Microbial safety of drinking water: assessing and reducing risks. Improved approach and methods

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Ensuring the microbiological safety of drinking water necessitates balancing water source protection and water treatment techniques for a water supply with management of the distribution system to prevent recontamination of treated water. Due to the intermittent and often unpredictable nature of pathogen contamination, water system managers must emphasize a “best practice” approach to produce water of consistent microbiological quality. This review intends to evaluate some available data, information and research results regarding the potential microbial health risks associated with the distribution system issues.

**Key words**: Drinking water; Human health; Microbiological safety; Pathogens; Water quality
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1. MICROBIAL RISK IN WATER AND ITS PREVENTION

The safety of drinking water depends on a series of factors, including quality of source water, effectiveness of treatments, reliability and integrity of engineered infrastructure and potential of microbial regrowth in the pipes. At each stage in the production and delivery of drinking water, hazards can potentially compromise the quality of the water.

Distribution systems may be less exposed to contamination than open surface water catchments. On the other hand, if piped systems become contaminated, there may be no treatment processes to reduce risks from the introduced hazards. In fact, biofilms, sediments and corrosion products may harbour pathogenic microrganisms introduced through inefficient treatment or breaches of the integrity of the distribution system. Hidden in the sediments or embedded in the biofilm and in tubercles, pathogens could be released during repairs and cleaning operations, by erosion caused by sudden changes in flow patterns or following the continuous natural detachment of biofilm. Pathogens’ survival depends on their nature, microbial activity in the biofilm and environmental factors. Only a few pathogenic bacterial species may multiply if favourable conditions, such as appropriate water temperature and nutrients are present.

Viruses and protozoa are obligate parasites and they need a human or animal host to multiply. If they enter the pipe network, they can only survive for a limited period; the infective dose for human hosts is likely to be reached only if large accumulations occur within system deposits. Such accumulations may occur as a result of cross-connections, backflow or contamination intrusion through pipe fractures.

Although there are currently no reports of health effects directly attributed to the long-term survival of pathogens within a distribution system, but Legionella, such organisms have been shown to persist within biofilms, thereby presenting a potential underlying health concern to consumers (Szewzyk et al., 2000).

Biofilms can bind and accumulate organic and inorganic contaminants, as well as particulate and colloidal matter (Flemming, 1995). Within biofilms, microbial pathogens can be protected from biological, physical, chemical and environmental stresses, including predation, dehydration and changes or fluxes in the environment (Buswell et al., 1998; Walker et al., 1995).

Bacterial pathogens such as Helicobacter pylori, enterotoxigenic E. coli, Salmonella typhimurium, Campylobacter and Pseudomonas species, mycobacteria can persist within biofilms, as well Legionella and amoebae (Szewzyk et al., 1994; Armon et al., 1997; Mackay et al., 1998; Buswell et al., 1998; Wingender & Flemming, 2004). The potential therefore exists for such pathogens to accumulate and persist within municipal distribution systems. Recently it has also been shown that biofilms are significant reservoirs for cysts, oocysts, viruses compared to abiotic surfaces (Searcy et al., 2006; Helmi et al., 2008). Pathogens that remain in the biofilm can have important public health implications because they may persist in the biofilm and eventually be released, resulting in potential human exposure.

1.1. Monitoring activities for microbial risks in water

Currently, routine monitoring of water quality is based on the detection of indicator organisms that are surrogate markers of contamination. In fact, it is still not possible to detect
pathogens even if in the recent years considerable advances in recovery methods have been made. Thus direct testing for pathogens has not been adopted for routine monitoring purposes.

The traditional indicator organisms (faecal coliforms and E. coli) provide a good measure of potential health risks from bacterial pathogens. However, they correlate less well with viral risks and rather poorly with risks from protozoa and toxic algae (Craun et al., 1997; Baker et al., 2001; Skraber et al., 2004).

The reasons for this relate to the biological and physicochemical properties of the different groups of pathogens. The bacterial pathogens are very similar to the faecal indicator bacteria in terms of their ability to survive in the environment and their response to water treatment processes. Therefore, contamination events or water treatment failures that lead to the appearance of these indicators in finished water supplies may easily result in the appearance of bacterial pathogens. In contrast, both viruses and protozoa tend to survive longer in the environment than do faecal bacteria. Thus, as they also have a higher degree of resistance to disinfection may be present in water when bacterial indicators, more susceptible, are not longer present.

Many studies were carried out with the aim to provide better markers of viral and protozoan risks, and work continues in this area. The emergence of protozoan pathogens as major causes of waterborne disease has prompted renewed interest in the direct detection of pathogens in water rather than in monitoring for indicators. Many alternative indicators to coliforms have been proposed, including enterococci, Clostridium perfringens, Bacteroides fragilis, bifidobacteria, bacteriophages, and non-microbiological indicators such as faecal sterols (Hussain et al., 2010). Of these alternative indicators, enterococci seems to have gained the most support. However, some studies have expressed similar consideration over low specificity for faecal contamination associated with the use of enterococci, as with coliforms (Sorensen et al., 1989; Sinton et al., 1993; Pinto et al., 1999; Edberg et al., 2000).

Thus, till now despite the investment of considerable resources in the investigation of potential new indicators, none has yet been accepted as being practical and broadly applicable for routine monitoring purposes.

It is important to realize that routine monitoring of treated water quality can trigger a corrective response only after a problem has occurred and affected the finished product. Microbiological tests generally require incubation times of several to 48 h, and if adverse test results are obtained, preliminary investigations to verify the problem are likely to take at least several hours more. Thus, by the time a public health response can be triggered (e.g., a boil water advisory, oblige not to use water for drinking), it is likely that people will have already consumed the affected water.

Routine monitoring is an essential component of water supply management, but it is not enough to protect public health. In fact, microbiological water quality can be highly variable and a single test only represents a snapshot of the system in time. The role of routine monitoring is to verify that the components of the water supply system are operating correctly and that water complying with specific requirements. Monitoring cannot be a preventive management tool because of the time delay between sampling and obtaining analysis results. For this reason, the scientific community agrees that implementing intensive microbiological monitoring schemes (more frequent sampling of finished water) may not be a cost-effective way to improve microbiological safety. More intensive microbiological sampling of finished water can improve future water quality only if it provides information that helps better system management.

A new approach can link monitoring frequency of finished water and sanitary surveys, as complementary activities.

Water quality testings creates accurate information regarding water quality, and sanitary surveys enable hazard identification (potential causes for contamination).
1.1.1. Monitoring programs and sampling procedures

Currently, water quality monitoring programs are designed to incorporate the following:

- **Frequency of samples**
  Sampling is generally correlated to the volume of water distributed or produced each day within a supply zone;

- **Location of samples**
  For compliance purposes, the number of samples should be distributed equally in time and location. In the case of water supplied from a distribution network, at the point at which it emerges from the taps that are normally used for human consumption;

- **Selection of test methods**
  In order to ensure comparability of results between water supplies and laboratories, standard test methods are specified;

- **Quality control of all stages of sampling, transport, testing, and reporting**
  Good Laboratory Practice (GLP) requires that testing be carried out in a uniform manner with adequate documentation and quality control. Again, this ensures accuracy and comparability of results;

- **Laboratory or method certification accreditation**:  
  Regulatory authorities may require or recommend that tests be carried out only by laboratories with specific certification to ensure an externally verified level of proficiency;

- **Costs and availability of resources**: 
  Monitoring programs are considered in the context of available resources, and are designed to provide necessary and useful information in a cost-effective manner.

Water quality standards and guidelines generally include requirements or recommendations on sample locations and frequencies, and on the manner in which compliance is assessed. In addition to carrying out the sampling program required for assessing regulatory compliance, water authorities may also choose to do additional monitoring of the same parameters or additional parameters for their own operational purposes according to specific site condition.

Water quality monitoring may be considered within a total quality management system framework. Ten elements are considered in a total quality management system framework:

- organization;
- personnel;
- equipment;
- purchasing and inventory;
- process control;
- documents and records;
- internal assessment;
- process improvement;
- service and satisfaction.

Within this framework, each laboratory test method may be considered in the following phases or stages:

- pre-analytical phase (sampling, transporting samples, ordering test);
- analytical phase (laboratory testing or testing);
- post-analytical phase (reporting, communicating).

Standardization of sampling, transport, and storage conditions is important in any analytical context, but it is particularly critical for microbiological testing. Some microrganisms are able to
grow in water samples, thus producing an erroneously high result, or false positive. Conversely, some microorganisms may die or deteriorate in samples, producing a spuriously low result, or false negative. The aim is to ensure that the sample reaches the analytical phase with as little alteration as possible in microbiological content from the time it was collected from the water supply. Generally, samples should be stored and transported at 4°C and tested within 24 hours of collection.

Detailed advice on the procedures to be used for sampling different sources of water or treatment plants and distribution systems and at the tap can be found in the Guidelines for drinking water quality and in the specific standards of the World Health Organization (WHO, 2008).

Care must be taken to ensure that samples are representative of the water to be examined and that no accidental contamination occurs during sampling and analysis. Sample collectors should, therefore, be trained and made aware of the responsible nature of their work. Samples should be clearly labelled with the site, date, time, nature of the work, and other relevant information and sent to the laboratory for analysis without delay (Bonadonna & Ottaviani, 2007).

If the water to be examined is likely to contain chlorine-based disinfectants, then sodium thiosulfate solution should be added to neutralize any residual disinfectant agent. A properly controlled concentration of thiosulfate has no significant effect on microorganisms, either in chlorinated or in unchlorinated water samples during storage.

When samples of disinfected water are taken, the concentration of residual disinfectant at the sampling point and the pH should be determined at the time of collection.

When a number of samples are to be taken for various purposes from the same location, the sample for bacteriological examination, rather than for chemical examination, should be collected first to avoid the danger of contamination of the sampling point.

Samples must be taken from different parts of the distribution system to ensure that all parts of the system are tested. When streams, lakes, or cisterns are being sampled, the water must be taken from below the surface, away from banks, sides of tanks, and stagnant zones, and without stirring up sediments. Taps, sampling ports, and the orifices of pumps should, if possible, be disinfected and a quantity of water run to waste to flush out the standing water in the pipe, before the sample is taken. Sampling ports in treatment processes and on water mains must be carefully sited, to ensure that samples are representative.

The changes that may occur in the bacterial content of water on storage can be reduced to a minimum by ensuring that samples are not exposed to light and are kept cool, preferably between 4°C and 10°C, but not frozen. Examination should begin as soon as possible after sampling and certainly within 24 hours. If samples cannot be cooled, they must be examined within 2 hours of sampling. If neither condition can be met, the sample should not be analysed. The box used to carry samples should be cleaned and disinfected after each use to avoid contaminating the surfaces of bottles and the sampler’s hands.

Especially time considerations are important as some bacteria will multiply over time and produce falsely elevated concentrations (also above acceptable standards) if they are delayed in transport to the laboratory.

For bacterial indicator testing, small volumes (usually 100 mL) are sampled. Aseptic collection techniques and sterilized collection bottles are required, and any disinfectant residual in the water must be neutralized (otherwise the disinfectant would continue to kill microorganisms during transport and storage). Specific pathogen sampling has the same constraints as bacterial indicator sampling and transport. A recommended schema for storage of water samples is reported in Table 1.1.
Table 1.1. Recommended time for storage of water samples to be analyzed for microbial parameters

<table>
<thead>
<tr>
<th>Organism</th>
<th>Time in h (maximum time allowed between sampling and analysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPC at 22°C or 36°C</td>
<td>8 (12)</td>
</tr>
<tr>
<td><em>Escherichia coli</em> and coliforms</td>
<td>12 (18)</td>
</tr>
<tr>
<td>Enterococci</td>
<td>12 (18)</td>
</tr>
<tr>
<td><em>Clostridia</em></td>
<td>48 (72)</td>
</tr>
<tr>
<td>Bacteriophages</td>
<td>48 (72)</td>
</tr>
<tr>
<td><em>Salmonella</em> and <em>Enterobacteriaceae</em></td>
<td>12 (18)</td>
</tr>
<tr>
<td>Enteroviruses</td>
<td>48 (72)</td>
</tr>
<tr>
<td>Cysts/oocysts of <em>Giardia/Cryptosporidum</em></td>
<td>48 (72)</td>
</tr>
<tr>
<td><em>Amoebae</em></td>
<td>48 (72)</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>8 (12)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>8 (12)</td>
</tr>
<tr>
<td><em>Legionella</em></td>
<td>48 (72)</td>
</tr>
<tr>
<td><em>Cianobacteria</em></td>
<td>48 (72)</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>6 (8)</td>
</tr>
<tr>
<td>Helminth eggs (at pH 2.0)</td>
<td>48 (72)</td>
</tr>
</tbody>
</table>

Samples are time sensitive. Delays in transport may result in the overgrowth of non-pathogenic bacteria and false negative results can be obtained. Thus, some pathogens that are present will not be detected. Time sensitivity is not as critical with parasites, such as *Cryptosporidium* and *Giardia*, because parasites do not multiply outside the host. Once present in the environment, their infective forms (oocysts and cysts) are persistent for long time. Concentrations of parasites are low in water, and large-volume samples are recommended. Transport of these large volumes makes filtration and collection of filtrates in the field necessary.

Very little is understood about the survival dynamics of pathogenic viruses in water: some viruses may survive for long time in the environment while other viruses last for shorter time.

Sample collection procedures should be specific and well documented. Staff must have access to these written procedures and should be adequately trained. For most microbiological testing, a sample is sent to the laboratory along with a requisition form. Accuracy is imperative, and sample information must include correct identification of the sample site and the collection time of the sample.

Analysis should be carried out by specified methods using appropriate materials and equipment that have been properly maintained and calibrated. Staff should be properly trained in laboratory methodologies and in the operation of equipment.

A number of different testing methods for indicator bacteria are available. Membrane filtration technique is the most commonly used laboratory method. Increasingly, laboratories are adopting more rapid methods using defined substrate technology systems and multiwell plates to obtain a result more quickly and to reduce costs for colony confirmation.

Laboratory methods to detect pathogens characteristically include three general steps: concentration, separation, and characterization:

- concentration: because the numbers of pathogenic microorganisms are generally low in water, techniques such as centrifugation, filtration, and chemical methods are used to concentrate the pathogens;
- separation: because pathogens may be mixed with numerous other non-pathogenic microorganisms or debris, techniques such as density gradients, differentiation growth
behaviours, and, most recently, antibody capture and flow cytometry are used to separate pathogens from other microorganisms;
- characterization: once the pathogen has been concentrated and separated from other components of the water sample, it can be characterized using techniques such as culture, biochemical differentiation, microscopy, and, most recently, nucleic acid methods.

The specifics of direct testing methods vary with each microbial group and genus. There is no single method that can be applied to all bacterial waterborne pathogens.

Staff should keep adequate records to track samples, such as recording when the sample was collected, when it arrived at the laboratory, and when it was tested. Records should also include the names of staff performing the tests and relevant information about materials and equipment used in testing that might help explain anomalous results if they occur.

1.1.2. Analytical methods: general approach

A number of methods to detect microorganisms in water has been developed for pathogens. Besides cultural methods for bacteria, a number of immunological and molecular techniques are available for detection and identification of viruses and protozoa as well as bacteria.

All of these tests have deficiencies that limit their use for routine monitoring of drinking water supplies and are not standardized (Allen et al., 2000).

One of the biggest limits is associated to the presence of pathogens that in source water supplies is often intermittent and/or undetectable.

While a large number of pathogens may be present in animal and human faeces, the number of people infected with a specific pathogen in a particular population will vary over time as a consequence of changes in exposure, population immunity, life style and seasonal factors. The potential for pathogens to enter water supplies will also be influenced by catchment management practices, protection of the site and precipitation patterns. Therefore, it is not possible to know which pathogens will be present at any given time, nor how abundant they will be.

When present, pathogens are usually in low numbers. Although an infected individual may excrete very large numbers of a pathogen, most individuals in a population will not be simultaneously infected. Thus, the infected material is likely to be “diluted” by large quantities of non pathogenic faecal and environmental microorganisms.

Conversely, faecal indicator organisms will always be present in much higher numbers in water contaminated by human or animal faeces. Pathogen numbers in treated water are generally lower. The degree of pathogen removal accomplished by water treatment depends on the specific system and the specific pathogen in question; however, reductions of several orders of magnitude are possible, especially for bacteria. Thus, reliable detection of pathogens in treated and disinfected water requires water samples of much larger volume than that required for pathogen detection in source water. This is technically more difficult and expensive and sampling generally has to be done in situ.

Test methods for environmental samples pose a particular challenge as the composition of samples is extremely variable, with many substances and contaminants that may potentially affect the test result. The difficulties experienced with methods for protozoa testing illustrate these problems (Clancy et al., 1994; Pontius and Clancy, 2000). There have been a number of incidents where public health alerts have been issued after the apparent detection of Cryptosporidium in water supplies, but investigations have thrown doubt on the test results, and no evidence of waterborne disease has been found (Clancy, 2000).
Small numbers of samples result in significant statistical limitations on the interpretation of the result. Microorganisms are not uniformly distributed in water, but follow a random statistical distribution. They may also exist as clumps rather than as single cells. One cannot measure a single sample and assume that the concentration detected is the average concentration of cells in the water source. Taking more samples can improve statistical accuracy. However, increasing the number of samples to detect pathogens is generally limited by cost and logistical considerations.

The primary test result does not necessarily indicate whether the organism is able to infect humans (i.e., viability and infectivity may be uncertain). For bacterial pathogens, detection methods generally require growth, or at least some metabolic activity, so there is a reasonable assumption that the cells are viable and able to cause infection. However, for viruses and protozoa, tests are based on detecting physical components of the cell (e.g., genetic material or cellular structures) and do not necessarily indicate viability. For these organisms, viability and infectivity can only be reliably assessed by more complex techniques (tissue culture, excystation and animal infection) which are beyond the scope of routine testing and are strictly related to the laboratory skill.

The delay between sampling and obtaining the result may be too long for timely public health action. Routine microbiological tests for indicators generally require a period of at least several hours (18 hours for Colilert), and more commonly one to two days to produce a result. Some specialized pathogen tests may take even longer.

Notifying health authorities, conducting preliminary investigations, and deciding on appropriate public health action are likely to take several more hours. This means that in the event that pathogens are found in treated water, the affected water will probably reach customers and be consumed by many of them before a jurisdiction can initiate an action, such as a boil water advisory.

