on a lawn of host bacteria in an agar medium, incubating to allow coliphages in the spots from enrichment cultures to infect and lyse host cells, and then estimating coliphage concentration based on which enriched and spotted sample volumes are positive and negative for lysis as an MPN. This enrichment culture MPN method for bacteriophage assay superficially resembles the enrichment broth culture MPN methods for bacteria assay. A more recent modification of the procedure to score for coliphage-positive enrichment cultures is to mix a drop of the enrichment culture with a drop of detector reagent containing plastic beads coated with antibodies directed specifically against the coliphages. The coliphages in the enrichment culture react with the antibodies on the beads, causing the beads to clump together and form visible aggregates. This procedure, referred to as particle agglutination, is both simple (mixing two drops of liquid, the enrichment culture and the detector reagent, together on a solid surface) and rapid (< 1 minute to detect agglutination or clumping) (Love & Sobsey, 2007).

Unlike the biosafety II level requirements for human enteric and other mammalian viruses, biosafety level I laboratories equipped with relatively basic facilities and equipment are adequate for working with these bacteriophages. Detailed methods for the propagation, storage and enumeration of these bacteriophages and their host cells have been provided by the USEPA in EPA Methods 1601/1602 for the analysis of F+RNA (male-specific) and somatic coliphages in water samples (USEPA, 2001a,b). The relevant Standard Methods for these are 9224B, C, D, E and F (Eaton et al., 2005). These are based on methods described by Adams (1959). Additional laboratory protocols for handling bacteriophages are available (Carlson, 2004).

A2.7.6 Protozoan parasites

The protozoan parasites often targeted for control in drinking-water are *Cryptosporidium parvum*, a zoonotic coccidian protozoan, and *C. hominis*, a species more commonly infecting humans. Both of the *Cryptosporidium* species are relatively small (3–7 µm in diameter) compared with other important waterborne parasites, relatively persistent in the environment and relatively resistant to chemical disinfection. They occur widely in humans and animals worldwide, causing gastrointestinal illness in healthy people and more severe and life-threatening illness in immunocompromised people. In some parts of the world, other parasites, such as *Giardia intestinalis* and *Entamoeba histolytica*, may be more appropriate protozoan pathogen targets for control in drinking-water because they are more prevalent in the population and more significant parasite contributors to community waterborne disease burdens. *Cryptosporidium* oocysts or other parasites for drinking-water treatment technology verification studies require a reliable source of the parasites, biosafety level II laboratory facilities and experienced laboratory staff. In the case of *Cryptosporidium* oocysts, the source is usually experimentally infected animal hosts such as newborn calves that shed high concentrations of the oocysts in their faeces. Producing stocks of *Cryptosporidium* oocysts is technically challenging and time-consuming because of the need for ethical treatment of experimental animals, careful collection of the animal faecal matter containing the oocysts and purification and appropriate storage of the oocysts. In some more technologically developed countries, *Cryptosporidium* oocysts and cysts of some other protozoan parasites, such as *Giardia intestinalis* (or the similar murine equivalent, *Giardia muris*), are commercially available. The costs of these commercially available protozoa are relatively high, and they require special

handling in shipment if they are still viable and infectious. However, *Cryptosporidium* oocysts and *Giardia* cysts that have been rendered non-viable and also stained with fluorescent dyes are commercially available. Such non-viable, fluorescent protozoan parasite cysts and oocysts are useful for performance evaluation of HWT technologies such as filters that function by physically removing the microbes.

For some drinking-water treatment technology performance evaluation or validation studies in which pathogen reduction is by physical removal, such as filtration, coagulation–flocculation and settling and sedimentation, the analytical methods to detect and quantify the parasites in water can be based on direct enumeration by microscopy. Such microscopic examination usually requires that the parasites in the water samples be further concentrated and then stained (usually by immunochemical methods such as fluorescent antibody staining) for subsequent microscopic enumeration, typically by epifluorescent UV microscopy. This is especially needed in samples of treated water where the remaining parasite concentrations may be very low due to their removal by the treatment process. Such fluorescent microscopy analysis is technically demanding, is time-consuming and requires immunofluorescent reagents, a high-quality epifluorescent microscope and a trained analyst.