The limitations of pathogen testing have resulted in regulatory authorities in most countries not recommending routine testing for these organisms. A notable exception has been the United Kingdom (Drinking Water Inspectorate, 1999) where a daily monitoring for Cryptosporidium on water supplies considered to be at risk of contamination was imposed (since 1999 until December 2007). Where water supplies exceeded the specified average concentration of oocysts (1 oocyst/10 L), water authorities could be subjected to prosecution. This legislation was developed primarily as a legal and political response to the failure of a court case against a water utility. The cost-effectiveness of such monitoring as a public health measure was very early questioned (Fairley et al., 1999) and in 2007 this duty was revoked.

In the United States, the US EPA mandated that large water authorities test source water for Cryptosporidium over an 18-month period. Such testing allowed the gathering of Cryptosporidium occurrence data. However, the specified test method was significantly flawed, and doubts have been expressed on whether the results have provided useful information for water quality management (Pontius and Clancy, 1999; Allen et al., 2000). A further round of source water testing was conducted and an improved method was used. The results of this testing program will be used to assign water sources to risk categories, and a graded range of treatment requirements will then be required depending on the source water category. Cryptosporidium testing will not be required after the initial sampling program according to the instructions of the US EPA (2001).

While pathogen testing is generally considered to be of doubtful value for routine monitoring, it can form an important part of investigative monitoring programs. Such programs should be aimed at assessing the relative importance of pathogen sources in catchments and characterizing the conditions (e.g., extreme weather events) that may lead to elevated numbers
of pathogens in source water. Investigative programs should also assess the impact of mitigation strategies, such as watershed protection and should optimize water treatment processes.

In addition to tests for indicator organisms, routine water monitoring generally tests for a range of other parameters that are relevant to assessing microbiological risks or provide information for day-to-day system management.

For example, the knowledge of water turbidity can be helpful as a measure of the fine suspended or colloidal material in water. Turbidity is dependent on the size, shape, and translucency of suspended particles in the sample. Such material tends to protect microorganisms in water from the disinfecting action of chlorine and to supply nutrients, but there is no direct and reproducible relationship between turbidity and microbiological water quality. Elevated turbidity is evaluated in conjunction with more direct measures of microbiological water quality, such as indicator organisms.

Turbidity is measured in Nephelometric Turbidity Units (NTU), and for this parameter the value of ≤1.0 NTU has to be applied when surface water is treated (European Directive 98/83/EC). However, well-operated modern filtration plants are able to produce water with very low turbidity in the range of 0.1 to 0.3 NTU.

Waterborne disease outbreaks have sometimes been associated with rises in turbidity, due either to highly contaminated source water overwhelming treatment capability, or failures in treatment processes that result in suboptimal removal of pathogens. Even short periods of suboptimal filter performance may be associated with health risks; therefore, emphasis on the need to maintain continuous filter performance has to be increased.

Frequent or continuous measurement of turbidity from individual filters in water treatment plants is now widely used to monitor the effectiveness of filtration and to maintain optimum operating conditions. In this context, increases in turbidity may be a sign of impending filter “breakthrough”, which may allow pathogens retained in the filter medium to enter the filtered water supply.

Filtration is presently the primary barrier against disinfectant-resistant pathogens such as Cryptosporidium, and filter performance is considered to provide a 2-log removal of such organisms. Filtration plants that consistently maintain even lower levels of turbidity (e.g., 0.1 NTU) would be expected to achieve a higher degree of removal.

Particle counting is not currently used on a routine basis in most water supplies. However, it has been increasingly recognized as a way to monitor the performance of water treatment plants, and – like turbidity monitoring – provides an early indication of problems with filtration processes.

### 1.2. Water safety plans

Measurements of water quality may be considered in a hazard analysis critical control point watershed-to-tap framework. The focus of this concept, as the HACCP (Hazard Analysis and Critical Control Points) approach, developed as a generic, scientifically based system to ensure safe food production (ILSI Europe, 1999), is on critical control points of the water production chain (Benford, 2001). Critical Control Points (CCP) are sites at which a failure will result in significant harmful events. The application of HACCP principles to drinking water supplies acknowledges the importance of water source quality, but identifies drinking water treatment as the prime CCP.

Identifying, prioritizing and preventing risk arising from hazards is the basis of the Water Safety Plan (WSP) system.
The objective of a WSP is to supply water of a quality that will allow health-based targets to be met. The success of the plan is assessed through surveillance and control (WHO, 2005).

The system assessment is meant to determine if the system is capable of delivering drinking water that meets the health-based targets. If the assessment finds that the system theoretically is capable of doing that, monitoring is the next step in ensuring that it actually meets the targets. If the system is not able to meet the health-based targets it has to be modified in some way to meet the targets. When the assessment is carried through it is important that all parts of the drinking water system are considered concurrently and that interactions and influences between each part and their overall effect are taken into consideration.

The operational monitoring aims to assess control measures in order to ensure that the drinking water system is operating properly. A control measure is an action that serves to reduce or eliminate contamination and is identified during the system assessment. The applied control measures in a system should together ensure that the drinking water meets the health-based targets and that the system is able to minimize risks.

The purpose of the management plans are to document and communicate all information regarding the management of drinking water quality. A management plan includes for example information regarding the system assessment and operational monitoring, and it also describes actions in both normal operation and during situations where control of the system is lost (Davison et al., 2002).

In a more detailed way it is possible distinguish three central components of a water safety plan:

- system assessment, which involves assessing the capability of the drinking water supply chain (up to the point of consumption) to deliver water of a quality that meets the identified targets, and assessing design criteria for new systems;
- identification of control measures in a drinking water system that will collectively control identified risks and ensure that health-based targets are met (for each control measure identified, an appropriate means of monitoring should be defined that will ensure that any deviation from required performance is rapidly detected in a timely manner);
- management plans that describe actions to be taken during normal operation or incident conditions, and that document system assessment (including upgrade and improvement), monitoring, communication plans and supporting programmes.

In general, water entering a drinking water distribution system should be safe to drink, without additional treatment, once it has reached the first consumer connection. Therefore, the management of distribution systems primarily involves maintaining water quality, and minimizing the risk of contamination and deterioration of quality during transport. However, many distribution systems are a complex array of pipes, pumps, tanks and valves, which means that risks are not always as easily identified as in other areas of drinking water supply.

The first step in developing a water safety plan is to assemble a multidisciplinary team with an understanding of the specific distribution system, to describe that system. The team would typically include managers, engineers (operations, maintenance, design and capital investment), water quality control staff (microbiologists and chemists) and technical staff involved in day-to-day operations. All members of the team should have a good knowledge of the system.

The next step is to document and describe the system. The description can include a basic flow diagram of the drinking water distribution system, and reference to maps showing water quality networks and zones.

It is important to acquire the elements of the water supply system in sufficient detail to allow risks to be assessed and control measures to be identified. Therefore, pressure, pumps,
connections, valves (and their status) and tanks need to be considered. Furthermore, other important features of the system can include:

- service reservoirs, balancing tanks, booster stations and break-pressure tanks;
- zones of supply from each source;
- layout of primary, secondary and tertiary pipelines;
- location of major valve boxes and junctions;
- flow within the system (for example, noting where there are areas of interconnection between different zones);
- numbers of consumer connections;
- hydraulic system flow rates and paths (including two-way flow);
- connections with high backflow hazard.

The representation of the system must be conceptually accurate, because the team should use the diagram as the basis for hazard analysis. Therefore, the team should validate the completeness and accuracy of the flow diagram and maps; for example, by visually checking against features observed on the ground. Proof of validation is typically recorded, together with an accountability.

The other step in risk assessment is to systematically evaluate the system’s potential vulnerability to external hazards, using the flow diagrams and system maps. Information that would normally be part of this assessment can includes:

- areas where soil moisture content or flooding makes it likely that contamination (e.g. faecal matter) from sources on the surface or shallow subsurface will enter the system;
- any other sources of contamination found in the urban area (e.g. animal husbandry);
- areas of high population density (used as a surrogate for faecal loading in the environment);
- areas of low pressure within the system;
- areas of intermittent supply and their likely recharging pattern;
- pipe material, age and condition (a vulnerability score can be developed based on likely risk of breaks or joint failure);
- cross-connections, proximity to sewers and high-hazard facilities, and the relative depth of water supply pipes and sewers;
- low-lying areas prone to flooding;
- depth to which pipes are buried (this differs from the point above concerning sewers, because it relates to the risk of accidental breakage by traffic, etc);
- condition and age of service reservoirs;
- areas where there are illegal connections;
- areas where a significant proportion of houses use household storage which may include the attachment of small pumps to the main, for pumping to roof tanks;
- areas of known high leakage;
- large buildings, such as hospitals or schools.

At each point, the objective is to identify how contamination could arise from the identified hazards, by considering the events that could lead to the presence of contamination.

The output from this collection of information is a list of hazardous events, their associated hazards and a reference to where in the system or process the risks are located.

This system involves identification of critical points to control hazards and maintain best management practices throughout production and distribution. Criteria are established for each control point, which are monitored, and corrective actions are established that should be carried out when critical limits are not met (ILSI Europe, 1997).

The working process in a HACCP system consists of consecutive steps that can be reassumed as follows:
– conduct a hazard analysis;
– determine the Critical Control Points (location in the process that a certain hazard can be controlled, either through total prevention, elimination or reduction);
– establish critical limits (a criterion that separates acceptability from unacceptability, for example a certain temperature, moisture level, pH, etc.) their exceeding can be an index of risk condition;
– establish a system to monitor control of the CCP;
– establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control;
– establish procedures for verification to confirm that the system is working effectively;
– establish documentation concerning all procedures and records appropriate to these principles and their application.

In large and complex systems, many risks may be identified and can be difficult to set priorities. Simple matrices for risk assessment typically combine technical information from guidelines, scientific literature and industry practice with well informed expert judgment. The risk ranking will be specific for any particular water supply system because each system is unique.

By using a semi-quantitative risk assessment, the WSP team can calculate a priority score for each hazardous event identified. The commonest way to describe hazard or hazardous events associates the likelihood of occurrence (e.g., certain, possible, rare) and the severity of consequences if the hazard occurred (e.g., insignificant, major, catastrophic).

Also the sanitary survey gathers field evidence to support the risk assessment. It includes systematic investigation of the complete distribution system, to identify all major hazards and vulnerable points. The survey deals mainly with the physical state of infrastructure, focusing primarily on external threats.

Urban piped water supplies can be difficult to survey, because most sanitary inspections are based on observation. Leaks associated with deep-laid pipes are often difficult to detect through observation, and contamination may occur a significant distance from a sample site. However, simple visual and question based approaches can still provide useful information about whether risks are at the level of the general supply or are localized. Local risks will include aspects such as the pooling of stagnant water around the joints between riser pipes and delivery mains. Tap leakage, pipe exposure and waste allowed to collect around the tap may be significant causes of contamination. Inspections are required at service reservoirs because these have the potential to cause widespread contamination.

In the context of a WSP, managing risks in distribution must focus on preventing recontamination or degradation of water quality caused by breaches in system integrity or difficult operational circumstances. It is useful to determine what contaminants are of concern (hazard assessment), and how they may reach unacceptable levels (risk characterization). This makes it easier to identify important potential contaminants (hazards) and the risk of events occurring that could cause these hazards to contaminate the system (hazardous events).

Risk management in distribution systems, as well in catchments, has the aim to prevent the introduction of hazards. However, a major difference is that distribution systems represent the final barrier before consumption in many supplies, whereas hazards arising in catchments may be reduced during storage and treatment.

Any risk management activity in a drinking water supply is considered to be a control measure. Examples of control measures in water distribution are positive pressure, intact pipe networks, backflow preventers and pest proofing on tanks.
Control measures are identified by considering the events that can cause contamination of water, both directly and indirectly, and the activities that can mitigate the risks from those events. Examples of control measures in the distribution system include:
- maintenance of the distribution system;
- availability of backup systems;
- maintenance of an adequate disinfectant residual in the system;
- presence of devices to prevent cross-connection and backflow;
- use of fully enclosed distribution system and storages;
- appropriate procedures, including disinfection of water mains after repairs;
- maintenance of adequate pressure in the system;
- maintenance of security to prevent sabotage, criminal damage, terrorism acts.

The WSP must be regularly reviewed through analysis of the data collected as part of the monitoring process but periodically, the WSP team should also meet and review the plan and learn from experiences and new procedures. The review process is essential in the overall implementation and provides the basis from which future assessments can be made. Following an emergency/incident/near miss risk should be reassessed and may need to be fed into the improvement/upgrade plan. A particular benefit of implementing the water safety plan framework is just a likely reduction in the number and severity of incidents, emergencies or near-misses affecting or potentially affecting drinking water quality.

1.3. Response to microbial water quality problems and emergencies

Surveillance is the continuous and vigilant public health assessment and overview of the safety and acceptability of drinking water supplies (WHO, 1976). Each component part of the drinking water production chain - the source, treatment, storage, and distribution - must function without risk of failure. A failure in one part will jeopardize and nullify the effects of other parts. Water is liable to contamination at all stages in the process of supply, hence the need for constant supervision. At the same time, careful assessment of likely sources of risk and breakdown are needed before a supply is planned and installed and, indeed, continuously thereafter, because of changing conditions and potential sources of contamination.

Environmental health hazards, threats to health from exposure to disease causing agents, are closely related to disasters and emergencies in a variety of ways.

During an emergency in which there is evidence of microbial contamination of the drinking water supply, it may be necessary either to modify the treatment of existing sources or to temporarily use alternative sources of drinking water.

If a good microbial quality cannot be maintained, it may be necessary to forbid by law the use for water for human consumption and advise consumers to boil the water during the emergency period. Initiating superchlorination and undertaking immediate corrective measures may be preferable where the speed of response is sufficient to prevent significant quantities of contaminated water reaching consumers.

When faecal contamination of a drinking water supply is detected or during an outbreak event, the concentration of free chlorine should be increased to greater than 0.2 mg/litre throughout the system as a minimum immediate response. It is most important that decisions are taken in consultation with public health authorities.

Water avoidance advisories, which share many features with boil water advisories but are less common, are applied when the parameter of concern, primarily chemical contaminants, is
not susceptible to boiling. Nevertheless this advice should be avoided as much as possible also be cause the use of alternative water source (e.g. water from water tanker and reservoir) could represent a major health risk respect to the use of boiled water. Boil water advisories should identify both affected and unaffected uses of the considered drinking water supplies. Generally, the advisory will indicate that unboiled water should not be used for drinking, preparing cold drinks, making ice, preparing or washing food or brushing teeth. Unless heavily contaminated, unboiled water will generally be safe for bathing (providing swallowing of water is avoided) and washing clothes. A boil water advisory could include specific advice for vulnerable groups, such as pregnant women, immunocompromised subjects, infant and elders.

Surely boil water recommendations are a serious measure that can have substantial adverse consequences. Advisories should normally include a description of the problem and details on potential health risks and symptoms, activities that are impacted, investigative actions and corrective measures that have been initiated, as well as the expected time to resolve the problem.

If the advisory is related to an outbreak of illness, specific information should be provided on the nature of the outbreak, the illness and the public health response. It should indicate that the water can be made safe by bringing it to a rolling boil. The types of event that should lead to consideration of boil water advisories include:

- substantial deterioration in source water quality;
- major failures associated with treatment processes or the integrity of distribution systems;
- inadequate disinfection;
- detection of pathogens or faecal indicators in drinking water; and
- epidemiological evidence suggesting that drinking water is responsible for an outbreak of illness.

Nevertheless advice to boil water can have negative public health consequences: an increase of the risk of scalding among the population and increased anxiety, even after the advice is revoked. In addition, not all consumers will follow the advice issued, even at the outset; if boil water advisories are issued frequently or are left in place for long periods, compliance will decrease. Hence, advisories should be issued only after careful consideration of all available information by the public health authority and the incident response team and conclusion that there is an ongoing risk to public health that outweighs any risk from the advice to boil water.

Where microbial contamination is detected in drinking water samples, factors that should be considered in evaluating the need for an advisory should take account of different information:

- reliability and accuracy of results;
- vulnerability of source water to contamination;
- confirmation of deterioration in source water quality;
- source water monitoring results;
- data from operational monitoring of treatment and disinfection processes;
- disinfectant residuals; and
- physical integrity of the distribution system.

The available information should be reviewed to determine the likely source of the contamination and the likelihood of recurrence or persistence.

During emergency circumstances, specific advice should also be provided to facilities such as hospitals, child care facilities, dental clinics, dialysis centres, doctors’ offices, schools, food suppliers and manufacturers, hotels, restaurants and operators of public swimming pools and spas.

Provision of alternative supplies of drinking water, such as bottled water or bulk water, should be considered with attention when temporary boil water or water avoidance advisories
are in place. The protocols should identify sources of alternative supplies and mechanisms for delivery.

When boil water and water avoidance advisories are revoked, information should be provided through same channels and to the same groups that received the original advice. In addition, operators/managers or occupants of large buildings and buildings with storage tanks should be advised of the need to ensure that storages and extensive internal distribution systems are thoroughly flushed before normal uses are restored.
2. METHODS FOR CHARACTERIZING MICROBIAL RISK

The essential objective for determining the microbiological quality of water is to identify, evaluate and through corrective actions, reduce the public health risk from consuming water intended for drinking and from exposure to recreational water.

Traditionally, microbiological and chemical contaminants have been regulated in very different ways. Rather than fixing a specific concentration for each type of microorganisms, regulators have established a zero tolerance goal for microbial contaminants and have used indicator organisms (E. coli, mainly) to show the possible presence of faecal contamination from warm blooded animals (man included). While this methodology has served well for indicating sewage contamination of surface waters and for controlling such diseases as cholera and typhoid fever, an increasing number of deficiencies with this approach have come to light in recent decades (WHO, 2008).

One lack in the current method used to regulate microorganisms is that, as a result of differences in survival capacity and transport, viruses and protozoa can be present and viable in raw waters in which indicator organisms are absent or viable but not culturable (VBNC), so assessments of the safety of raw waters fail and underestimate the potential health risk. A second problem is that some bacteria, many viruses and protozoa show greater resistance to many conventional treatment processes included the disinfection, than do the traditional bacterial indicators; also in this situation, assessment of the safety of treated water fails. The third limitation is that an increasing number of such pathogens as Legionella, mycobacteria, etc. can originate from sources other than faecal pollution. Thus, the faecal indicator strategy is less relevant for these types of microorganisms.

At the end of last century, WHO began to set goals for microbial acceptable risk, researchers have begun to publish on methods of risk assessment for microbiological contaminants, and new techniques have evolved that suggest that the development of an occurrence database for pathogens may become possible (WHO, 1996).

Choice of microbial contaminants for development of regulations and standards has been based on reported waterborne disease outbreaks. Formal risk assessment methods utilizing occurrence databases and exposure assessment were used only recently.

The approach of microbial quantitative risk associated with drinking water is based on dose-response modelling (Haas, 1983). Mathematical models can be use for best estimating the probability of infection from the existing databases associated with human exposure experiments. Exponential models can also be used in order to evaluate daily and annual risks of protozoan infections from exposure to contaminated water after various levels of reduction through treatment (Rose et al., 1991). This approach applied at Giardia, requires a safety goal of achieving 99.9% reductions of Giardia cysts through filtration and disinfection in all surface water systems. It has been accepted that this level of pathogen removal would correspond to an annual risk of no more than 1 infection per 10,000 people exposed over a year from drinking water (United States Environmental Protection Agency, 1989).

It has been shown that the β-Poisson distribution best describes the probability of infection from enteric viruses. This model has been used to estimate the risk of infection, clinical disease, and mortality for hypothetical levels of viruses in drinking water. Meanwhile, the development and availability of new detection techniques for viruses have allowed the creation of a meaningful occurrence database that can be used in these types of risk estimates (Haas, 1997).

Analytical methods remain a critical issue for assessment of exposure to microbiological contaminants. There has been limited development and standardization of processes, however,
for laboratory approval and appropriate application of both established methods (e.g., microscopy) and newer methods. In fact, in recent years the development of newer methods has allowed to obtain faster and more reliable information on the pathogens in water (e.g., immunomagnetic capture and molecular techniques). Most available detection methods may be able to address some aspect of microbial occurrence (i.e., identification, quantification, viability, virulence, source, transport), but no single analytical method can be used to address all the needs of the exposure assessment (Table 2.1).

Table 2.1. Exposure factors related to risks of microbial contaminants in drinking water

<table>
<thead>
<tr>
<th>Exposure factor</th>
<th>Info requested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmission</td>
<td>Define oral-faecal, respiratory, contact, or multiple exposure routes</td>
</tr>
<tr>
<td>Environmental source</td>
<td>Determine levels found in human waste, animal waste, sediments, biofilm, and potential loading to a water system</td>
</tr>
<tr>
<td>Survival ability</td>
<td>Estimate inactivation in waste, soil, groundwater, surface water, sediments, biofilm, and determine effects of temperature, sunlight and dehydration</td>
</tr>
<tr>
<td>Regrowth potential</td>
<td>Determine growth in waste, soil, groundwater, surface water, sediments, biofilm and effects of temperature and nutrients</td>
</tr>
<tr>
<td>Occurrence in raw water</td>
<td>Estimate raw water type and level of contamination in different raw waters and determine spatial variations</td>
</tr>
<tr>
<td>Resistance to treatment</td>
<td>Determine reduction by waste treatment, drinking water treatment, and distribution; consider resistance to disinfection, removal by filtration, etc., and adequacy of surrogates (indicator bacteria, turbidity) to evaluate removal</td>
</tr>
<tr>
<td>Availability of methods</td>
<td>Develop methods for assessing source water, identifying environmental sources, quantifying organisms, determining viability, and assessing treated water</td>
</tr>
</tbody>
</table>

2.1. Exposure and health effects data

Data on both exposure and risk to human health are needed for assessing the risk associate to microorganisms in drinking water. However, many causes of waterborne disease are still unknown; thus, the disease potential for microorganisms occurring in water needs to be examined carefully (Table 2.2).

Outbreak survey should be a significant component of the health effects assessment. This is the result of the extreme costs associated with outbreaks, not only in medical care and days lost from work but in costs accrued in assessment of the outbreak, boil orders, communication efforts, remediation, and future safety efforts. The waterborne disease outbreak in Milwaukee USA in 1993, associated to the presence of *Cryptosporidium* in drinking water, cost the community many billion dollars, not including subsequent costs of aversion behaviour because of loss of confidence in the water supply (e.g., purchase of bottled water and point-of-use devices to further treat the water) (MacKenzie *et al.*, 1994).

Abatement of microorganisms by water process maintains a significant role in exposure assessment. While water treatment, such as chlorination, may readily control some microbial risks, such as *Shigella, Vibrio or Campylobacter*, the reliability of treatment and the potential for growth of microbial pathogens in the water distribution system must be included in any risk assessment.
### Table 2.2. Health factors associated with risks of microbial contaminants in drinking water

<table>
<thead>
<tr>
<th>Health effects</th>
<th>Info requested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimation of waterborne outbreaks</td>
<td>Magnitude of community impact, attack rates, hospitalization and mortality, sensitive populations, grade of contamination, duration, medical and community costs, course of immune response and secondary transmission</td>
</tr>
<tr>
<td>Estimation of endemic disease</td>
<td>Incidence, prevalence, geographic distribution, temporal distribution, percentage associated with various transmission routes (i.e., water versus food), demographics, sensitive populations, hospitalization, individual medical costs, antibody prevalence, infection rates, and illness rates</td>
</tr>
<tr>
<td>Immune status</td>
<td>Protection of sensitive populations, lifetime protection versus temporary protection, effects of age</td>
</tr>
<tr>
<td>Information on microbial pathogens</td>
<td>Mechanism of pathogenicity, virulence factors, virulence genes, antibiotic resistance</td>
</tr>
<tr>
<td>Disease description</td>
<td>Types of disease, duration, severity, medical treatment and costs, days lost, chronic sequelae, contributing risks (i.e., pregnancy, nutritional status, lifestyle, immune status)</td>
</tr>
</tbody>
</table>

Given the high risk of overcoming of the coliform standard limits (primarily in small public water systems), if disinfection failure continues to occur in a large percentage of facilities using highly polluted water supplies, the risk could become significant. It is critical that occurrence databases are developed for microorganisms that may exhibit a high level of virulence in water in order to determine the potential effects of treatment failures.

### 2.2. Health risk from distribution system contamination

The identification of microbial hazards associated with drinking water has been accomplished in the same manner since the first documented occurrence of a waterborne disease outbreak: a cholera outbreak that was associated with contamination of the Broad Street pump in London, England, in 1855 (Snow, 1855). The cause of this outbreak (contaminated drinking water) was determined through an epidemiological study. Since then, epidemiology has been the major science used to study the transmission of infectious disease through drinking water.

Epidemiological studies attempt both to show the influence of environmental factors on human health and to describe as applying knowledge of prevention and control to health problems. Furthermore, epidemiological studies can help control the occurrence of future waterborne disease outbreaks (Hunter et al., 2002).

Waterborne disease outbreaks have been investigated and initial efforts to control microbial pathogens focused on bacteria. Virus outbreaks began emerging in 1950 with hepatitis A being the primary concern. Several hundred enteric viruses are possibly important agents of waterborne disease (Fong & Lipp, 2005). However, information regarding the incidence of viral infections and the role of contaminated water in acquiring these is limited. Virus contamination of groundwater is of great concern because of the resistant nature of the viral structure, which interferes with disinfection, and the colloidal size of viruses, which makes them easily transported through soil systems (Crist et al., 2005). Viruses can survive for months in groundwater. Several studies have found viruses in high percentage of groundwaters (Yates et al., 1985; Schijven et al., 2006).
The most challenging aspects of reducing the risk of water contaminated distribution systems remain the possibility to quantify the existing risk. This is made complicated not only by the numerous factors that can constitute public health risks, including the multiplicity of microbial pathogens and chemical compounds, their distribution into the system, their concentration, etc., but also by the varying response that a given individual will have when exposed to those different factors.

Methods of risk assessment involve determining the likelihood and severity of different adverse impacts given exposure of a population to a hazard (Bartram et al., 2001). Risk analysis includes the process of risk assessment, as well as risk management activities to decide what an acceptable risk level is and to take actions to reduce it. Risk assessment requires the activities of hazard identification, exposure assessment, and dose-response (or exposure-response) assessment (Haas & Eisenberg, 2001).

Hazard identification is a process used to identify all possible situations where people may be exposed to injury, illness or disease, the type of injury or illness that may result from these and the way in which work is organised and managed. Differently, exposure assessment is the quantitative determination of the levels of contaminants (in the case of environmental exposures) individuals may consume/inhale/contact over a specific time period. Dose-response assessment is the quantitative determination of the likelihood of an individual having a particular adverse effect from a given exposure. Alternatively, this can be viewed as the proportion of persons in a population who are expected to have the adverse effect were they to have the particular exposure (Benford, 2001).

Several international agencies have developed specific guidelines and procedures for performing risk assessment, particularly for carcinogens and for substances that result in non-carcinogenic toxic effects. In the case of infectious agents (which are frequently the concern in drinking water), methodologies are still at a developmental stage and time has to be spent before obtaining a clarification of this issue.

One of the goals of performing risk assessment within a regulatory framework is to develop regulatory guidance or standards (or decide not to undertake such action) based on the results. This process, is part of risk analysis, and requires additional considerations such as cost and equity.

Drinking water can act as a transmission vehicle for a variety of hazardous agents: enteric microbial pathogens from human or animal faecal contamination (e.g., noroviruses, E. coli O157:H7, Cryptosporidium), aquatic microrganisms that can cause harmful infections in humans (e.g., nontuberculous mycobacteria, Legionella, Pseudomonas), toxins from aquatic microrganisms (such as cyanobacteria), and several classes of chemical contaminants (organic chemicals and various pesticides; inorganic chemicals; metals; disinfection byproducts or DBPs and radioactive compounds).

Contaminants in drinking water can produce adverse effects in humans due to multiple routes of exposure. In addition to risk from ingestion, exposure can also occur from inhalation and dermal routes. For example, inhalation of droplets containing respiratory pathogens (such as amoebae, Legionella or Mycobacterium) can result in illness.

It has been recognized for some years that consumers face risk from multiple hazards, and that action to reduce the risk from one hazard may increase the risk from other hazards given the same exposure. There are prominent examples of this phenomenon in the drinking water arena that have greatly complicated efforts to reduce overall risk from distribution systems. Havelaar et al. (2000) assessed the relative changes in risk from switching to ozone treatment of drinking water in the Netherlands. In this case, there was a projected reduction in risk from waterborne infectious disease (such as Cryptosporidium) while there was a projected increase in risk from DBP formation.
When risk is assessed for microbial exposure, it should be considered that not all segments of the population are at the same degree of risk. This may be due to differences in exposure in terms of either consumption or in concentrations (due to heterogeneity in the environment, e.g., in the distributed water), or to intrinsic differences in susceptibility. Unfortunately, the ability to assess quantitative differences in intrinsic susceptibility remains poor, and therefore protection of susceptible subpopulations often relies upon the imposition of safety factors.

2.3. Methods for characterizing health risks

Characterization of human health risks may be performed using an epidemiological approach or using a risk assessment approach. These schemes are complementary and have different strengths and shortcomings, and each has been used for assessment of drinking water risks in various applications.

Epidemiological approaches investigate the relationship between exposures and disease in actual populations. Descriptive, correlational, or analytic studies can be carried out. These different kinds of studies show the advantage of involving human populations, often experiencing the exposure of interest and representing a range of variability in susceptibility and behaviour. However, very large sample sizes, and consequently considerable expenses and efforts, are required to detect a small increase in risk from the baseline during the epidemiological studies.

Risk assessment methods, on the other hand, follow the hazard identification, dose-response assessment, exposure assessment, and risk characterization paradigm noted above. Frequently, but not always, the dose-response assessment is based upon extrapolation from results of trials in animals. Risk assessment approaches show the big advantage of being flexible in their application to potential (but not yet experienced) circumstances. A risk assessment can be performed even when the risk from a particular exposure or change of exposure is very small. This approach has the disadvantage of requiring extensive measurement or modelling to ascertain exposure, and also of the need for dose-response studies. Often these dose-response studies are in animals or at higher doses; that requires extrapolation with respect to dose and/or between species. Commonly, whether animal or human data are used to establish the dose-response relationship, the range in variability in susceptibility is small and therefore some margin of safety may need to be explicitly used to account for more susceptible subpopulations.

2.3.1. Data from pathogen occurrence measurements

The risk assessment approach relies on being able to measure or predict (e.g., by modelling) the concentration of an etiologic agent in the water supply.

Certain microbial pathogens are significant of distribution system contamination arising from both internal and external sources. These include microrganisms known to form biofilms – a complex microbial aggregation growing on solid surface characterized by structural heterogeneity, genetic diversity, complex community interactions, and an extracellular matrix of polymeric substances – and microrganisms that indicate an external contamination event. In distribution systems, the interior pipe walls, storage tanks, sediments and other surfaces in contact with finished water are colonized by bacteria, which can survive, grow, in some circumstances also multiply, and detach depending on environmental factors and on the phase of the bacterial life cycle. The microbiology of distribution systems can be influenced by a variety of factors (e.g., poor quality source water, inadequate treatment, unsanitary activity,
backflow, contamination intrusion in the system, negative pressure). Virtually any microorganism in close enough proximity to a vulnerable part of the distribution system (e.g., a cross connection, main break, or leak) could enter during an external contamination event. Control of these events is important for reducing the risks of not only microbial pathogens but also chemicals that might enter distribution system.

2.3.1.1. Microbiology of bulk water

The microbiology of distribution systems essentially consists of two different environments - microorganisms in the bulk water column and those, in higher numbers, in biofilms attached to the surfaces of pipes, sediments, and other materials or harboured within amoebae. Microorganisms in the bulk water column originate from either the source water, from bacterial growth within the treatment process (e.g., filters), from biofilms within the distribution system, or from recontamination of the water from cross connections, intrusion, pipe breaks, or other external sources.

Many groups of microorganisms are able to survive in water distribution systems and some of them represent part of the natural microbial flora of drinking water. However a part of them can pose a public health threat.

Some groups of microorganisms and species of concern in drinking water are described here below.

Heterotrophic bacteria

Heterotrophic bacteria are organisms (bacteria, yeasts and moulds) requiring organic compounds as sources of carbon and energy. The Heterotrophic Plate Count (HPC) is a non-specific term for the measure of growth of viable, naturally occurring bacteria in water. They are commonly found in the bulk water of distribution systems because they readily form biofilms in such systems. Bacteria are known to maintain their activity in distribution system biofilms exposed to chlorine disinfectants, though free floating bacteria are much more sensitive to disinfectant exposure (Williams and Braun-Howland, 2003). The presence of a disinfectant residual in drinking water has also a selective effect, particularly on gram-negative bacteria, which are relatively sensitive to inactivation by disinfectants.

The term may encompass bacterial strains of Arthrobacter, Aeromonas, Alcaligenes, Chromobacterium, Pseudomonas, Sarcina, Micrococcus, Flavobacterium, Proteus, Bacillus, Acinetobacter, Sphingomonas spp. and Klebsiella spp. as predominating genera. Other gram-positive bacteria found in disinfected drinking water can include Staphylococcus spp. and Bacillus spp. form environmentally resistant spores that can withstand prolonged contact with chlorine. Some strains of Bacillus and Staphylococcus aureus can produce toxins when contaminated water is used in food preparation (LeChevallier & Seidler, 1980).

Recent studies have shown that α-proteobacteria was the predominant phylogenetic group observed in the treated distribution water, suggesting that these organisms are well suited to survive in potable water supplies (William et al., 2004).

For the most part, the HPC have limited public health significance. In fact, some of the confusion over the significance of HPC bacteria in water results from the fact that waterborne pathogens are almost always heterotrophic; however, isolation of pathogenic microbes, often in a VBNC stage, usually involves use of specific growth conditions and big water volumes not found in the general HPC test. Even if some HPC bacteria, such as Pseudomonas, Aeromonas can be associated with illnesses in human, generally the correlated infections are nosocomial, acquired in the hospital environment or due to the equipment use or wound infections. Cases of infection due to HPC bacteria outside of hospitals are very rare, and even fewer are suggested to be of an oral route of infection. According to the WHO (WHO, 2002) the available body of
evidence supports the conclusion that, in the absence of faecal contamination, there is no direct relationship between HPC values in ingested water and human health effects in the population. Nevertheless it is not uncommon to note a significant increase in HPC bacteria when point-of-use (POU) treatment devices are used; in fact, post treatment, the increase often is several-fold higher than the influent water. Reports of high levels of HPC bacteria in such treated water fueled the debate of their health significance.

**Coliform bacteria**

Coliform bacteria are used primarily as a measure of water treatment effectiveness and can occasionally be found in distribution systems. Coliform bacteria, belonging to the Enterobacteriaceae family, can originate from untreated surface water and groundwater, vegetation, soils, insects, and animal and human faecal material.

The classification of the group has long been based on criteria defined in the last century (Breed & Norton, 1937). Nevertheless, with this classification, some strains of coliforms were classified as atypical or irregular because the conventional analytical techniques could not distinguish and catalog them accurately (Cenci et al., 1990).

In recent years, the taxonomy of the species representing Enterobacteriaceae has undergone a series of revisions which has also lead to a distinction in the meaning of the different species in the family. Currently, all the species are consistently recognized as β-galactosidase-positive and E. coli is also β-glucuronidase-positive. Furthermore now it is well-known that a good percentage of the species, as some E. coli strains, can neither ferment lactose nor produce gas. Some E. coli strains can even give a negative reaction for the production of indole (Manafi, 1996).

Most coliforms are not pathogenic, but they can indicate the potential presence of contamination. In particular *Escherichia coli* can indicate the presence of faecal pathogens, especially, and thus in the absence of more specific data may be used as a surrogate measure of public health risk (Bonadonna, 2003). In fact, *Escherichia coli* is considered to be more directly related to faecal pollution as it is commonly found in the intestinal tract of warm-blooded animals. Conversely, the presence of coliforms is the distribution system is usually interpreted to indicate an external contamination event, such as injured organism passage through treatment barriers or introduction via water line breaks, cross connections, or uncovered or poorly maintained finished water storage facilities. However, biofilms within distribution systems can support the growth and release of coliforms, even when physical integrity (i.e., breaches in the treatment plant or distribution system) and disinfectant residual have been maintained. Coliform regrowth in the distribution system is more likely during the summer months when water temperatures are higher.