For drinking-water treatment technologies relying on parasite inactivation by a physical or chemical disinfection process but not physical removal, such as thermal treatment, UV irradiation or chlorination, reductions of parasite infectivity are the basis for evaluating performance. Infectivity assays for parasites based on infecting an experimental animal or mammalian cell cultures are technically challenging, require specialized facilities and equipment and are expensive to perform. These animal or cell culture infectivity assay capabilities may not be available in some countries or parts of the world. Many parasites can be assayed for "viability" and changes in viability (due to a treatment process), which are based on exclusion or uptake of chemical dyes or other vital staining properties. However, it is now well documented that such viability assays are unreliable predictors of infectivity or changes in infectivity due to a treatment process. Such viability assays should not be used to determine protozoan parasite infectivity reductions in drinking-water treatment technology verification studies.

Where possible and appropriate, *Cryptosporidium* should be used to challenge HWT technologies. Because the resources for parasite production and their analyses by microscopy or infectivity may not be available in some countries or regions of the world, alternative approaches are needed for drinking-water treatment technology evaluation of protozoan parasite reductions. A practical and reasonably reliable alternative to using the parasites themselves is to use a microbial indicator for them. The most widely used and best documented microbial indicator for protozoan parasite reduction by water treatment processes is spores of the anaerobic bacterium *Clostridium perfringens* or spores of (aerobic) *Bacillus* spp. *Clostridium perfringens* spores are small (about 1 µm in diameter), environmentally stable and persistent, and relatively resistant to physical and chemical disinfection processes. *Clostridium perfringens* can be obtained from reference collections or can be isolated from water, wastes or soils by culture using differential and selective media and then further biochemical confirmation. Reference strains of *C. perfringens* that are known to be efficient at spore production (sporulation) are preferred over primary isolation of naturally occurring unknown strains from environmental media. This is because *C. perfringens* is generally inefficient at sporulation, and therefore many separate isolates may need to be screened to identify one that is efficient at producing spores.

Bacillus spp. spores (e.g. *Bacillus subtilis*, *Bacillus atrophaeus*) may be harvested from natural waters, are relatively easy to grow to high titre, are easy to enumerate and present many of the advantages of *C. perfringens* with respect to modelling treatment processes. However, *Bacillus* spores may germinate under some environmental and testing conditions, resulting in the formation and growth of vegetative cells. Therefore, precautions should be taken to maintain *Bacillus* spore integrity and prevent spore germination and proliferation of vegetative cells. The potential for spore germination and vegetative cell growth in the test system should be considered and controlled for, and challenge test samples should be assayed as soon as possible after collection. Similar concerns about spore germination and vegetative cell proliferation also apply to spores of *C. perfringens*, but the potential for this is less likely than for *Bacillus* spores, because *C. perfringens* is an anaerobe.

Where the technology's mechanism for protozoan reduction is physical removal based on size exclusion, other synthetic surrogate particles may be used. Such particles may be fluorescent beads of the same size, density and shape as the protozoan (e.g. *Cryptosporidium* oocyst) that may be enumerated via a number of methods, such as fluorescent microscopy.

A2.7.6.1 Clostridium perfringens spores as an indicator for Cryptosporidium spp. and other protozoa

Clostridium perfringens spores have been suggested as experimental surrogates for *Cryptosporidium* oocysts in treatment process and transport modelling, owing to their resistance to chemical disinfection and environmental stability (Venczel et al., 1997; Sartory et al., 1998). In filtration processes relying on physical straining, the 1 µm spores may provide a conservative indicator of the behaviour of the larger (5 µm) oocysts (Schijven et al., 2003). They may also be the best available surrogate for *Cryptosporidium* inactivation by chemical disinfection as a result of their relative resistance to chlorine inactivation. Venczel et al. (1997) found that *C. perfringens* was inactivated by 1.4 $\log_{10}$ over 4 hours compared with no measurable inactivation of *Cryptosporidium* oocysts exposed to free chlorine over 4 hours, although inactivation of the two microbes was similar for a mixed oxidant disinfectant. Payment and colleagues (Payment et al., 1985; Payment & Franco, 1993) found that *C. perfringens* and coliphages correlated well with *Cryptosporidium*, *Giardia* and human enteric viruses in removal by water treatment processes. UV radiation and heat inactivation of microbes are less effective treatments against bacterial spores than against protozoa as well as vegetative bacteria and viruses. Therefore, *C. perfringens* spores may provide a conservative indicator of the effectiveness of these technologies against waterborne protozoa such as *Cryptosporidium*, *Giardia* and *Entamoeba*.