**Aeromonas**

*Aeromonas* spp. is a gram-negative bacterium widely associated with environmental waters; only some strains seem likely to be pathogenic (Albert *et al.*, 2000; Borchardt *et al.*, 2003). The epidemiological relationship between aeromonads isolated from humans and those isolated from water has been studied by typing (Havelaar *et al.*, 1992; Bonadonna *et al.*, 2002). These investigations demonstrated conclusively that the aeromonads isolated from the public water supply were unrelated to those isolated from patients with gastroenteritis.

The ability of these microorganisms to grow at low temperatures and low nutrient conditions are important in their occurrence in drinking water supplies (Chauret *et al.*, 2001). However, aeromonads appear to survive poorly in nutrient-poor waters in comparison with other autochthonous oligotrophic bacteria even if these bacteria can also be recovered in drinking water, especially when temperature is about 20°C. Some countries (such as the Netherlands)
have set standards for aeromonads in drinking water leaving the treatment plant (<20 CFU/200 mL) and in the distribution system (<200 CFU/100 mL).

**Pseudomonas**

*Pseudomonas* is a genus of gamma proteobacteria, belonging to the larger family of pseudomonads. Pseudomonads are ubiquitous bacterial inhabitants of soil and surface water with a variety of metabolic capacities (Filip & Hermann, 2001). These bacteria are highly versatile and can adapt to a wide range of habitats and grow in distilled water and disinfectants. Some species are known to participate in the plant nutrient cycling, degradation of pollutants, and biofilm formation (Bonadonna *et al.*, 2005). Some of them may also represent a pathogenic risk to humans, animals and plants (Filip & Hermann, 2001). In fact, pseudomonads are known as opportunistic pathogens and may cause also severe systemic infections associated with a high mortality in immunocompromised patients. In healthy individuals *Pseudomonas* can be responsible of primary skin infections, usually a self-limiting skin rash or folliculitis. Environmental isolates of *Pseudomonas* can infect humans, undergo rapid adaptation, and cause nosocomial pneumonia, sepsis in burn wounds, urinary-tract infections and chronic pulmonary inflammation in hosts rendered susceptible by cystic fibrosis. *P. aeruginosa* is often associated with nosocomial infections. Its infective dose for healthy individuals ranges between $10^6$ and $10^{10}$ CFU/mL. This dosage may become much less in the case of immunocompromised individuals.

In-plant water treatment and delivery systems may actually encourage the growth of *Pseudomonas* since the organism can adhere to the walls of piping systems, inside filters (especially carbon filters, which provide a food source), and on other surfaces. This bacterium is even often recovered in biofilm at the point of use (POU) (Bonadonna *et al.*, 2008).

The risk of colonization from ingesting *P. aeruginosa* in drinking water is low. The risk is slightly higher if the subject is taking an antibiotic resisted by *P. aeruginosa*. Nevertheless, the use of POU-devices may amplify the numbers of bacteria present in tap water by promoting biofilm formation. Thus, based on a daily ingestion of two liters of POU treated water, *P. aeruginosa* could have a probability of less than $10^{-6}$ of colonizing the gut; however, annual risks could be as much as 100-fold greater.

In the last few years there has been considerable progress in the study of the regulation of quorum sensing systems of several *Pseudomonas*, especially of *P. aeruginosa*. These systems can regulate the production of multiple virulence factors and biofilm formation.

**Stenotrophomonas**

*Stenotrophomonas* is a genus of Gram-negative bacteria, phylogenetically placed in the Gammaproteobacteria, c-subclass of Proteobacteria. The genus name was intended to highlight the limited nutritional range of the bacterium. However, several studies subsequently demonstrated that the genus is capable of great metabolic versatility and intraspecific heterogeneity. *Stenotrophomonas* species play an important role in nature, especially in the element cycle; however, nowadays, they are also frequently used in applied microbiology and biotechnology (Suckstorff & Berg, 2003). The genus currently comprises eight species, *S. maltophilia*, *S. nitritireducens*, *S. rhizophila*, *S. acidaminiphila*, *S. koreensis*, *S. chelatiphaga*, *S. terrae* and *S. humi*. Although *Stenotrophomonas* spp. occurs ubiquitously in the environment, soil and plants are their main environmental reservoirs. Members of the genus have an important ecological role in the nitrogen and sulphur cycles and several *Stenotrophomonas* species, especially *S. maltophilia* and *S. rhizophila*, can engage in beneficial interactions with plants (Ryan *et al.*, 2009).

*S. maltophilia* is a typical, often dominant member of the microbial communities that are found on or in plants and has a worldwide distribution (Ryan *et al.*, 2009).
Stenotrophomonas maltophilia is a Gram-negative, nonfermenting environmental bacterial species often isolated from the rhizosphere and from water sources (Bonadonna et al., 2008). This species is also a relevant human opportunistic pathogen associated with a broad spectrum of clinical syndromes, such as bacteremia, endocarditis, infection in cancer patients and respiratory tract infections, including those suffered by cystic fibrosis patients. One of the most problematic characteristics of S. maltophilia is its intrinsic high resistance to several antibiotics. This intrinsic antibiotic resistance is at least partly due to the presence in its genome of genes encoding antibiotic-inactivating enzymes and multidrug resistance efflux pumps (Turrientes et al., 2010).

Legionella

Legionella is a Gram negative bacterium, responsible for Legionnaires’ disease and Pontiac fever; it is ubiquitously, found in a variety of natural reservoir (e.g., lakes, rivers, thermal springs and soils). The bacterium can enter and colonize man-made water supply systems (e.g., water distribution plants, tanks, cooling towers) and even HVAC systems and from there into humans (Bonadonna et al., 2009a). Although no direct correlation has been demonstrated between Legionella load and risk of legionellosis, determination of the microbial load underlies the analysis of water samples taken from distribution systems (Ditommaso et al., 2010). Legionella pneumophila cause severe pneumonia in persons with weakened immune defenses. The bacterium’s survival and spread depend on the ability to replicate inside eukaryotic phagocytic cells like amoebae or other protozoa (Cazalet et al., 2008).

Currently, the Legionella genus includes 52 species and more than 70 different serogroup. More than 20 species have been proven to be causative agents of Legionnaires’ disease (Lee et al., 2010). In developed countries, Legionella has the highest prevalence in drinking water respect to other microbes.

National and international guidelines for Legionella prevention and control set risk and intervention threshold levels in water distribution systems based on the Legionella load detected in the samples. The most commonly used method for environmental surveillance of Legionella is the standard culture technique by which Legionella organisms can be isolated and their number in environmental samples estimated. The International Organization for Standardization (ISO) recommends concentrating a sample by filtering it through a polycarbonate or nylon membrane (porosity 0.22 µm) or by centrifugation (6000 g for 10 min). For analysing samples that are difficult to filter, the guidelines recommend using membranes with larger pores (0.45 µm). Nevertheless the US Centers for Disease Control and Prevention recommends the use of polycarbonate membranes (porosity 0.22 µm) to recover Legionella from water samples (Ditommaso et al., 2010).

Non-tuberculosis mycobacteria

Mycobacteria are a large group of microorganisms that inhabit a diverse range of natural environments. Environmental mycobacteria, also called Non-Tuberculosis Mycobacteria (NTM), are a frequent cause of opportunistic infection in human beings and livestock. There is growing recognition in recent years that water is an important vehicle of transmission of environmental mycobacteria. Mycobacteria is found in the natural water environment as well as in engineered water systems such as plumbing systems, cooling towers, swimming pools. The persistence of mycobacteria in drinking water, especially in tap water, is attributed to their high resistance to disinfectants commonly used in water treatment (Briancesco et al., 2009). Indeed, it is postulated that they may be selected for in distribution systems as a result of their resistance to chlorine. Results obtained from a wide investigation in Italy evidenced a constant presence of NTM species in drinking water (Briancesco et al., 2009). Falkinham et al. (2001) reported that 20% of the water isolates and 64% of the biofilm isolates were identified as M. avium or M. intracellulare and 8% were identified as M. kansasii. The greatest increase in NTM infections have been with acquired...
immunodeficiency syndrome (AIDS) patients; approximately 25 to 50 percent of these patients suffer debilitating and life-threatening NTM infections, although the availability of highly active antiretroviral therapy has reduced the incidence of these bacteria in AIDS patients in recent years.

**Free-living protozoa**

Free-Living Amoebae (FLA) including *Acanthamoeba* spp. are commonly found in natural aquatic systems, water supplies, and cooling systems, usually feeding on bacteria (Bonadonna *et al.*, 2006). It was shown that amoebae host several intracellular pathogens including *Legionella* spp., *Pseudomonas* spp., *Chlamydia* spp., *Parachlamydia* spp., *Listeria* spp., *Burkholderia* spp., *Campylobacter jejuni*, *Helicobacter pylori*, *Pasteurella multocida*, *Salmonella enterica*, *Francisella tularensis*, and *Simkania negevensis*.

The term amoeba covers a heterogeneous of diverse unicellular eukariotes that share common morphological and behavioural characteristics. More than 11,300 amoebal species have been identified up to now, of which only very few are recognized as human pathogens. Most of them present two developmental stages: a vegetative stage, called trophozoite, and a resting form, the cyst, which allows them to survive in hostile or oligotrophic environments. FLA are present worldwide and live more especially at the contact of biofilms where they can feed on smaller microrganisms like bacteria, fungi and algae (Loret & Greub, 2010).

FLA have been isolated from various domestic water system, such as cooling towers and hospital water networks. In addition to their own pathogenicity, FLA can also act as Trojan horses and be naturally infected with Amoeba-Resisting Bacteria (ARB) that may be involved in human infections. Moreover, amoebae are a reservoir for ARB. The internalised bacteria may be protected from adverse conditions, particularly from agents used for water disinfection since amoebae are resistant to most of these disinfectants, especially when they are encysted (Thomas *et al.*, 2006).

Members of the genera *Acanthamoeba* and *Naegleria* have been recognized as opportunistic human pathogens, capable of causing infections of the Central Nervous System (CNS) in both immunocompetent and immunocompromised hosts. *Naegleria* is the causal agent of the primary amebic meningoencephalitis, a fulminating, rapidly fatal infection of the CNS; *Acanthamoeba* spp is responsible for a more chronic and insidious infection of the CNS termed granulomatous amebic encephalitis. *Acanthamoeba* spp is also responsible of amebic keratitis. Amebic keratitis occurs in contact lenses users when, due to improper maintenance and poor sanitary precautions, amoebas proliferate in the ophthalmic solutions or in the lens cases and are transferred to the corneal surface when the lens is inserted (Schuster, 2002).

Routine monitoring for free-living protozoa is rarely done even if they are ubiquitous. Isolation and identification of these organisms are accomplished only when there is evidence for disease outbreak or when research studies are being conducted.

**2.3.1.2. Microbiology of distribution system biofilms**

Biofilms in drinking water pipe networks contain a variety of microrganisms that can be found in bulk distribution system water. The microbial composition of any given pipe segment can be highly variable, and in most cases is poorly, if ever, characterized. The pipe surface itself can influence the composition and activity of biofilm populations. Studies have shown that biofilms developed more quickly on iron pipe surfaces than on plastic PVC pipes, despite the fact that adequate corrosion control was applied, that the water was biologically treated to reduce AOC levels, and that chlorine residuals were consistently maintained.

In addition to influencing the development of biofilms, the pipe surface has also been shown to affect the composition of the microbial communities present within the biofilm. Iron pipes supported a more diverse microbial population than did PVC pipes. Undoubtedly part of the
reason that certain bacteria associate with certain pipe types is because materials may leach compounds that support bacterial growth. For example, pipe gaskets and elastic sealants (containing polyamide and silicone) can be a source of nutrients for bacterial proliferation (Colbourne et al., 1984). Organisms associated with joint-packing materials include populations of *Pseudomonas aeruginosa*, *Chromobacter spp.*, *Enterobacter aerogenes*, and *Klebsiella pneumoniae*. Coating compounds for storage reservoirs and standpipes can contribute organic polymers and solvents that may support regrowth of heterotrophic bacteria. Liner materials may contain bitumen, chlorinated rubber, epoxy resin, or tar-epoxy resin combinations that can support bacterial regrowth. PVC pipes and coating materials may leach stabilizers that can result in bacterial growth. Studies performed in the United Kingdom reported that coliform isolations were four times higher when samples were collected from plastic taps than from metallic faucets.

For both bulk drinking water and biofilms, the identification of microorganisms typically relies on culturing bacteria from potable supplies, which has important limitations. Culture methods do not detect all microbes that may exist in water, such that only a fraction of viable organisms is recovered. In addition, most culture methods only detect relatively rapidly growing heterotrophic bacteria, and slowly growing organisms, fastidious or autotrophic organisms, and anaerobes are generally not investigated.

Molecular methods offer the promise of a more complete determination of the microbiology of water. DNA extraction coupled with Polymerase Chain Reaction (PCR) amplification can be used to identify waterborne microbes. These procedures can be combined with quantitative real-time PCR, fluorescence in-situ hybridization, or flow cytometry to provide quantitative assessments of bacterial populations. However, careful quality assurance is necessary to ensure complete extraction and recovery of environmental DNA. Martiny et al. (2003) utilized terminal restriction fragment length polymorphisms to identify members of a biofilm consortium over a three-year time period. In this study, several organisms were identified (*Pseudomonas, Sphingomonas, Aquabacterium, Nitrospira, Planctomyces, Acidobacterium*).

The analytical methods for the microflora detection in distribution systems are expensive, time consuming and require optimization for specific conditions. As a consequence, there is a lack of information about the types, numbers, and activities of autotrophic microorganisms in drinking water. It is also unknown how the ecology of the main distribution system is related to that in premise plumbing. Furthermore the presence of microorganisms can vary between distribution systems in different locations, and the microbial populations can differently respond to water quality changes within a distribution system. This translates into a lack of understanding about whether organisms of potential public health concern may be present in water systems and further complicates the ability to assess risk due to their presence.

It can be hard to determine whether the detection of frank or opportunistic pathogens in drinking water poses an unacceptable risk. In addition to the monitoring techniques being difficult, time-consuming, expensive, and of poor sensitivity, the methods do not detect specific virulence determinants, such that many environmental isolates (e.g., *E. coli*, *Aeromonas, Legionella*, etc.) are indistinguishable from their clinical strains. Therefore even when monitoring for potentially pathogenic organisms is done, the public health significance of the results is often in question. Furthermore, there is insufficient supporting information (occurrence data for exposure assessment, dose-response data, health effects, and models predicting pathogen occurrence for different distribution system contamination scenarios such as contamination via cross connections, main breaks, or intrusion) to conduct a risk assessment for many waterborne microbes. For all these reasons, measurement of the microbe itself is typically insufficient to make a public health determination. Until better monitoring methods, pathogen occurrence models, dose-response data, and risk assessment data are available,
pathogen occurrence measurements are best used in conjunction with other supporting data on health outcomes. Such supporting data could include enhanced or syndromic surveillance in communities, as well as the use of microbial or chemical indicators of potential contamination.

2.4. Concepts of microbial risk assessment and management of drinking water

The purpose of drinking water treatment and drinking water hygiene is to minimize the adverse health effects of hazards on the consumer. Nevertheless in practice it is impossible to reduce the risks to zero under all circumstances.

The WHO introduced the international concepts of HACCP and WSP with the aim to improve drinking water safety and security.

The WSPs draw on many of the principles and concepts from other risk management approaches, in particular from the multi-barrier approach and from HACCP. The general principles of the WSPs should be developed and implemented for individual drinking water system. The key steps in the WSPs are similar to those of HACCP. The current WSP guidelines are primarily directed at risk identification and qualitative risk assessment for ranking of risks. This approach implies that the microbial risks and adverse health effects to which a population is exposed through drinking water should be minimized, be very low and not exceed the tolerable risk suggested by WHO Guidelines for drinking water quality (Havelaar & Melse, 2003). Some risk must be accepted or tolerated and several approaches can be applied to estimate what the acceptable level of risk may be in a given situation. A risk may be acceptable when:

- it falls below an arbitrarily defined probability, a level that is already tolerated, an arbitrarily defined attributable fraction of the total disease burden in the community,
- the cost of reducing the risk would exceed the costs saved, including those saved when the costs of suffering are also factored in,
- the opportunity costs would be better spent on other, more pressing, public health problems or
- public health professionals, the general public or politicians say the risk is acceptable.

Each of these points could lead to a different definition of the acceptable risk, even in the same population.

The acceptability of risk is dependent on the given population, circumstances and time; a risk accepted somewhere is not necessarily accepted elsewhere (Fewtrell & Bartram, 2001).

The incidence of waterborne illness in the population or the occurrence of waterborne outbreaks are direct triggers for curative risk management. A more precautionary incentive for assessing the water-related health risks and the installation of risk management is to demonstrate that the water supply is providing an adequate level of protection of public health.

The level of water safety is another valuable consideration and its definition has to be provided by regulator. In fact, he can translate this information on risk estimate and level of risk that is considered tolerable into a health target for drinking water, considering other factors such as relative contribution of drinking water transmitted disease to the overall health burden and the economic climate. Thus health targets could be a tolerable disease burden or this could be translated into water quality targets or performance targets.

The health target is defined as the level of a tolerable risk level for drinking water, which could be expressed as the tolerable risk of infection through drinking water (i.e., risk of
infection <10^{-4} per person per year) or the tolerable amount of disease burden (i.e., <10^{-6} Disability Adjusted Life Years, DALY, per person per year).

Health targets may be different in different health status situations and countries. Setting the health target, that have to be realistic under local operating conditions, is the responsibility of the regulator and the target they set for drinking water is the starting point for risk management by the water supplier. They need to design, operate, control and maintain their system in a way that ensures that the health target is met at all times also taking in account the cost-benefit.

The health target could be also translated into water quality targets for pathogens. In this case, rather than producing a standard and monitoring requirement for all pathogens that could be transmitted through drinking water, the use of a suite of “index pathogens” is advisable.

Establishment of adequate control against this suite of pathogens should offer protection against the other known and unknown pathogens.