Method of production

Clostridium perfringens spores are produced by culturing the bacteria in sporulation media under conditions that promote spore formation. There are several sporulation media for propagating *C. perfringens* spores, but Duncan-Strong medium and variations of it are widely used and recommended (Duncan & Strong, 1968; Labbe, Somers & Duncan, 1976; Labbe & Rey, 1979; Hsieh & Labbe, 2007). The resulting spore crops can be stored refrigerated for extended periods of time (weeks), or they can be frozen (months to years).

Analysis for *Clostridium perfringens* spores

Spores are usually quantified or enumerated by culture methods in selective and differential media. To assay only spores and not vegetative cells that might also be present in samples, the samples are pretreated by heat exposure, typically 70 °C for 15–30 minutes, before culturing. For quantal assay by broth culture methods of multiple sample volumes to obtain MPN estimates of concentration, a preferred culture medium is iron milk, with incubation at 41 °C. In this medium, *C. perfringens* growth is detected by "stormy fermentation", which occurs when the medium clots and entraps gas bubbles produced by the growing bacteria. Alternatively, *C. perfringens* in water samples can be assayed by membrane filter methods with incubation on selective media such as mCp as modified by Armon & Payment (1988) from the original formulation of Bisson & Cabelli (1979) and tryptose-sulfite-cycloserine (TSC) medium (Sartory et al., 1998; Adcock & Saint, 2001). Recent studies suggest that TSC medium is equivalent to or better than mCp for enumeration of *C. perfringens* (e.g. Araujo et al., 2001).

ASTM Method D5916-96(2002), Standard Test Method for Detection and Enumeration of *Clostridium perfringens* from Water and Extracted Sediments by Membrane Filtration (MF) (ASTM, 2002), is one commonly used method for quantification of *C. perfringens* based on membrane filtration and incubation on selective media. The United Kingdom's Health Protection Agency (2004) has developed a standard method for *C. perfringens* enumeration using incubation on TSC medium.

A2.7.6.2 Bacillus spp. spores as an indicator for Cryptosporidium and other protozoa

Bacillus spp. spores have been suggested as experimental surrogates for *Cryptosporidium* oocysts in treatment process and transport modelling. *Bacillus* spp. and other aerobic spore-forming bacteria populations are relatively resistant to disinfection, are environmentally stable and are also often found in natural waters in high enough concentrations to be useful in calculating multiple $\log_{10}$ reductions (Dey et al., 1998; Nieminski, Bellamy & Moss, 2000; Chauret et al., 2001; Verhille et al., 2003).

Method of production

Bacillus spp. spores are produced by culturing bacteria in sporulation media under conditions that promote spore formation. There are several sporulation media for propagating *Bacillus* spores, but AK Agar #2 (Sporulating Agar) medium is widely used and recommended. The resulting spore crops can be stored refrigerated for extended periods of time (weeks), or they can be frozen (months to years) if stored in 7–10% glycerol at –80 °C. Methods for spore production are fully described in Dey et al. (1998) and Chauret et al. (2001). A note of caution when using *Bacillus* spores for technology performance evaluation is the ability of the spores to germinate into vegetative cells and reproduce (multiply). The use of these spores in technology challenge studies where the test conditions may lead to spore germination and propagation is not recommended. Physical treatment processes that involve biological activity or in which there are long contact times could lead to *Bacillus* spore germination and multiplication of vegetative cells. The use of *Bacillus* spores should be avoided where there is a risk of such conditions in the test procedure. In general, *Bacillus* spores in test water samples from technology performance studies should be assayed as soon as possible after the samples are collected to avoid spore germination and

vegetative cell propagation. If the samples must be held prior to assay, they should be kept cold (preferably 4 °C).

Analysis for *Bacillus* spp. spores

Spores are usually quantified or enumerated by culture methods in either non-selective or selective and differential media. The commonly used non-selective medium is nutrient agar (plates), but care must be taken to distinguish the *Bacillus* spores from other bacteria that will grow on this medium. The addition of bromothymol blue at 0.005% (weight per volume) concentration into nutrient agar facilitates colony counting (Francis et al., 2001). To assay only spores and not vegetative cells that might also be present in samples, the samples are pretreated by heat exposure, typically 70 °C for 15–30 minutes, before culturing on media at 37 °C for 24 hours. An accessible membrane filtration–based assay has been developed that allows for rapid enumeration of *Bacillus* spp. spores in waters of different types (Francis et al., 2001).

A2.8 Effectiveness of HWT technologies

Estimated reductions of waterborne bacteria, viruses and protozoan parasites by several of the above-mentioned HWT technologies are summarized in Table A2.4. The table has been extracted from the fourth edition of the GDWQ (WHO, 2011). Reductions are based on the results of studies reported in the scientific literature. Two categories of effectiveness are reported: baseline reductions and maximum reductions. Baseline reductions are those typically expected in actual field practice when done by relatively unskilled persons who apply the treatment to raw waters of average and varying quality and where there are minimum facilities or supporting instruments to optimize treatment conditions and practices. Maximum reductions are those possible when treatment is optimized by skilled operators who are supported with instrumentation and other tools to maintain the highest level of performance in waters of predictable and unchanging quality (e.g. a test water seeded with known concentrations of specific microbes).

Table A2.4. Estimates of baseline and maximum effectiveness of selected HWT technologies against microbes in water

Treatment process	Enteric pathogen group	Baseline removal (LRV[a])[b]	Maximum removal (LRV[c])	Notes
Chemical disinfection				
Free chlorine disinfection	Bacteria	3	6	Turbidity and chlorine-demanding solutes inhibit this process; free chlorine × time product predicts efficacy; not effective against *Cryptosporidium* oocysts
	Viruses	3	6	
	Protozoa, non-*Cryptosporidium*	3	5	
	Cryptosporidium	0	1	
Membrane, porous ceramic or composite filtration				
Porous ceramic and carbon block filtration	Bacteria	2	6	Varies with pore size, flow rate, filter medium and inclusion of augmentation with silver or other chemical agents
	Viruses	1	4	
	Protozoa	4	6	
Membrane filtration (microfiltration, ultrafiltration, nanofiltration, reverse osmosis)	Bacteria	2 MF; 3 UF, NF or RO	4 MF; 6 UF, NF or RO	Varies with membrane pore size, integrity of filter medium and filter seals, and resistance to chemical and biological ("grow-through") degradation
	Viruses	0 MF; 3 UF, NF or RO	4 MF; 6 UF, NF or RO	
	Protozoa	2 MF; 3 UF, NF or RO	6 MF; 6 UF, NF or RO	
Fibre and fabric filtration (e.g. sari cloth filtration)	Bacteria	1	2	Particle or plankton association increases removal of microbes, notably copepod-associated guinea worm (*Dracunculus medinensis*) and plankton-associated *Vibrio cholerae*; larger protozoa (> 20 µm) may be removed; ineffective for viruses, dispersed bacteria and small protozoa (e.g. *Giardia intestinalis*, 8–12 µm, and *Cryptosporidium* 4–6 µm)
	Viruses	0	0	
	Protozoa	0	1	
Granular media filtration				
Rapid granular, diatomaceous earth, biomass and fossil fuel–based (granular and powdered activated carbon, wood and charcoal ash, burnt rice hulls, etc.) filters	Bacteria	1	4+	Varies considerably with media size and properties, flow rate and operating conditions; some options are more practical than others for use in developing countries
	Viruses	1	4+	
	Protozoa	1	4+	
Household-level intermittently operated slow sand filtration	Bacteria	1	3	Varies with filter maturity, operating conditions, flow rate, grain size and filter bed contact time
	Viruses	0.5	2	
	Protozoa	2	4	
Solar disinfection				
Solar disinfection (solar UV radiation + thermal effects)	Bacteria	3	5+	Varies depending on oxygenation, sunlight intensity, exposure time, temperature, turbidity and size of water vessel (depth of water)
	Viruses	2	4+	
	Protozoa	2	4+	

Table A2.4. (continued)

Treatment process	Enteric pathogen group	Baseline removal (LRV[a])[b]	Maximum removal (LRV[c])	Notes
UV light technologies using lamps				
UV irradiation	Bacteria	3	5+	Excessive turbidity and certain dissolved species inhibit process; effectiveness depends on fluence (dose), which varies with intensity, exposure time, UV wavelength
	Viruses	2	5+	
	Protozoa	3	5+	
Thermal (heat) technologies				
Thermal (e.g. boiling)[d]	Bacteria	6	9+	Values are based on vegetative cells; spores are more resistant to thermal inactivation than are vegetative cells; treatment to reduce spores by boiling must ensure sufficient temperature and time
	Viruses	6	9+	
	Protozoa	6	9+	
Sedimentation				
Simple sedimentation	Bacteria	0	0.5	Effective due to settling of particle-associated and large (sedimentable) microbes; varies with storage time and particulates in the water
	Viruses	0	0.5	
	Protozoa	0	1	
Combination treatment approaches				
Flocculation plus disinfection systems (e.g. commercial powder sachets or tablets)	Bacteria	7	9	Some removal of *Cryptosporidium* possible by coagulation
	Viruses	4.5	6	
	Protozoa	3	5	