2.5. Evidence from outbreak data

In Europe waterborne disease surveillance systems are non adequately developed, at least in some countries. In the United States a surveillance system for waterborne disease outbreaks started in 1971 and now it is a collaboration between the Centers for Disease Control and Prevention (CDC), the USEPA, and state and regional epidemiologists. In addition to the waterborne disease outbreak surveillance system, there is also a national system of notifiable diseases in the United States that mandates that health care providers report specific infections, including a number of potentially waterborne infections such as cholera, cryptosporidiosis, E. coli O157:H7, giardiasis, hepatitis A virus, legionellosis, poliomyelitis, salmonellosis, shigellosis, tularemia, and typhoid fever. Like the outbreak surveillance system, the surveillance for notifiable diseases is a voluntary passive surveillance system with low sensitivity and reporting delays.

Also in Europe there are national systems of notifiable diseases including a variable number of infections diseases. Nevertheless the WHO underlines that all the surveillance systems has big limits, independently on the development level of the country.

A waterborne disease surveillance system should include outbreaks associated with both drinking and recreational water, and outbreaks due to both microbial and chemical agents. The objectives of the surveillance system should be to (1) characterize the epidemiology of waterborne disease outbreaks, (2) identify the etiologic agents that cause the outbreaks, (3) determine the risk factors that contributed to the outbreak, (4) inform and train public health personnel to detect and investigate waterborne disease outbreaks, and (5) collaborate with local, regional, national and international agencies on strategies to prevent waterborne diseases.

From 1971 until 2002, 764 drinking water outbreaks have been reported through the US surveillance system. Although this is believed to be an underestimate of the true number of outbreaks that occurred during this period, the information collected in this surveillance system can be extremely valuable for improving the understanding of the agents that cause waterborne disease and the risk factors involved in waterborne disease outbreaks.

The data collected in a surveillance system should include:
- type of exposure (drinking water or recreational water);
- location and date of outbreak;
- actual or estimated number of persons exposed, ill, hospitalized, dead;
- symptoms, incubation period, duration of illness;
- etiologic agent;
- epidemiological data (attack rate, relative risk or odds ratio);
- clinical laboratory data (results of faecal and serology tests);
- type of water system;
- community, non-community, or individual homeowner drinking water supply;
- swimming pool, hot tub, water park, or lake for recreational water;
- environmental data (results of water analyses, sanitary survey, water plant inspection);
- factors contributing to contamination of water.

Data obtained from the US surveillance system indicate three main trends in the considered period:
- The overall number of reported waterborne disease outbreaks associated with drinking water is declining from a peak of over 50 reported outbreaks in 1980 to eight reported outbreaks in 2002.
- For a substantial portion of drinking water outbreaks, the pathogen is not identified and the outbreaks are classified as “acute gastrointestinal illness of unknown etiology” (AGI). From 1986 through 2002, approximately 41 percent of the over 250 outbreaks reported during this period were classified as AGI, and this proportion varies by reporting period from a peak of 68 percent in 1991-1992 to 17 percent in 1993-1994. Overall, *Giardia* and *Cryptosporidium* are the most commonly reported etiologic agents of waterborne disease when a pathogen is identified and are associated with about 20 percent of reported outbreaks associated with drinking water since the mid-1980s. However, *Legionella* is now the single most common cause of outbreaks involving drinking water.
- Most drinking water outbreaks involve groundwater systems, especially untreated groundwater systems. Forty percent of the 25 drinking water outbreaks reported between 2001 and 2002 involved untreated groundwater systems.

On the other hand, the unique sure data obtained through the European Surveillance Systems, support the opinion that *Legionella* is the most common pathogen associated with drinking water.

### 2.5.1. Etiologic agents associated with drinking water outbreaks

The agents responsible for waterborne disease outbreaks are generally predominantly undefined, microbial (parasitic, bacterial, or viral), or chemical.

Indeed, surveillance data on waterborne disease outbreaks associated with drinking water in the United States from 2001 to 2002 indicate that almost 30% of reported outbreaks were due to bacterial agents, 16% were due to protozoa, 16% were due to viral agents, 16% were due to chemical contaminants, and 23% had an unidentified etiology.

Relatively few outbreaks due to viruses have been reported, in part because of the difficulty of the detection methodologies for these organisms. However, the number of reported viral outbreaks has increased significantly since 1999 with the development of better diagnostic techniques for noroviruses. Nine of the 15 drinking water outbreaks associated with noroviruses that have been reported since 1986 occurred between 1999 and 2002.

#### 2.5.1.1. Outbreaks associated with groundwater systems

In recent years, as treatment of surface water supplies improved, waterborne outbreaks have increasingly involved groundwater supplies. There is increasing recognition that many groundwater supplies have microbial contamination. A survey of 448 wells in 35 US states reported that 31% of the sites were positive for at least one virus, and enterovirus RNA was
detected in approximately 15%, rotavirus RNA in 14%, and hepatitis A virus RNA in 7% of the wells by reverse transcription polymerase chain reaction (RT-PCR). Fout et al. (2003) examined 321 samples from 29 groundwater sites by RT-PCR and reported that 72% of the sites were virus positive. Borchardt et al. (2004) collected monthly samples from four municipal wells in one city in Wisconsin for a 12-month period and detected enteric viruses by RT-PCR in 50% of the samples. Two studies in Ontario, Canada examined the relationship between E. coli in well water and acute gastroenteritides in households using the water for drinking. In the first study of 181 households with untreated well water, water samples were collected five times during the one-year study, and E. coli was detected in 20 percent of the household wells. The second study included 235 households in four rural communities and reported that 20 percent of the households had at least one water sample that exceeded the national standards for total coliforms or E. coli.

2.5.1.2. Outbreaks associated with distribution systems

Some data showed that among the seven outbreaks associated with community water systems in 2001-2002, 57.1% were related to problems in the water distribution system. Other data collected in 2003-2004 indicated that distribution systems were associated with 38% of the outbreaks associated with drinking water systems. Other epidemiological and outbreak investigations conducted suggested that a substantial proportion of waterborne disease outbreaks, both microbial and chemical, is attributable to problems within distribution systems. Craun et al. (2001) examined causes of reported waterborne outbreaks from 1971 to 1998 and noted that, in community water systems, 30% of 294 outbreaks were associated with distribution system deficiencies, causing an average of 194 illnesses per outbreak. Distribution system contamination was observed to be the single most important cause of outbreaks in community water systems.

The reason for the apparent increase in the proportion of outbreaks associated with water distribution systems is not entirely clear. Outbreaks associated with distribution system deficiencies have always been reported. However, there may be more attention focused on the distribution system now that there are fewer outbreaks associated with inadequate treatment of surface water. Also, better outbreak investigations and reporting systems may result in increased recognition and reporting of all the risk factors contributing to the outbreak, including problems with the distribution system that may have been overlooked in the past.

The U.S. CDC surveillance system for waterborne disease outbreaks collects information on outbreaks and their contributing causes. For example, from 1981 to 1998, the CDC documented 57 waterborne outbreaks related to cross-connections, resulting in 9734 detected and reported illnesses. Contamination from cross-connections and backspinhage were found to cause the majority of the outbreaks associated with distribution systems (51%), compared with contamination of water mains following breaks (39%) and contamination of storage facilities (10%). A separate compilation by the EPA of backflow events revealed many more incidents of backflow and resulting outbreaks – a total of 459 incidents resulting in 12093 illnesses from backflow events from 1970 to 2001 (US EPA, 2002). The situation may be of even greater concern because incidents involving premise plumbing are even less recognized.

Most reported outbreaks associated with distribution systems occur in community water systems because of their greater size and complexity. For example, from 1999 to 2002 there were 18 reported outbreaks in community water systems, and nine (50%) of these were related to problems in the water distribution system. However, there have been a number of reported outbreaks associated with noncommunity water systems that have been attributed to deficiencies in the distribution system. Finally, the magnitude and severity of reported outbreaks associated
with distribution systems vary, with an average about almost 200 illnesses per outbreak and a total of 13 deaths.

2.5.2. Extent of underestimation

The number of identified waterborne disease outbreaks is considered an underestimate because not all outbreaks are recognized, investigated, or reported to health authorities.

Factors influencing whether a waterborne outbreak is recognized include awareness of the outbreak, availability of laboratory testing, and resources available for surveillance and investigation of outbreaks. The detection and investigation of waterborne outbreaks is primarily the responsibility of the local, state, and territorial public health departments with varying resources and capacities. Differences in the capacity of local and state public health agencies and laboratories to detect an outbreak might result in reporting and surveillance bias, such that the states with the majority of outbreaks might not be the states with the majority of waterborne disease (Bonadonna et al., 2009b) Outbreaks are more likely to be recognized when they involve acute illnesses with symptoms requiring medical treatment, or when sensitive laboratory diagnostic methods are readily available.

Although in some countries the surveillance system generally includes also outbreaks associated with individual homeowner water systems, it is likely that most sporadic cases and small clusters of waterborne disease associated with individual homeowner water systems are not recognized or reported because small numbers of people are involved. Furthermore, a cluster of cases of gastroenteritis within a single household may easily be attributed to food contamination or person-to-person transmission, such that the possibility of waterborne transmission may not be considered or investigated.

Adverse health effects associated with premise plumbing problems are less likely to be recognized and reported in surveillance systems, especially if they occur within a single household. However, outbreaks associated with drinking water have been reported from public building settings such as schools, restaurants, churches, factories, and apartment buildings. Some of these outbreaks were due to contamination of a private well that serves the building. Other outbreaks in public buildings were classified as due to distribution system deficiencies and appeared to involve cross-connections and/or backsiphonage problems.

The WHO-United Nations Economic Commission for Europe (UNECE) Protocol on Water and Health includes legally binding targets covering the prevention of waterborne diseases. In addition to general targets concerning access to safe water and provision of sanitation, common requirements for surveillance systems and contingency plans for detection and prevention of waterborne outbreaks are specified.

Surely, surveillance systems for waterborne diseases are important components of public health policy.

In Europe, a specific compulsory system for Water-Related Diseases (WRDs) surveillance is still missing even if many legislative systems have been set with regards to the surveillance of communicable diseases.

In Italy, although a surveillance system for many communicable diseases exists since 1990, a separate monitoring system for cases and outbreaks of diseases specifically attributable to water does not yet exist. The current Italian Surveillance System on Communicable Diseases is defined by a Ministerial Decree which identifies 5 classes of priority diseases including totally 47 diseases. Any new case of communicable disease is notified by the physician to the Local Health Unit of the National Health System. Each Local Health Unit forwards collected data to the Regional Health Authority which is responsible for their transmission to Ministry of Health. This latter provides reports of confirmed cases to the National Institute of Statistics.
Among the diseases included in the 5 classes of priority diseases, some pathologies such as salmonellosis, Hepatitis A virus and non-A and non-B hepatitis, diarrhoeal infections, typhoid fever, toxinfecctions, legionellosis, amoebiasis, directly or indirectly associate with water, are encompassed.

Some Italian studies reported cases of WRDs; nevertheless a specifically focussed picture of this issue has never been elaborated. In the light of that, an investigation on reported cases of WRDs was carried out.

Some data were collected in the period 2001-2006 in a district of Rome, Italy (area with about one million and an half of inhabitants) on the basis of notified cases of diseases within the current Italian surveillance system on communicable diseases (Bonadonna, Di Porto, 2009). Data were inclusive of all the pathologies directly and indirectly associated with water and their trend during the whole period was examined. Shortcomings and weakness of the national surveillance system as it is at the moment arranged were also evaluated.

Seventeen different diseases, directly and indirectly associated with water, were recognized and selected among the notified data and a total of 2928 cases (with an average of 172 cases/year) were collected and elaborated (for year and origin).

In the examined period, the total number of cases of WRDs slightly and progressively decreased: 505 cases were notified in 2001, while in 2006, 400 cases were recorded. Conversely, the notified cases of legionellosis (218 total cases), rotavirus (175 cases) and hepatitis E (50 cases) infections increased; these yields may suggest that a greater attention was focussed on these specific diseases in last years. Hepatitis A infections, with a total of 679 cases, non-serologically typized salmonellosis (538 cases) and acute gastroenteritis (496 cases) showed a higher incidence respect to the other diseases, while Salmonella parathyphi B (7 cases) and Salmonella serotype E (4 cases) were the less recorded etiological agents.

A total of 75 confirmed cases was directly associated with water and, in this instance, Legionella (58%) and hepatitis A virus (16%) were the most frequently notified etiological agents. Nevertheless, if many cases of legionellosis were acquired in Italy (hospital, hotel, spa, swimming pool), almost all the cases of hepatitis A were associated with travel abroad.

The causes of the more recurrent diseases were represented, in decreasing order, by consumption of shellfishes, use and consumption of water and consumption of contaminated agricultural products.

The obtained outcomes represent an underestimation of the real situation also in the light of shortcomings and weakness emerged from the study. In fact, among several failings, except that many notification forms were not fully filled, thus causing loss of data (e.g., the age of the patient), it could be assumed that many people (essentially adult subjects) do not seek medical attention also because many enteric infections are self-limiting. That implies that these cases were not calculated in the surveillance system on communicable diseases.

This investigation was the first study focussed on data selected from the notification forms concerning WRDs in Italy, whose results show that a situation of risk does occur and that an underestimation of cases is very probable. Inevitably the current system fail to pick up many cases and outbreaks and complementary approaches are needed to improve outbreak surveillance.

The understanding of the actual impact of water quality on human diseases is difficult to reach. A comprehensive public health surveillance strategy could be the most efficient approach to better understand and control the impact of water quality on WRDs. Thus it is be hoped that a continuous and stricter interaction among epidemiologists and microbiologists will be set up.
2.6. Conclusions

Accurate estimates are not yet available for the prevalence of adverse health effects attributable to deficiencies in distribution systems from pathogen occurrence measurements, waterborne disease outbreak surveillance, or epidemiological studies. Pathogen occurrence measurements are rare due to limitations in detection methods, sensitivity of analytical techniques and cost issues. Models to quantitatively predict pathogen occurrence in distribution systems (e.g., by cross-connections, main breaks, or intrusion) have not yet been developed (Haas et al., 1999) or difficulties can be encountered in their evolution. Despite under-reporting and limited data on risk factors, the waterborne disease outbreak surveillance system can provide the best available evidence of public health risks associated with distribution systems. Data suggest that about one-third to one-half of reported waterborne disease outbreaks can be associated with distribution system problems. To date, only few epidemiological studies have been specifically designed to examine the contribution of the distribution system to endemic disease occurrence. Nevertheless some conclusions and recommendations can be provided.

The distribution system is the remaining component of public water supplies yet to be adequately addressed in national efforts to eradicate waterborne disease. Data indicate that although the number of waterborne disease outbreaks including those attributable to distribution systems is decreasing, the proportion of outbreaks attributable to distribution systems seems to be increased. Most of the reported outbreaks associated with distribution systems have involved contamination from cross-connections and backsiphonage. Furthermore, *Legionella* appears to be a continuing risk and is the single most common etiologic agent associated with outbreaks involving drinking water. Additional research is necessary to determine the relationship between disinfectant usage and the risks of *Legionella* and other pathogenic microorganisms.

Distribution system ecology is poorly understood. There is very little information available about the types, activities, and distribution of microorganisms in distribution systems. Limited HPC data are available for some systems, but these data are not routinely collected, they underestimate the numbers of organisms present, and they include many organisms that do not necessarily present a health risk. Investigation on water in distribution systems evidenced a constant occurrence of environmental bacteria and *Pseudomonadaceae*. To more adequately assess risk, more information on the microbial ecology of distribution systems, including premise plumbing, is needed.

There is inadequate investigation of waterborne disease outbreaks associated with distribution systems. Data on outbreaks due to many etiologic agents generally would rarely implicate premise plumbing because backflow and regrowth events likely would not be recognized and reported unless an institutional building with large numbers of people is affected.

Epidemiology studies that specifically target the distribution system component of waterborne disease are needed. Surveillance systems help to follow trends in the causes and risk factors of waterborne disease, but they are not very sensitive and cannot serve as a rapid warning system of a water-related health problem in a specific community because of reporting delays.

Nevertheless recently completed epidemiological studies have either not focused on the specific contribution of distribution system contamination to gastrointestinal illness, or they have been unable to detect any link between illness and drinking water. Thus epidemiological studies of the risk of endemic disease associated with drinking water distribution systems need to be performed and must be designed with sufficient power and resources to adequately address the deficiencies of previous studies (Hellard et al., 2001).
3. REQUIREMENTS FOR MICROBIOLOGICAL ANALYSIS

Carrying out microbiological tests aimed at determining microbial entities is subject to conditions of variability. This possible occurrence can arise from internal factors, i.e., those related to the process of development and growth of microorganisms and to the model used to estimate the “possible and acceptable” dispersion of the microorganisms in the sample to be analyzed, and external factors, due to contingent conditions, such as work environment, equipment and materials, analytical technique, personnel, etc. Each type of factor, alone or in synergy or antagonism with others, may contribute to the overall variability of the experimental result and its influence should be controlled in a manner defined by the laboratory by assessing the level of its contribution to overall uncertainty of test result compared to the acceptable confidence level.

Specific activities taking place during the process are added to the duties performed by personnel who is trained and authorized to the execution of analysis and all support activities (Figure 1).

![Flowchart](Figure 1. Description of the different phases of microbiological process)
The requirements mentioned below must integrate the general aspects of managerial and functional nature, listed in the previous section, and all other requirements provided in the specific analytical methods.

For a laboratory performing analytical tests on the basis of legal provisions, the central objective must be to provide reliable results. It is therefore necessary to establish procedures that ensure:

- goal setting, at long and short term;
- definition of measures for ensuring the quality of results;
- organization and personnel management;
- supervision of technical performance;
- opportunity to evaluate procedures and personnel;
- possibility to interrupt the procedures in case of discrepancies and deviations from the standard.

3.1. Staff

The technical operator embodies two important stages of the process of investigation, preparation and analysis and performs various tasks and various testing phases:

- sampling;
- sample storage;
- preparation and handling of growth media;
- sample preservation, preparation of diluted solutions, inoculation;
- incubation.

The laboratory, according to the staff entrusted with the task of performing the tests, on one hand, therefore, shall provide objective evidence of the analyst's operational capacity through provision of appropriate documentation to prove that the training provided and on the other hand ensure that the achieved operational capability is maintained over time.