LRV, $\log_{10}$ reduction value; MF, microfiltration; NF, nanofiltration; RO, reverse osmosis; UF, ultrafiltration

[a] $\log_{10}$ reduction value, a commonly used measure of microbial reduction, computed as $\log_{10}$ (pretreatment concentration) – $\log_{10}$ (post-treatment concentration).

[b] Baseline reductions are those typically expected in actual field practice when done by relatively unskilled persons who apply the treatment to raw waters of average and varying quality in developing countries and where there are minimum facilities or supporting instruments to optimize treatment conditions and practices.

[c] Maximum reductions are those possible when treatment is optimized by skilled operators who are supported with instrumentation and other tools to maintain the highest level of performance in waters of predictable and unchanging quality.

[d] Heat pasteurization is another example of a thermal technology. For further explanation of the process and references, refer to section A2.5.6.

Source: WHO (2011)

APPENDIX 3. ADDITIONAL FACTORS TO CONSIDER IN NATIONAL ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAMMES

The extent to which a water treatment process reduces microbial pathogens is critically important in determining how useful it will be in reducing the risks of waterborne disease and providing safe water. Because of the diversity of microbial pathogens and their properties, it is especially important to understand and quantify the effectiveness of individual HWT technologies in reducing all classes of pathogens in waters of diverse quality. Recent experiences highlight the importance of this. For example, the widely used disinfection technology of chlorination was found to be ineffective in reducing the infectivity of a previously overlooked but widespread waterborne protozoan parasite, *Cryptosporidium*, in the 1990s. In part because of such experiences, there are now strict guidelines, performance standards and protocols for validation of pathogen reductions by drinking-water treatment processes in the technologically more developed world (USEPA, 1987; NSF, 2003). These are not, however, the only relevant considerations in evaluating technologies for local use. A wide range of additional factors not related to laboratory-demonstrated effectiveness against microbial pathogens may be locally important in technology verification as well. The following is an overview of additional factors that may be considered in local technology verification programmes, although there are many others. Technology verification should respond to local needs and resources to ensure the protection of public health and the responsible use of local resources.

A3.1 Field microbiological performance

Microbial effectiveness of HWT technologies is often observed to be reduced under field use conditions compared with laboratory challenge studies (Baumgartner, 2006; Brown, Sobsey & Loomis, 2008; Brown & Sobsey, 2010). This observation highlights the need to both replicate actual use conditions as closely as possible when performing laboratory testing and track continued performance of technologies after their laboratory performance characterization and during actual field use. The differences in performance may be a consequence of user behaviour, caused by aspects of the technology itself or associated with household environmental conditions (i.e. hygienic conditions). Household use data can play an important role in technology verification programmes by providing an in situ measure of the intervention's potential for improving and protecting water quality. Specific indicators to measure field microbiological performance are outside the scope of this document but should be considered carefully in concert with HWT stakeholders in order to provide a realistic assessment of technology effectiveness.

A3.2 Health impact

There is a recognized need to incorporate outcomes of epidemiological studies in evaluating the effectiveness of household-based water quality interventions. Some stakeholders consider this to be essential information for their policy decisions on selection and implementation of household and other small-scale technologies. While

a substantial evidence base for health impacts of water quality improvements has been established for some water treatment technologies, more and better quality studies are needed. Novel or unproven technologies should, in addition to extensive laboratory testing for effectiveness in reducing waterborne pathogens, be subjected to field testing, specifically for the reduction of infectious diseases associated with drinking-water. There is a clear need for additional controlled trials of small-scale water treatment devices or methods that are rigorous, long-term, randomized and blinded.