It is important that all staff know their responsibilities and the limits of their jurisdiction and they also have to know to whom they can delegate responsibilities.

Here below a prospectus for the grouping of tasks with the level of skills and training of laboratory personnel is proposed:

- staff for cleaning and disinfection of equipment and glassware, and for maintaining hygiene in the laboratory;
- staff for the preparation of the growth media and processing of samples who should have followed at least one training in the workplace;
- experienced staff, with adequate training and knowledge of safety requirements who can also be charged with simple analytical operations;
- personnel with basic technical skills and training specific to the routine tests in accordance with standard procedures;
- staff for conducting more complex examinations with qualifications of laboratory technician.

A document stating the competence of academic and professional qualifications, training and experience relevant to all employees in charge of the microbiological examinations should therefore be kept up to date.

Training activities undertaken in the laboratory can afford to acquire expertise and knowledge of internal procedures. When appropriate, staff turnover in different areas of the laboratory should be encouraged to gain knowledge and experience to do all the procedures.
relating to the specific level of expertise and training. Following this practice the replacement of absent staff or the intervention of additional staff in the case of an unexpected work increase will be assured.

Continuous assessment of staff performance is essential to maintain an adequate level of quality in laboratory activities. The main objective is to improve the performance of work, but the assessment may also serve to motivate staff and recognize when it is necessary to organize training courses for staff in order also to facilitate the improvement of operational capacity and efficiency of individual members of staff.

At the end of training period, gained expertise should be verified by the head of the laboratory also in view of a possible revision of the training program.

3.2. Safety

To ensure a safe work environment in a microbiology laboratory it is necessary to both activate a system of prior surveillance to prevent the spread of infection, and minimize the risk of accidents and eliminate possible contamination of samples which should be examined.

Security procedures should be well defined and documented and will be appointed by a safety officer. All staff should know the procedures to be implemented in the event of an accident, know who should be contacted and how to operate in emergency conditions. It is necessary to draw up operational protocols to be adopted in case of accidents of varying severity and in different conditions of risk and it is necessary to be provided with materials, equipment, protective clothing (coats, gloves, face masks, goggles) and chemical disinfectants and neutralizing (emergency kits) for use in case of leakage of microbiological or chemical fluids.

Staff should be aware of the need for containment facilities for particular groups of organisms according to biological risk and should learn the procedures to be followed in the use of such resources and access limits. Therefore written protocols should be defined for each level of containment in relation to the risk classification of the particular group of biological pathogens. You must remember that most of the routine microbiological tests for water are carried out with microorganisms of Risk Group 2 (moderate individual risk, low risk to the community), unless that the examination is intended to isolate bodies of a higher class of biological risk (e.g., risk group 3, high individual risk, low risk to the community, or even risk group 4).

With an established frequency, out internal and external safety checks should be carried and for all the staff the importance of respecting the code of safe laboratory must be clear. The main goals of a safety program are related to the protection and safety of staff, but also to guarantee the production of valid and reliable analytical results.

Each laboratory should also have written response plans with regard to the possibility of fire and for all staff an annual fire training should be provided. Safety equipment including fire extinguishers and fire hoses, must still be checked regularly to ensure the proper functioning and correct positioning.

The laboratory should have a facility that is designed to provide appropriate levels of security, safety, and minimize contamination. Laboratory management should ensure that:

- the access to laboratory exclusively to be restricted to authorized persons;
- the use of lab coats to be in the workspace;
- the use of lab coats and other protective clothing to be only in the laboratory, avoiding to wear them in public areas or where people consume meals;
- the use of additional personal protective equipment (gloves, goggles, masks, etc.) where required by specific activity;
- windows and doors to be closed at work;
- the use of devices to pipette, avoiding to perform the operation with the mouth;
- no eating, drinking or smoking inside the laboratory;
- the use of disinfectants to clean counters, cleaning at least once a day and according to specific circumstances;
- ban on the production of aerosols during the handling of microorganisms;
- the use of procedures for disinfection / sterilization of waste and efficient laboratory glassware before cleaning, reuse or disposal;
- the use of specific procedures for the transportation of contaminated materials (growth media, waste) sealed in airtight containers;
- ban on producing air currents in the laboratory;
- proper use of chemical hoods, areas of inoculation and laminar flow hoods;
- the use of safety cabinets to store chemicals, substrates and reagents for microbiology;
- periodic scheduled maintenance of equipment such as chemical hoods, laminar flow hoods, autoclaves;
- the use of specific containers for the disposal of infectious waste, use separate containers to throw broken glass, laboratory needles (syringes, etc..), solvents and other hazardous waste.

### 3.3. Documents of the laboratory

Each laboratory should develop manuals describing analytical procedures to be performed for the examination of different samples. Furthermore, procedures are necessary for the samples acceptance, for daily use of equipment and special care, cleaning and maintenance of such equipment and for measures to be taken in case of change from normal working operations.

All operating procedures shall be readily accessible to the employees.

The criteria for acceptance of test samples should be established by laboratory and, in principle, can follow a procedure that describes the various steps that have been held since the time of receipt of the samples in the laboratory.

Containers for the sample with the appropriate label, showing the information provided in the operating procedure of sampling, has to be delivered upon arrival in the laboratory to the administrative staff office with minutes of sampling. The delivery of the sample must be formalized by completing a form of acceptance and samples delivered to the staff immediately should be stored in refrigerated conditions. After having stored the samples, the administrative staff must complete the worksheet based on the type of sample and analysis that the laboratory will perform.

Subsequently, the spreadsheet and the sample must be delivered to the laboratory personnel responsible for conducting the analysis depending on the information contained on the worksheet and according to the methods used by the laboratory.
3.4. Methods used by the laboratory

The laboratory must use appropriate methods for the analysis being performed. Non-standard methods or in-house methods must be validated by the laboratory before being used for the analysis of test samples. Validation data must be made available to regulatory authorities upon request.

Established standard methods are available for microbiological tests, e.g. those of the International Organization for Standardization (ISO), the European Committee for Standardization (CEN) and the American Public Health Association (APHA). Methods for the detection and enumeration of indicator bacteria (Escherichia coli, etc.) and some pathogenic or opportunistic bacteria (Salmonella, Pseudomonas, etc.) are so widely needed that international standardization is well underway.

Methods must be documented in sufficient detail to provide clear, stepwise instructions to staff and should contain the following information:
- unique identification;
- scope (including reference to standard methods if appropriate);
- type of sample to be tested;
- dates of sample receipt, when analysis was conducted, and test report issue date;
- apparatus and equipment required including performance requirements (i.e. water bath running at 36 ± 1°C);
- reference standard and quality control required;
- environmental conditions required and any stabilisation required (i.e. tempering of molten agar prior to use);
- description of procedure including labelling of samples, check of equipment to be performed, recording of observations, calculation of results (including a worked example) and reporting; and
- acceptance and rejection criteria for relevant aspects of the analysis, i.e. sample receipt.

The laboratory shall also have appropriate documentation and procedures for monitoring the validity of test results including media quality control and verification of test results. It shall have a documented procedure for handling, preparation and use of control cultures. Control cultures for each test being performed must be included with each batch of tests or run at least daily. Moreover, the laboratory shall participate in external proficiency testing programs for all analytes where available, at a minimum frequency of 6 months.

The laboratory shall have appropriate documentation and procedures for the reporting of results. All reports issued by the laboratory should have as a minimum the following information:
- unique report number;
- laboratory/institute name;
- date of report;
- identification of the method used;
- identification of any ambiguous conditions associated with the test;
- date of receipt of the sample including unique sample identification;
- date of testing;
- test results including units, i.e. CFU/mL, etc.

Furthermore, the laboratory may use alternative methods rather than reference methods. Nevertheless, methods other than those standardized have to be showing that results obtained
are at least as reliable as those produced by the methods specified by a standard or a technical regulation.

Laboratories which have recourse to alternative methods shall provide with all relevant information concerning such methods and their equivalence. In fact, a laboratory that decide to use alternative methods should perform comparison tests and to collect in a document the specific protocol, the procedure, data and statistical analysis performed for the calculation of equivalence between the two methods compared. The procedure and calculations to be made for assessing the equivalence between methods are set out in EN ISO 17994 (Water quality - Criteria for establishing equivalence between microbiological methods).

The basic requirements for this review are those established by an European microbiological technical group and include:

- selecting a number (minimum 6) of significant laboratories distributed in a wide land area and distributed in different geographical areas;
- analysis of a sufficient number of samples, initially not less than 50, which should be a function of the techniques used (e.g., MPN, qualitative or quantitative methods) and on relative mean differences in statistical analysis;
- confirmation of all presumptive target microorganisms and a sufficient number of interfering organisms; statistical calculation of the values, as dictated by EN ISO 17994, performed by a statistician.

### 3.5. Characteristics of the microbiological methods

A reference method is a method which is internationally recognised and accepted (e.g., ISO, CEN and AOAC International methods, methods given in EU/national legislations and certain national standards of equivalent standing).

If a laboratory uses in-house methods or adopt standardized methods but outside the specific purpose and scope of the methods, it is necessary to demonstrate that the method fit for purpose.

It can be shown through the process of validation.

The validation process is intended to demonstrate the suitability of the method used by the evaluation of all relevant parameters that characterize the technical characteristics, applicability, performance analytics, etc. It is a procedure that allows to confirm, through the examination and the contribution of objective evidence that, for the intended use, the individual requirements of the method are met.

In this case, the characteristics of a method should be stated according to technical parameters (scope, accuracy, precision, etc.) and also in economic terms (availability of adequate human and material resources, cost, time, etc.). The method performance criteria and the requirements are defined according to the validation phase. The definition of the performance characteristics of a method is dependent from the definition of an application range.

Validation of a microbiological method can be carried out at two levels: i) preliminary validation, ii) secondary validation.

Developmental validation should be appropriately documented and should address specificity, sensitivity, reproducibility, bias, precision, false-positives, false-negatives, and determine appropriate controls. Any reference database used should be documented. Generally speaking, the procedure should be tested using known samples. The laboratory should monitor and document the reproducibility and precision and define reportable ranges of the procedure using control(s).
Before the introduction of a new procedure into sample analysis, the analyst or examination team should successfully complete a qualifying test for that procedure.

The preliminary validation is a exploration procedure in order to define the limits and possibilities of a new, modified, or not sufficiently characterized method. It is the acquisition of limited test data to enable an evaluation of a method. Numerical results of the trial, changes to the test procedure, etc. that may affect the performance of the method should be described in detail and unambiguously in a separate document at the end of validation.

A laboratory that develops a method in-house (internal method) or edits an existing standard method is required to validate the analytical procedure following the same steps involved in the preliminary validation.

The secondary validation or verification is required when a laboratory proceeds to implement a method already developed and validated. Its aim is therefore to provide evidence that the laboratory, using a validated method, is able to meet the requirements set forth in the preliminary validation.

The secondary validation usually uses and simplifies the procedures used in preliminary validation, for example, by providing appropriate control charts.

Currently, most of the standardized methods does not report specifications required by the preliminary validation or only a few of them; in this case, participation with other laboratories, to external circuits could be a first step for the completion of secondary validation.

For the definition of the performance of an analytical method at least the repeatability (r) and reproducibility (R) should be stated.

The following are the method parameters and their definitions:

- **Repeatability**
  Precision under repeatability conditions, i.e. conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time. This quantity can be expressed in terms of close repeatability or intermediate repeatability. The first is significant, for example, in estimates of duplicate analyses. By the standard deviation calculated in these conditions, we obtain the repeatability limit “r”, which allows the operator to decide if the difference between repeated measurements in duplicate on the same sample is or is not significant. To assess the correlation of data in a long period of time, you use the term intermediate repeatability, by measuring variables by the same analyst, same equipment in the same laboratory, while the only different parameter is the time.

- **Reproducibility**
  Precision under reproducibility conditions, i.e. conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment. If the measure is carried out by different laboratories using the same analytical method, the result expresses the reproducibility which takes into account all the possible variability in the laboratory. This is a significant measure of repeatability and is used to calculate the uncertainty associated to a measure, if you use a standard method or standard that contains this information.
  From the standard deviation of reproducibility it is possible to derive the reproducibility limit R, which allows the analyst to decide if the difference between analysis of duplicate samples under reproducibility conditions is or is not significant.

- **Sensitivity**
  Fraction of the total number of positive cultures or properly assigned colonies in the examination. Therefore, the parameter is the probability, expressed as a percentage, that a positive result is actual.
– Selectivity
It is usually assessed by verifying its ability to measure the target organism at all stages of the procedure and it is connected with the method response to the presence of interfering organisms.

– Robustness
It is in relation with the stability of results against changes in the environment (including physical, chemical and human). It is therefore the ability of a method to be not influenced significantly by changes, introduced in its implementation stages.

– Accuracy
The parameter quantitatively measures how close the true value is respect to the average of a series of measures and it is usually expressed in terms of deviation. For the assessment of the accuracy you can proceed in two ways: one is to evaluate it by using certified reference materials, the other is to determine the values of an unknown matrix, using a reference method. In any case, the laboratori should consider the values of precision, a priori established in terms of repeatability and reproducibility.

– Recovery
It allows to verify the efficiency of the method in determining the concentration of the target organism present. The best approach to assess the recovery, although this is not always possible, is to operate using certified reference materials. The alternative is to proceed with known addition of microorganisms of interest or aliquots of samples containing microorganisms.

– Uncertainty
Uncertainty of measurement according to ISO is a parameter, associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand (measurand is a general term for any particular quantity subject to measurement). The parameter may be a standard deviation or a given multiple of it, or a half-width of an interval having a stated level of confidence.

3.6. Quality control

The analytical quality assurance is part of the overall program of the laboratory activities and must be introduced and evaluated in an integrated manner.

It is the responsibility of management of the laboratory to select and justify an appropriate level of quality control based on evaluation of the results and the possible risks, bearing in mind the criticalities of the work, the feasibility, reliability of the method and the possibility of repeating the analysis. The validation of an analytical method, besides to determine its performance, provides the parameters to be used to set quality control during the routine application of the method.

Quality control can be performed at different levels, such as internal quality control and external quality control. The type of quality control to implement depends on the kind, criticality and frequency of the analysis, the number of samples to be analyzed over day, the possible automation and the difficulty of the analysis and, finally, on the operator judgment based on experience in the specific method.

An example of an appropriate control is based on the verification of compliance with the limit of repeatability of the method. In this case, duplicate determinations are performed and it
should be verified if the difference between the values exceeds the limit of repeatability obtained during method validation. If this occurs, it means that the method does not meet the validation criteria. The operator will then identify the causes of non-compliance and adopt the most appropriate procedures to fit the method within the specified requirements. If this does not happen, it must be carried out a further study and possibly a new validation study.

Another control strategy can be to systematically measure a sample with a known content and comparing the data obtained with those expected according to a default criterion of acceptability (according to the requirements of the method), and check if unexpected changes of the method performance were observed during the analysis.

The external quality control is generally accomplished through participation in interlaboratory trial for proficiency testing. These trials allow the laboratory to state the reproducibility and accuracy and to highlight possible systematic errors and provide a mean for monitoring the method reliability.
4. WATERBORNE PROTOZOA

Waterborne protozoan infections can affect immunocompetent people, and may cause life-threatening diseases among immunocompromised and immunosuppressed populations. These infections are common, easily transmissible, and maintain a worldwide distribution. Waterborne protozoan infections remain common in both developed and developing countries and their transmission is facilitated via contacts with recreational and surface waters, or via consumption of contaminated drinking water.

The growth in the number of severely immunocompromised individuals, as a result of the AIDS epidemic, cancer chemotherapy, and organ transplants, has been paralleled both by the increasing prevalence of opportunistic infections and by greater recognition of the disease-causing potential of various intestinal protozoan parasites, such as Cryptosporidium, Giardia, Cyclospora, and microsporidia, as human pathogens. The transmissive stages of human protozoan parasites are small, shed in large numbers in feces of infected people or animals and resistant to environmental stressors. Cryptosporidium oocysts are also hardly able to resist standard disinfection applied to water.

Cryptosporidium was initially thought to be an opportunistic pathogen of immunocompromised persons, but a number of waterborne outbreaks, plus frequent cases in immunocompetent individuals, have disproved this (Arrowood, 1997).

4.1. Cryptosporidium and Giardia

Cryptosporidium is a small protozoan parasite that infects the microvillus region of epithelial cells in the digestive and respiratory tract of vertebrates. The parasite has been first described in mice in 1907 but was not recognized as a causative agent for human illness until 1976. It was first associated with disease in severely immunocompromised individuals, especially AIDS patients with low CD4-counts, but is now also recognized as widespread, general pathogen of immunocompetent humans. It is an obligate intracellular parasite of man and other mammals, birds, reptiles and fish. It requires its host to multiply. Environmentally robust oocysts are shed by infected hosts into the environment. These oocysts can survive the adverse conditions on the environment for months until they are ingested by a new suitable host. In the new host, the life cycle starts again and multiplication occurs, using resources of the host.

The symptoms of cryptosporidiosis are diarrhoea (92% of patients), mild abdominal pain (45% of patients), nausea and vomiting (51% of patients), mild fever (63% of patients) and fatigue. The incubation period of the disease is between 4 and 28 days with an average of 7 days. No drug has been to be effective against Cryptosporidium infection and recovery from the illness is dependent on the body’s immune system. Although the disease is not usually fatal it can be life-threatening for individual with weakened immune systems.

In stool surveys of patients with gastroenteritis, the reported prevalence of Cryptosporidium is 1-4% in Europe and North America and 3-20% in Africa, Asia, Australia, and south and central America (Fricker et al., 1999).

In industrialised countries, the prevalence is high in children under 5 years of age and in young adults. In developing countries, infection is common in infants less than 1 year, but is rarely seen in adults. The prevalence of cryptosporidiosis in AIDS patients in industrialized countries is around 10-20%.
Asymptomatic carriage, as determined by stool surveys, generally occurs at very low rates in industrialised countries (<1%); high rates of asymptomatic carriage (10-30%) are common in non-industrialised countries. Seroprevalence rates are generally higher than faecal carriage rates, from 25-35% in industrialised countries up to 68-88% in Russia and 95% in South America (Fricker et al., 1999).