When diarrhoeal disease burdens are high, measuring health impact may be more practical and cost-effective than obtaining location-specific pathogen occurrence and disease burden data for a detailed QMRA analysis, such as that suggested in the GDWQ. It is also likely to provide better estimates of waterborne disease risks than using faecal indicator microbes alone as proxies for waterborne pathogens in QMRA analysis. This is because studies examining the relationship between measured microbial indicators in drinking-water and health outcomes may reveal only limited or inconsistent associations (Moe et al., 1991; Brown, Proum & Sobsey, 2008) or no apparent association (Jensen et al., 2004). Ideally, health impact data should complement water quality data from laboratory and field studies that quantify reductions in microbes through use of a specific technology or method for water treatment. Carefully collected epidemiological evidence can be an important consideration in technology selection and local verification. Priority should be given to rigorous study designs, particularly those that are blinded and randomized.

A3.3 Correct, consistent and continuous use

A number of studies of household water treatment have measured long-term use of water quality interventions (Luby et al., 2001; Parker et al., 2006; Brown, Sobsey & Proum, 2007; Arnold et al., 2009; Mäusezhal et al., 2009; Jain et al., 2010). Existing evidence suggests that declines in use of water treatment devices or practices may occur after implementation programmes or pilot studies are over and that such declines are linked to a broad range of factors. Technologies with a high user burden, with recurrent costs or that involve substantial behaviour change may be especially susceptible to appreciable declines in use after introduction of the technology. Extended field studies and data on economic and consumer preferences may be needed to know whether the technologies can be viable long-term options for HWT. Post-implementation assessments are a critical feedback mechanism to identify and address challenges faced by small-scale water treatment devices in field use.

A3.4 Safe storage

Ideally, HWT methods or technologies can also safeguard against contamination of water stored in the home through unsafe water handling practices, known to be a major cause of degraded drinking-water quality. For this reason, safe storage is an important aspect of some technologies used for drinking-water treatment, or safe storage containers may be used as a stand-alone technology for protecting water quality where the main source of contamination is improper handling. Devices that store water safely prevent users from dipping hands or other potentially contaminated objects into the water container, acts that may introduce disease-causing microbes.

Safe storage containers thus usually have a narrow mouth (so that water is obtained by pouring, not dipping) or a tap that dispenses the stored water into a cup for drinking. Technologies using disinfection may be designed to maintain a disinfectant residual to protect against recontamination. Verification programmes may choose to include the safe storage element in laboratory technology testing.

A3.5 Chemical contaminants and toxicity

The greatest risks of waterborne disease globally are from microbial pathogens (Prüss et al., 2002), although chemical contaminants are locally or regionally significant risks to public health. These guidelines do not address the potential problem of anthropogenic or naturally occurring radiological or chemical contaminants that may be present in drinking-water, including, but not limited to, pesticides, arsenic, fluoride, heavy metals, nitrate, excess salts, disinfection by-products, pharmaceuticals and others (Thompson et al., 2007). However, national technology verification programmes may choose to develop and implement effectiveness testing protocols where HWT is proposed as a solution to chemical contamination of water.

Alternatively, there have been concerns that technologies may leach chemical contaminants during use, especially filters that use raw materials with potentially high concentrations of arsenic, technologies using photodegradable plastic compounds, technologies incorporating silver and/or iodine as disinfectants or technologies using post-consumer recycled materials. If this is suspected, testing of leachate is recommended to ensure that treated water does not pose additional risks to users. Recommended limits on chemical contaminants in drinking-water are indicated in the GDWQ (WHO, 2011). Technologies that use metal-based (e.g. copper, silver, iodine, bromine) or other novel chemical disinfectants that are potentially toxic at concentrations in water above WHO guideline values should be subjected to testing for their concentrations in treated water according to standard methods to ensure that the chemical quality of the treated water is within acceptable limits.

A3.6 Acceptability of existing published or unpublished performance data or other evidence

Many HWT technologies are now supported by published and unpublished studies demonstrating microbiological performance and/or providing data regarding health impacts, cost-effectiveness, sustainability in long-term use or other factors. In some cases, these technologies may have demonstrated acceptable performance according to other evaluation programmes. Technology performance evaluation or verification programmes will need to determine whether and under what conditions existing evidence may be used to meet performance requirements for national verification, certification or product labelling.

A3.7 Other factors that may be considered important in environmental technology verification programmes

A number of other factors can help form the basis for technology selection or local/national approval. Some of these have been proposed or used in technology selection

frameworks or national technology performance evaluation programmes. These factors include the following:

Likely to be of use in verification programmes

- Flow rate or volume treated per day (BETV-SAM[2])
- Verification of manufacturer claims (BETV-SAM)
- Technology-specific field performance/operation guidelines in place (BETV-SAM)
- Time elapsed/volume of water treated before media or element replacement (BETV-SAM) or useful life of device
- Ability of user to assess performance or expiry of technology
- Reduction of turbidity or other indicators of water treatment (technology specific)
- Achievement of a measurable chemical residual as evidence of proper dose and to protect treated water (technology specific, such as chlorine disinfection)

Other potentially useful factors to consider

- Potential for and risk of operator error
- Technology dependence on electricity or water pressure
- Acceptability to target population
- Educational or training support required for implementation
- Cost, cost-effectiveness and affordability
- Cost and local availability of replacement products, parts, media or other consumables
- Environmental factors such as waste produced (in manufacturing or in product use), disposal of waste, waste recyclability, carbon footprint, use of local materials
- Durability under typical use conditions (materials, moving parts, consumables)
- Aesthetic qualities of treated water (reduction of iron, improvements in taste, colour, smell)
- Population-specific factors such as susceptibility to specific pathogens known to be present
- Local use and demand already demonstrated
- For emergency or relief use: ability to deploy technology for rapid response

[2] The Bangladesh Environmental Technology Verification – Support to Arsenic Mitigation Project (BETV-SAM). This is the arsenic reduction technology verification programme in place in Bangladesh (http://www.betv-sam.org/).

APPENDIX 4. BASIS FOR USE OF QMRA

Obtaining reliable data on the prevalence of diarrhoeal disease from countries and attributing a percentage of the disease burden to ingesting unsafe drinking-water are challenging and in many instances impossible. The reasons for this are many and detailed elsewhere but, in short, include the multiple transmission pathways of intestinal pathogens, a lack of proper recording and reporting of disease by health authorities, heterogeneities in disease prevalence among districts/provinces and those sick not seeking care at health facilities (WHO, 2009). Furthermore, few existing epidemiological research studies have been designed to estimate the real incidence of acute diarrhoeal disease (the dominating disease burden arising from ingestion of unsafe water) in a population (WHO, 2009).

Epidemiological studies on the health impacts of HWT play an important contributing role in informing evaluations of HWT technology performance. Yet relying solely on existing studies to assess the efficacy of HWT technology performance is problematic. Epidemiological studies on HWT typically assesses water quality through use of faecal indicators. Unfortunately, as described in Appendix 3, current evidence does not demonstrate a fixed relationship between indicators of microbial water quality and pathogens. The functional objective of HWT methods is to reduce pathogens, and thus determination of pathogen removal is an essential, direct measure of performance. Because epidemiological studies typically do not measure pathogen concentrations before and after treatment, it is difficult to attribute pathogen reduction to health effects and consequently to set pathogen performance targets.

It is now increasingly recognized that drawing conclusions from the current epidemiological evidence base addressing HWT, especially for developing countries, is premature. Great heterogeneities exist among studies, making it difficult to draw conclusions across regions, and the effect of the health benefits associated with HWT may be exaggerated due to the lack of blinded trials and a decline in health impact (e.g. magnitude of diarrhoeal disease reduction) over time (Hunter, 2009; Schmidt & Cairncross, 2009). Many HWT intervention studies have demonstrated significant diarrhoeal disease reductions (e.g. 20–40%), despite the different capabilities of the HWT technologies to reduce common waterborne pathogens, and despite being inconsistently applied or practised (e.g. no residual chlorine found, or observations of householders regularly drinking untreated water). Therefore, making quantitative estimates of impact and drawing conclusions on acceptable performance of HWT technologies by only relying on epidemiological studies would be imprudent. Furthermore, longer-term HWT epidemiological studies have not convincingly demonstrated reductions in diarrhoeal disease, suggesting that there are many factors influencing their results beyond whether or not a technology "works". These include acceptability, consistent use and varying sources and pathways of faecal–oral contamination (Arnold et al., 2009; Mäusezhal et al., 2009; Boissone et al., 2010).

QMRA, through its application of pathogen-specific dose–response information, provides a direct method of linking reductions in waterborne pathogens as a result of water treatment to health impacts. It is an increasingly common method for assessing the microbial safety of drinking-water. The framework is being applied in the development of risk-based drinking-water standards in Australia, the European Union (MICRORISK) and the Netherlands, among other places.