Oocysts, with a diameter of 4-6 µm, the environmentally resistant transmission stage of the parasite, are shed by infected hosts with their faeces and are immediately infectious. They may remain in the environment for very long periods without loss of infectivity: a very robust oocyst wall protects the sporozoites inside against physical and chemical damage.

Differently, *Giardia* has been known as a human parasite for 200 years. After ingestion and excystation of cysts, the trophozoites attach to surfaces of the gastrointestinal tract. Infections in both children and adults may be asymptomatic. In day care centres, as many as 20% of children may carry *Giardia* and excrete cysts without clinical symptoms.

The symptoms of giardiasis generally include diarrhoea and abdominal cramps; in severe cases, however, malabsorption deficiencies in the small intestine may be present, mostly among young children. Giardiasis is self-limiting in most cases, but it may be chronic in some patients, lasting more than 1 year, even in otherwise healthy people. Studies on human volunteers revealed that fewer than 10 cysts constitute a meaningful risk of infection (Fricker et al., 1999).

### 4.2. Taxonomy

Several species of *Cryptosporidium* have been described and appear to be specific for a class of vertebrates: *C. parvum, C. muris, C. felis,* and *C. wrairi* infect mammals, *C. baileyi* and *C. meleagridis* infect birds, *C. serpentis* infects reptiles, and *C. nasorum* infects tropical fish.

Considering that two host specific genotypes were identified within the species of *C. parvum* (a human genotype H-type or type 1 and a cattle genotype C-type or type 2), a revision of the taxonomy has allowed to include a new species, named *C. hominis,* which appears to be more specific to humans, has been identified. The majority of human infections are caused by *C. hominis* and the cattle genotype of *C. parvum.* Other *Cryptosporidium* species that occasionally infect immunocompetent humans are *C. meleagridis, C. felis* and *C. canis.* Species that have been reported only in immunocompromised individuals are *C. muris/andersoni* and a cervine and pig genotype (Xiao et al., 2004).

The genus *Giardia* consists of a number of species, but human infection giardiasis is usually assigned to *G. intestinalis,* also known as *G. lamblia* or *G. duodenalis.* *Giardia* has a relatively simple life cycle consisting of a flagellate trophozoite that multiplies in the gastrointestinal tract and an infective thickwalled cyst that is shed intermittently but in large numbers in faeces. The trophozoites are bilaterally symmetrical and ellipsoidal in shape. The cysts are ovoid in shape and 8-12 mm in diameter (Fricker et al., 1999).

### 4.3. Routes of transmission

Transmission occurs through direct or indirect contact with faeces of infected shedders. Different routes of transmission have been individuated: person-to person spread in institutions, animal contact during farm visits, contact with recreational waters, swimming pool visits, municipal drinking water and food.
A relevant route of infection with Cryptosporidium is person-to-person transmission, as illustrated by outbreaks in day-care centres and the spread of infection within the households of children attending these centres. Sexual practices involving oro-anal contact also involve a high risk of exposure to the organism. Cryptosporidium can also be transmitted from mammals to humans, and many infections have been derived from contact with infected calves and lambs. Domestic pets can be infected with oocysts, but do not appear to be important sources of human infection (Angus, 1990).

Indirect person-to-person or zoonotic transmission may occur through contaminated water used for drinking or recreation (e.g. swimming pools) or through food and drinks.

Infection studies in healthy human volunteers demonstrated a clear relationship between probability of infection and the ingested oocyst dose of a bovine C. parvum strain. At the lowest dose (30 oocysts), the probability of infection was 20%; at a dose of 1000 oocysts, probability increased to 100%.

Waterborne outbreaks of cryptosporidiosis have been attributed to contaminated drinking water, from both surface-water and groundwater sources and to recreational water, including swimming pools. Outbreaks caused by drinking water have been attributed to contamination of the source water by heavy rainfall or snow-melt, to sewage contamination of wells, to inadequate treatment or treatment deficiencies. Leakages and cross-connections in water-distribution systems have also caused outbreaks of cryptosporidiosis.

Although water is probably not the most important route of transmission, the largest outbreaks of cryptosporidiosis were attributed to contaminated drinking water, both from surface water and groundwater sources (LeChevallier et al., 1991; Mac Kenzie et al., 1994).

By far the most common route of transmission of Giardia is person-to-person contact, particularly between children. Contaminated drinking water, recreational water and, to a lesser extent, food have been associated with outbreaks. Animals have been implicated as a source of human infectious G. intestinalis, but further investigations are required to determine their role.

4.4. Characteristics of Cryptosporidium and Giardia favouring waterborne transmission

Cryptosporidium oocysts have high persistence in the environment and can survive for months in surface water. They also survive well in estuarine waters (over 12 weeks at 20°C and a salinity of 10‰), but less in seawater (4 weeks at salinity of 30‰). Long survival (120 days) of oocysts in soil has also been reported (Robertson et al., 1992).

Due to their smaller size, oocysts are less efficiently removed during soil passage, in bank filtration and in rapid or slow sand filtration in drinking water treatment.

Another factor which influences Cryptosporidium water spreading is oocyst shedding in high numbers. During acute infection, oocysts can be found in high numbers in the faeces of the host. At the peak of the infection, infected humans shed up to $10^5-10^7$ per gram of faeces.

Unlike coccidian parasites and helminths, Cryptosporidium oocysts do not require a period of maturation of the oocysts after shedding with faeces. They are immediately able to infect a new host.

Giardia can multiply in a wide range of animal species, including humans, which excrete cysts into the environment. Numbers of cysts as high as 88,000 per litre in raw sewage and 240 per litre in surface water resources have been reported. These cysts are robust and can survive
for weeks to months in fresh water. The presence of cysts in raw water sources and drinking water supplies has been confirmed.

The currently available standard analytical techniques for the enumeration of these parasites provide an indirect measure of viability and no indication of human infectivity. Cysts also occur in recreational waters and contaminated food.

4.5. Environmental spreading of Cryptosporidium

Cryptosporidium is widespread in the environment, particularly in aquatic environments, as surveys of waters in both temperate and tropical countries have shown. It can be found in effluent from sewage treatment plants, which passes into rivers and water reservoirs, but dissemination into the environment of organism from agricultural sources (manure and slurry) and wild animals is also significant (Briancesco and Bonadonna, 2005). A study of irrigation water used for production of crops traditionally eaten raw has shown widespread presence of protozoan parasites in the United States and Central America.

Cryptosporidium has been found in very many surface waters worldwide, but depending on the level of faecal pollution only few or almost all samples are found positive. The contamination level is related to the presence of the sources of faecal contamination, especially human sewage and run-off from agricultural land with cattle or sheep manure, in the watershed.

The concentration of oocysts at a surface water site varies considerably. An overview of surface water surveys in the US showed that Cryptosporidium was found in 9.1-100% of the surface water samples in geometric mean concentrations of 0.003-1920 oocysts per litre (Le Chevallier et al., 1991). Events such as rainstorms and snowmelt lead to run-off from agricultural lands and overflow of sewage systems. This gives rise to a sudden, sharp increase in the pollution of surface water with human and animal excreta and to a rapid travel of oocysts from source to drinking water or groundwater wells (Atherholt et al., 1998). Several authors have shown that during these events the concentration of Cryptosporidium can be 10-100-fold higher than during non-event situations. Historical data on rainfall, snowmelt and river flow and turbidity may help to identify the conditions that lead to peak events.

Groundwaters that mix with surface water or other sources of contamination (e.g. surface run-off) may contain low levels of Cryptosporidium and give rise to waterborne illness.

Despite the overall relation with the level of faecal contamination, the correlation between Cryptosporidium concentration and the concentration of faecal indicator bacteria is usually low. In waste water only spores of Clostridium perfringens are reported to correlate with Cryptosporidium concentrations in some studies (Payment & Franco, 1993).

4.6. Cryptosporidiosis outbreaks

Cryptosporidium spp. and Giardia duodenalis account for the majority of water-associated outbreaks of parasitic protozoan disease (50.8% and 40.6% respectively) (Karanis et al., 2007).

Numerous outbreaks have been linked to contaminated water from various sources (van Asperen et al., 1996).

Waterborne cryptosporidiosis was first identified in the United States and the United Kingdom in the mid-1980s, and many outbreaks have been recognised since then, particularly in these two countries. In the USA in 1993, the Milwaukee waterborne out break involved more than 400,000 people. It was caused by spring runoff into the intake of two waterworks
combined with their inefficient operation, was the largest on record; approximately 54 people (85% of whom were immuno-deficient) died as a consequence. Both treatment works extracted water from Lake Michigan and treated it by the addition of chlorine and polyaluminium chloride coagulant, rapid mixing, mechanical flocculation, sedimentation, and rapid sand filtration. The filters, cleaned by backwashing the water. Although no specific failure was identified, it was noted that unusually high turbidity values were recorded in the treated water at the start of the outbreak (MacKenzie et al., 1994). In other incidents, cases have been identified in different ways, including routine or enhanced surveillance or special studies. Molecular typing of *Cryptosporidium* has implicated both human and animal strains.

Many waterborne outbreaks of cryptosporidiosis have been reported in industrialized countries. In these outbreaks, oocysts have entered the drinking water because of surface-water treatment failure, contamination of the source water, and leakage into the distribution system. In a significant number of these outbreaks, the drinking water implicated as the cause complied with the WHO Guidelines for *Escherichia coli* levels and turbidity, but, in many cases, deviations from normal raw water quality or treatment operations recommended in the guidelines were identified. However, in other outbreaks that were traced to drinking water, no abnormalities in treatment operations or in the quality of raw or treated water were detected.

Many outbreaks of cryptosporidiosis appear to be associated with swimming pools (Drinking Water Inspectorate, 2000; Anonymous, 1998). In the UK, in the period January 1999 to December 2000 there was a total of 18 outbreaks of cryptosporidiosis of which 14 were attributed to swimming pools.

Several outbreaks have occurred in close social groups such as households, nurseries, and hospital or nursing home settings. *Cryptosporidium* transmission occurs frequently in nurseries, where infants are clustered within classrooms, and share toilets and play areas.

Conversely, few outbreaks of foodborne cryptosporidiosis have been recorded, and those that have were probably due to environmental contamination.

### 4.7. Water treatments for *Cryptosporidium* removal

Preventing *Cryptosporidium* entering a drinking water supplies depends firstly on catchment control measures to minimise pollution risks, secondly physical removal in a well-operated treatment plant, and thirdly by disinfection or inactivation of the organisms. Filtration processes are important barriers for *Cryptosporidium* in water treatment (Adham et al., 1998). Oocysts are sensible to UV, but filtration processes can optimise oocyst removal. Full scale conventional treatment with coagulation, flocculation removal and rapid granular filtration removes >2.3 logs. Other filtration processes, such as slow sand filtration and diatomaceous earth filtration give similar removal efficiencies for oocysts. Membrane filtration can provide even higher removals of more than 4 logs (Akiba et al., 2002).

A well-operated water treatment plant using conventional coagulation and filtration can reduce oocysts by as much as 99% or even 99.8%. Contrary to received opinion, oocysts, although small can be removed by well-operated sand filters. However, sudden changes in flow rates dislodge some the solids retained within the filters, including oocysts, so the careful operation of the filters is of great importance.

The high resistance of *Cryptosporidium* oocysts against chlorine disinfection renders this process ineffective for oocyst inactivation in drinking water treatment. Chlorine dioxide is slightly more effective, but still requires a high CT product of 75-1000 mg min/l for 99% inactivation of oocysts. Ozone is the most potent chemical oocysticide: at 20°C, the CT for 99% inactivation of *C. parvum* oocysts is 3.5 mg min/L. The effectiveness of ozone reduces at lower
temperatures and the CT values required for inactivation of oocysts at low temperatures are high. CT values are limited, however, since high CT’s can give rise to formation of high concentrations of (geno)toxic by-products (Korich et al., 1990).

Although older literature suggests that UV systems have a limited effect on Cryptosporidium viability, more recent work shows that medium-pressure UV is effective against Cryptosporidium; they obtained 99.98% inactivation at UV-doses as low as 19 mJ/cm². The use of UV light for disinfection of water and inactivation of oocysts can be helpful especially on waters taken from sources which are known (or suspected) to be susceptible to contamination by Cryptosporidium.

4.8. Regulations

UK has been the only country which has adopted a legislative measure to control and reduce the risk of waterborne Cryptosporidium outbreaks, considering the numerous events happened in the last decade. The Drinking Water Inspectorate in the UK has also produced detailed protocols for the approved methods of analysis for Cryptosporidium oocysts (Drinking Water Inspectorate, 1999).

The UK regulations required not more than one oocyst in 10 litres and taken no account of whether the oocysts were viable or not; thus a sample would fail even if the oocyst were non-viable. However, these regulations were not a health standard but an operational standard to ensure that the processes for physical removal of particles (including oocysts) are working efficiently at the supplier’s treatment works. The regulation was based on an analysis of waterborne outbreaks of cryptosporidiosis which showed that in all cases there was either some failure in the effective operation of the treatment plant or inadequate physical barriers in place. For these reasons it was considered important to have a monitoring system which checked on the efficacy of treatment for sites at risk of contamination. The treatment standard was that there should be less than 1 oocyst in 10 litres with a sample flow-rate of at least 40 litres per hour taken over a day. This mean in practice, a sample of around 1000 litres a day passed through a continuous sampling cartridge.

This regulation is not more in force since 2007.

At the moment, the European Directive on drinking water (98/83/EC) mentions Cryptosporidium and suggests to determine its presence when Clostridium perfringens is recovered.

4.9. Methodology for analysis

Analytical methods for identifying protozoan parasites have been developed as their public health significance has been realized (Fricker & Crabb, 1998). Initially, much of the work in this area was done in North America and then in the United Kingdom.

Detection of Cryptosporidium in stool samples during acute infection is relatively easy because of the high concentration of parasites, although mistakes in identification can be made. Also, intermittent shedding can occur, in which case the organism may escape detection. Direct examination of stained or unstained stool preparations is generally used for diagnosis in the clinical laboratory.

Isolation from water, on the other hand, requires more complex methods involving concentration and purification. A complicating matter is that parasites are not evenly distributed,
and so large samples are required. Because of the generally small number of organisms which may be present relative to the amount or volume of interfering material, and the fact that the organisms do not grow on laboratory culture media, the methods are inherently more difficult than many bacteriological methods (Bonadonna et al., 2004).

The methodology for the detection of Cryptosporidium oocysts in water is completely different from that traditionally used for quantification of faecal indicator bacteria in the water industry.

The isolation of Cryptosporidium from water requires the use of a procedure which allows the volume of the sample to be reduced whilst retaining any oocysts.

Cause of the similar route of transmission, the way of spreading and the common ability of their resistant stages to survive to environmental stresses and to disinfection procedures, methods are often developed for the simultaneous detection of Cryptosporidium and Giardia.

The relevant laboratory methods can be divided into several stages: concentration, elution, purification, and detection of organisms (Fricker & Crabb, 1998).

The concentration procedure used, however, is dependent upon the water type which is to be analysed, the volume of sample and the amount of particulate material in the sample.

There are two standardized methods for Cryptosporidium and Giardia enumeration, but currently available methods are characterized by low and variable recovery and it is not possible to differentiate viable oocysts of strains that are infectious to humans. Many factors, such as water quality and age of the oocysts, can have significant effects on the overall efficiency of recovery, and it is almost impossible to compare the effectiveness of methods used in different laboratories unless these factors are standardized. There is considerable interest in determining whether oocysts recovered from the environment are viable and potentially infectious.

Mechanical elution from a filter into an appropriate wash buffer containing detergent is followed by concentration, usually by centrifugation. Purification of target organisms from non-target organisms and other debris is increasingly done with paramagnetic beads coated with antibody to the target organisms. The paramagnetic bead/organism complex is then removed from the sample matrix with a magnet, and the beads are disassociated from the organisms, providing a purified concentrate. The technique is known as ImmunoMagnetic Separation (IMS).

A number of different detection mechanisms may be applied to purified concentrates. Epifluorescence microscopy is currently the method of choice for food, water, and environmental samples; monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC) are available for Cryptosporidium and Giardia. The technique is known as fluorescence immunoassay (IFA).

The size, shape, and morphology of cysts or oocysts are important in distinguishing these organisms from other organisms (e.g., algae, yeasts, pollen, etc.) that may be co-purified with the target. Specialised microscopic techniques such as differential interference contrast (DIC), which elucidates internal features, are useful in identification. Sporozoite nuclei within Cryptosporidium oocysts may be stained with a DNA binding dye such as 4′,6-diamidino-2-phenylindole (DAPI) and viewed with fluorescence microscopy.

Genetic amplification detection techniques, particularly polymerase chain reaction (PCR), can be applied for both detection and characterisation. With molecular techniques, oocysts/cysts containing genetic material are detected, whereas immunofluorescence will detect empty oocyst/cysts shells. Notably, neither reliably distinguishes live from dead organisms.

Molecular characterisation of isolates in environmental matrices can greatly improve our understanding of contamination routes.
4.9.1. Quality assurance

Care must be taken to ensure that the particles being counted are oocysts or cysts, to determine whether or not they contain sporozoites, and to exclude algae and yeast cells from any counts that are made. The criteria used for determining that a particle is in fact a *Cryptosporidium* oocyst or a *Giardia* cyst vary between laboratories. Some workers use only the fact that oocysts/cysts fluoresce when labelled with a fluorescein isothiocyanate-tagged anti-*Cryptosporidium* or anti-*Giardia* monoclonal antibody and that it is in the proper size range for a cyst or oocyst. Others will additionally use differential interference contrast microscopy or nucleic acid stains to ascertain that the particles counted are indeed oocysts/cysts. This more detailed analysis allows the confirmation of the counted particles as presumptive oocysts/cysts.

Many factors influence the microscope counts: the amount of background debris and background fluorescence, the experience and alertness of the technician who performs the count, the intensity of fluorescence after staining with the monoclonal antibody, and the quality of the microscope. Quality assurance protocols should define how these factors are addressed.

4.9.1.1. Recovery efficiency

In view of the low and variable efficiency of recovery in the methods used for monitoring *Cryptosporidium* and *Giardia*, it is essential that laboratories collect their own data on recovery efficiency in the different water types they monitor. This can be achieved by seeding a second water sample with a known number of oocysts and cysts and determine the percentage of these recovered by the total protocol for sampling, processing, and counting of environmental samples. However, this assay is influenced by the number, age, and storage conditions of the seeding oocysts/cysts, all of which should be standardized (at least within a particular laboratory) if recovery data are to be meaningful. The recovery efficiency should be assessed sufficiently often to reveal how its variation influences the uncertainty of the monitoring data. This is essential for the interpretation of environmental monitoring data.