QMRA is not without limitations, and the mathematical models on which it is based rely on several assumptions. First, the dose–response values are derived from

studies on human volunteers who may differ in immunity and health status from those populations to which the method is applied. Second, the assumptions for background water quality can be questioned. Third, QMRA is essentially a mathematical model, with the high level of uncertainties inherent in predictive modelling.

QMRA is complementary to epidemiological approaches. If robust epidemiological data indicate health benefits from HWT devices and the HWT devices meet the "protective" target for two pathogen classes, then such HWT devices can be recommended. As the amount of robust epidemiological evidence increases, especially from randomized, controlled, blinded HWT trials over longer periods of intervention time, such data will play a larger role in evaluating the efficacy and effectiveness of HWT technologies. This is especially true given that the HWT performance guidance is not prescriptive, but rather meant to provide a framework from which national guidelines and regulations can be developed in the context of local conditions and evidence.

The HWT health-based performance requirements pertain to three pathogen classes (viruses, bacteria and protozoa). Epidemiological evidence and population and environmental trends suggest that the occurrence of different types of waterborne pathogens is varied and in many cases difficult to predict or reliably measure. Globally, water-related pathogens that have emerged or re-emerged recently include bacteria (e.g. *Vibrio cholerae*, *Salmonella typhi*, *Salmonella* spp., *Shigella* spp., *Legionella* spp. and pathogenic *Escherichia coli*), protozoa (e.g. *Cryptosporidium parvum*, *C. hominis*, *Giardia intestinalis* and *Entamoeba histolytica*), helminths (e.g. *Ascaris lumbricoides*), viruses (noroviruses, rotaviruses, hepatitis A and E viruses and adenoviruses) and fungi (UNEP/GEMS, 2008). The temporal and spatial variations in pathogen concentrations suggest the need to reduce all three major classes for which there is clear evidence of waterborne disease: bacteria, parasites and viruses. Selected field studies illustrate the importance of protecting against all three types of pathogens, especially in the absence of timely, seasonal, local data. For example, the global enteric multi-centre study taking place in seven developing countries has isolated all three classes of pathogens in children under five years of age (Levin, 2009). Elsewhere, a recent childhood diarrhoeal study in Yaoundé, Cameroon, found that of those cases of infectious diarrhoea, 59.2% were caused by pathogenic parasites, 36.9% by pathogenic bacteria and 3.8% by pathogenic viruses (Yongsi, 2008). An etiological study of diarrhoea in Tanzania, however, found that in the dry season, pathogenic bacteria (37.4%) followed by pathogenic viruses (23.6%) dominated as causes of disease in children, whereas in the rainy season, protozoa became more prevalent and viruses diminished in importance (Vargas et al., 2004). Other studies indicate, in developing countries, the asymptomatic faecal shedding of intestinal protozoan pathogens, complicating and sometimes compromising a purely epidemiological approach based on assessing only cases of diarrhoea (Checkley et al., 1997; Esteban et al., 1998; Ramos et al., 2005; Wongstitwilairoong et al., 2007). The relative importance of each pathogen class may be locally variable. However, in the absence of strong evidence to negate the use of all three and given the global nature of this document, the most prudent approach is to base the performance targets on removal of bacteria, viruses and protozoa.

Evaluating household water treatment options: Health-based targets and microbiological performance specifications

Household water treatment (HWT) is increasingly being promoted as a rapidly implementable and cost-effective interim approach to improve water quality. It is a key preventive component of the WHO/UNICEF comprehensive strategy on diarrhoea control.

This document, for the first time, sets forth global criteria that enable users to evaluate whether an HWT option reduces waterborne pathogens sufficiently to protect health. Through use of a risk-based framework and by emphasizing the philosophy of incremental improvement, it is intended to provide implementers and policy-makers with an evidence-based and pragmatic approach to select options suited to local conditions.

The document provides a range of technical recommendations, including:

- a step-by-step overview of how to evaluate HWT microbiological performance;
- elaboration of health-based water quality targets ranging from interim to highly protective, including establishment of default targets for use in data-scarce settings;
- description of technology-specific laboratory testing protocols and guiding principles;
- considerations relating to developing national technology evaluation programmes.

This document is especially intended for resource-scarce settings where water quality laboratories may have limited capacity and incremental improvements of HWT performance could have a substantial, positive impact on public health.

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