4.9.1.2. Specificity

The specificity of the immunofluorescence assay is based on the specificity of the monoclonal antibody-antigen reaction. Although this is highly specific, non-specific binding is observed in natural samples. Many of the particulates that react with the monoclonal antibody can be discriminated from oocysts by a trained observer, but occasionally particles (algae) that are very difficult to discriminate from oocysts occur in the sample. This may lead to false-positive results. The immunofluorescence method is also not specific to *Cryptosporidium* species and genotypes that are infectious to human and animal can be detected and not distinguishable each other. Molecular techniques (PCR, genotyping) are rapidly evolving and some laboratories are now using these methods for environmental monitoring.

4.10. Standardized methods

Currently validated methods for detection of *Cryptosporidium* oocysts from water are the U.S. Environmental Protection Agency 1622 method (January 1999; EPA-821-R-99-001), the USEPA 1623 Method (April 1999; EPA-821-R-99-006 revised in 2001 as EPA-821-R-01-025 and EPA-821-R-01-026) and the ISO 15553:2006.
The protocol of all three methods consists of filtration, immunomagnetic separation, staining with a fluorescent antibody, and microscopic analysis. Microscopic analysis includes detection by fluorescent antibody and confirmation by the demonstration of 1-4 sporozoites or nuclei after staining with DAPI.

4.10.1. ISO 15553

The ISO 15553 specifies a method that is applicable for the detection and enumeration of Cryptosporidium oocysts and Giardia cysts in water. It is applicable for the examination of surface and ground waters, treated waters, mineral waters, swimming pool and recreational waters.

It is not applicable to the identification of the species of organism, the host species of origin or the determination of viability or infectivity of any Cryptosporidium oocyst or Giardia cyst which may be present.

The ISO 15553 describes the use of two alternative concentration techniques for varying volumes of water using cartridge filtration and elution followed by low speed centrifugation.

The first concentration technique requires Pall Envirochek STD and Pall Envirochek HV filters depending upon the volume to be analysed (10-200 l and 10-1,000 l samples of water, respectively). The second concentration technique requires IDEXX Filta-Max compressed foam filters for concentration of 10-1,000 l samples of water.

The Envirochek STD filter consists of a 1 μm pore size pleated polyether sulphone membrane sealed in a polycarbonate shell. The filter is supported on a loose polypropylene support. The flow through the filter should not exceed 2.3 litres per min and the differential pressure across the filter should not exceed 2.1 bar.

The Envirochek HV filter consists of a 1 μm pore size polyester track-etched membrane permanently enclosed in a polycarbonate housing. The polyester membrane is directly laminated to a polypropylene support which offers a significant strength improvement over the standard.

The capsule housing burst strength exceeds 10 bar and the differential pressure across the filter membrane is rated to 4.1 bar. The flow through the Envirochek HV should not exceed 3.4 litres per min.

The IDEXX Filta-Max filter consists of a foam filter module comprising 60 open cell reticulated foam discs with an external diameter of 55 mm and an internal diameter of 15 mm. The discs are sandwiched between two retaining plates and compressed by tightening a retaining bolt to give a nominal porosity of 1 μm. The flow through the filter should not exceed 4 litres per min.

4.10.1.1. Sampling

The size of the sample is dependent on the type of water being sampled, the purpose of the analysis, the sensitivity to be achieved and the speed with which the result is required. Small volume samples (10 l) will give an indication of water quality at the time of sampling whereas large volumes (1 000 l) will give an indication of water quality over an extended period.

Small volume samples (10-100 l) can be collected in the field, transported to the laboratory and then concentrated. For large volume samples (1000 l), concentration has to be performed \textit{in loco}. A filtration device has to be connected in-line with the water supply and a flow meter should be included with the filter and this should be read before and after sampling.
Filters and small volume grab samples have to be transported to the laboratory in the dark at ambient temperature. Once at the laboratory, samples should be stored at 4°C unless they are to be analysed immediately. Samples should be analysed preferably within 24 hours of collection and no longer than 4 days.

4.10.1.2. Elution

For Envirochek STD and Envirochek HV capsule oocysts/cysts are eluted with Laureth 12 elution buffer and wrist action agitation.

IDEXX Filta-Max foam filters are eluted in an elution apparatus (IDEXX Wash Station) which allows them to expand and contract mechanically to release oocysts/cysts in PBS-Tween; eluates are then concentrated through a 3µm membrane filter under vacuum conditions.

Eluates from all filter systems are further concentrated by centrifugation at 1100 x g for 15 minutes and clarified.

4.10.1.3. Clarification by immunomagnetic separation procedure

The IMS procedure has to be performed as follows. Briefly, each 10 mL sample concentrate is added to a Leighton tube containing 1 mL of 10x SL buffer A and 1 mL of 10x SL buffer B (Dynal, A.S., Oslo, Norway). One hundred microliters of Cryptosporidium and Giardia IMS beads is added to each tube, and samples are incubated for 1 h at room temperature with constant rotation. The Leighton tubes are then placed in a magnetic particle concentrator and gently rocked for 2 min through a 90° angle. The supernatant is decanted, the tubes are removed from the magnetic particle concentrator, and 1 mL of 1x SL buffer A is added to each tube. The tubes are gently rocked to resuspend the bead-oocyst/cysts complexes, a Pasteur pipette is used to transfer the suspension into a 1.5 mL polypropylene tube, and the tubes are placed in a second magnetic particle concentrator and rocked for 1 min. Supernatants are aspirated, the magnet is removed, and 50 µL of 0.1 N HCl is added to each sample, which is vortexed for a minimum of 10 s. Samples are allowed to stand for 10 min in the upright position and vortexed for a further 10 s, and magnets are inserted. The tubes are allowed to stand undisturbed for 2 min. The resulting supernatant is placed in the center of a well of a three-well microscope slide containing 5 µL of 1 N NaOH.

4.10.1.4. Staining procedure for recovered oocysts/cysts

The samples are dried in an incubator at no higher than 42°C, fixed in methanol, and air dried, and 50 µL of fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies (MAb) against Cryptosporidium and Giardia are placed onto each well. The slides are placed in a humidified chamber and incubated at 37°C for 30 min, and excess FITC-MAb is aspirated. Any remaining FITC-MAb is removed by adding 50 µL of Phosphate Buffered Saline (PBS) to each well, allowing the slides to stand for 1 min, and aspirating the excess PBS. A 50 µL aliquot of 4',6'-diamidino-2-phenylindole (DAPI) solution (0.4 µg/mL in PBS) is introduced into each well. The slides are allowed to stand at room temperature for 2 min, and excess DAPI solution is removed by washing the slides twice in 0.01M PBS and deionised water. The slides are placed in the dark until dry, a 10 µL aliquot of mounting medium (2% diazabicyclooctane [DABCO] in 60% glycerol-40% PBS) is introduced onto each well, and slides are sealed for subsequent examination under epifluorescence optics.
4.10.1.5. Microscopic examination

Slides are examined by using an epifluorescence microscope fitted with DIC and objectives and eyepieces to a total magnification of 200x or 400x and 1 000x.

Fluorescence microscope equipped with a blue filter block (excitation wavelength, 490 nm; emission wavelength, 510 nm) is used for detection of FITC-MAb-labeled oocysts at a magnification of 400x. Confirmation of oocysts/cysts is achieved at a magnification of 1000x by using a UV filter block (excitation wavelength, 400 nm; emission wavelength, 420 nm) for visualization of DAPI-stained nuclei, and the internal morphology of oocysts/cysts is confirmed under differential interference contrast microscopy.

The majority of *Cryptosporidium* oocysts appear spherical or slightly ovoid with apple green fluorescence with brighter even staining around the entire circumference. Some oocysts can deviate from this description. Those which have been in the environment for some time can be weakly stained or appear fuzzy. They may still have contents and sporozoite nuclei can be identified. Sometimes oocysts may have ruptured during drying on the slide and sporozoite nuclei may be evident adjacent to the oocyst. In addition, oocysts, especially those without contents, may appear to be distorted or partially folded.

The majority of *Giardia* cysts appear ovoid (8-12 μm X 7-10 μm) with apple green fluorescence, however, on occasion, cysts may appear spherical with dimensions of approximately 10 μm. Cysts which have been in the environment for some time may stain weakly and be badly distorted, especially those without contents. Because of other objects (e.g. algae) may mimic the size, structure and staining of *Cryptosporidium* and *Giardia*, when an apple green fluorescent event is observed which is characteristic of a *Cryptosporidium* oocyst or *Giardia* cyst, the object has to be examined with the UV filter block for DAPI staining and subsequently with DIC.

The nuclei of DAPI stained oocysts and cysts appear sky blue upon examination with the epifluorescence microscopy (DAPI UV filter block).

An object that exhibits up to four distinct, sky blue nuclei within a single body, or characterised by nuclear material slightly diffuse, giving it a fuzzy or ragged appearance, or without internal staining could be considered a *Cryptosporidium* oocyst or a *Giardia* cyst and has to be examined by DIC.

Using DIC the size and internal contents of cysts and oocysts can be confirmed.

Internal morphological characteristics typical of a *Cryptosporidium* oocyst include four elongated sporozoites and a cytoplasmic residual body, whereas internal contents or *Giardia* cyst include four nuclei and discernable residua of trophozoite microfilaments and microtubules.

4.10.2. Methods for determining oocyst viability

The significance of finding oocysts in treated and raw waters is not always clear, since some of the organisms that are detected may be nonviable and thus pose no threat to public health. Consequently, there has been considerable interest in developing in vitro methods capable of determining oocyst viability.

The most widely accepted in vitro procedure for determining oocyst viability, excystation, has not been used with the IFA method, because it is difficult to incorporate into the IFA protocol. However, it has been used in combination with molecular technique (e.g., PCR) to detect the presence of viable *Cryptosporidium* oocysts. The sensitivity of this method in environmental samples needs further research. Excystation has also been used in survival and disinfection studies; in the latter, the technique appears to yield a lower inactivation rate than the neonatal mouse infectivity assay.
Some authors developed a procedure to detect viability of *Cryptosporidium* oocysts based on the exclusion of the vital dye Propidium Iodide (PI). They used DAPI as supporting stain, which gave a good correlation with in vitro excystation. Four classes of oocysts can be identified using the assay: those that are viable and include DAPI but exclude PI, those that are non-viable and include both DAPI and PI, and two classes that include neither DAPI nor PI, those with internal contents (sporozoites) and therefore potentially viable, and those without and therefore non-viable, as determined by DIC microscopy. The DAPI/PI procedure is simple to perform and, despite some workers’ reservations about its applicability, can be used for routine environmental work. The incorporation of DAPI into the nucleic acid acts as a further criterion for determining whether a particle is an oocyst or not.

Attempts have been made to develop in vitro models of infectivity using tissue. For these assays, water samples are concentrated by normal procedures and bacteria may be removed by exposure of the concentrate to chlorine at levels that are lethal to bacterial cells but that are thought not to affect oocysts. The concentrates are then inoculated onto the tissue-culture monolayer and left in contact for a period to allow potentially infectious oocysts to infect cells before the remaining debris is washed away. The monolayer is then left for 24-48 hours before being examined for the presence of intracellular parasite antigen or nucleic acid. Immunofluorescent techniques have been used to identify cells that have become infected.

This method offers a means of quantifying infection, although it is not clear whether the presence of a single infectious oocyst will lead to one or more infected cells. In theory, an oocyst that excysts successfully would be expected to produce 4 infected tissue culture cells but initial results have not demonstrated that this can be consistently achieved.

The RT-PCR methods that amplify induced mRNA coding for heat shock proteins can also be used to indicate viability of *Cryptosporidium* oocysts. In combination with the reported sensitivity and specificity, these methods may in the future prove to be very valuable for the water industry (Amar et al., 2004).

### 4.11. Risk assessment

The extreme resistance of some protozoa implies that a “zero risk” is no longer achievable (Teunis & Havelaar, 1999). Measures should be designed to reduce oocyst/cysts concentrations in raw water as far as possible, and treatment should preferably include filtration steps.

This implies that information on parasite concentrations in the raw water is necessary, as well as information on the removal efficiency of treatment processes (Teunis et al., 1997). Quantitative risk assessment provides a tool for combining information on raw water quality (concentrations detected, recovery of the detection method, viability) and treatment efficiency (removal by different steps in the treatment) (Medema et al., 2003).

The definition of maximum acceptable concentrations of pathogens in drinking water based on a maximum acceptable (infection) risk level has become possible with data from studies in volunteers and from dose-response models. An annual infection risk level of $10^{-4}$, as proposed by the United States Environmental Protection Agency (1998), is currently used in Canada, the Netherlands and the USA as the basis for determining the appropriate removal efficiency of surface-water treatment systems.

The exposure of humans to infective *Cryptosporidium* oocysts through drinking water is determined by the concentration of viable and infective *Cryptosporidium* oocysts in drinking water at the point of consumption and by the consumption of drinking water without further treatment (i.e. boiling) by the population.
The concentration of Cryptosporidium in drinking water can be directly assessed by monitoring drinking water for the presence of Cryptosporidium or indirectly by monitoring source water for the presence of Cryptosporidium and assessment of the efficacy of the water treatment processes in removing Cryptosporidium oocysts. Combining source water concentration and treatment efficacy yields an estimate of the concentration of Cryptosporidium in drinking water.

In the UK, direct monitoring of drinking water was embedded in the drinking water regulation (Drinking water Inspectorate, 1999). Water supply systems that are at risk to Cryptosporidium were obliged to sample their treated water continuously.

Although not put in place to assess exposure of consumers to Cryptosporidium, but rather as a treatment standard, the data that were collected under this regulation could be applicable for risk assessment. There are several major drawbacks associated with direct monitoring.

The actual concentration of Cryptosporidium oocysts in drinking water is in most cases very low. In the absence of information on viability, infectivity and genotypes it is difficult to interpret monitoring data in terms of health risk.

A very extensive monitoring programme is required to establish accurate information about the concentration. In the indirect approach, the occurrence of Cryptosporidium oocysts in drinking water is calculated from their occurrence in source water and the removal efficacy of the treatment process. Source water monitoring for Cryptosporidium is applied in several countries, although usually in research rather than in routine monitoring.

If no data are available on the presence of Cryptosporidium in a watershed, the average concentration of oocysts can be estimated very roughly from information on the level of faecal pollution of the watershed (WHO, 2006). This can be assessed by a sanitary survey and available data on faecal indicator bacteria (E. coli, enterococci).

Many studies have indicated that Cryptosporidium concentrations may peak during storm events or snowmelt, and that peak concentrations in source waters may be much higher than the concentration in non-event situations. The potential occurrence of peak events should be taken into account in the sanitary survey and the estimation of the Cryptosporidium levels on the basis of watershed use.

If a watershed lies in an area without human settlements and agriculture activities with E. coli concentrations typically below 1/100 mL, Cryptosporidium oocysts may sporadically be present. Average concentration of oocysts can be estimated at 0.001 oocysts per litre.

If the watershed lies in an area with little human settlement, wildlife and agricultural activities with E. coli concentrations in the range of 1-10/100 mL, Cryptosporidium is infrequently present. The average Cryptosporidium concentration can be estimated at 0.01 oocysts per litre.

If the watershed lies in an area with villages and extensive agricultural activities but faecal wastes are collected and treated before discharged into the watershed, with an average E. coli count estimated at 10-100/100 mL, Cryptosporidium is occasionally present. The average Cryptosporidium concentration can be estimated at 0.1 oocysts per litre.

If small cities, villages and agricultural areas are present in the watershed but wastewater is collected and treated before discharge in the watershed, E. coli counts are typically around 100/100 mL. Cryptosporidium is generally present and the average Cryptosporidium concentration can be estimated at 1 oocyst per litre.

When many and large urbanised areas and intensive agriculture are present in the watershed, average E. coli counts lies around 1000/100 mL. Cryptosporidium is generally present, in an estimated concentration of 10 oocysts/L.
If large urbanised areas and intensive agriculture are present in the watershed and wastewater is generally not treated and/or manure is discharged into the watershed, the *E. coli* counts are usually above 1000/100 mL. *Cryptosporidium* is very generally present in average concentrations of 100 oocysts/L and higher.

### 4.11.1. Scoring system methodology

In 1999 in Northern Ireland a methodology developed to assess risk, which is based on a scoring system, was introduced (Environmental Heritage and Services, “Guidance for the monitoring of *Cryptosporidium* in treated water supplies in Northern Ireland”, 2002, Regulation 27).

It has been developed from the methodologies and protocols issued by the Drinking Water Inspectorate for England and Wales, and from the Direction issued by the Scottish Executive.

The system assesses the risk by identifying the potential for *Cryptosporidium* oocysts to be present in water supplies, after considering a list of factors (Table 4.1) which includes: the degree of exposure of the catchment to *Cryptosporidium* oocysts, agricultural practices, sewage inputs, water source type, river and intake management, *Cryptosporidium* monitoring and epidemiology, water treatment and water treatment monitoring.

A possible score has been assigned to every factor which characterises the water supply. A score classification is provided to understand the level of the risk and the type of action to be taken:

- **High risk level**
  Where a risk assessment score is greater than 100, as calculated from the classification score, a “high risk” level has to be applied. Measures to reduce the risk level from “high” to “significant” are: implementation of continuous monitoring of treated water for *Cryptosporidium* as soon as practicable, and put in practice measures to reduce the risk assessment score to less than 100.

- **Significant risk level**
  Where a risk assessment score is between 50 and 100, as calculated from the classification score, a “significant risk” level has to be applied. Measures to reduce the risk level from “significant” to “low” are: implementation of continuous monitoring of treated water for *Cryptosporidium* as soon as practicable, and put in practice a plan of action to reduce the risk assessment score to less than 50.

- **Low risk level**
  Where a risk assessment score is less than 50, as calculated from the classification score, a “low risk” level has to be applied. In this case monitoring of treated water is not required but shall be carried out if there is a significant deterioration of raw water or a disturbance at a water treatment plant. If an outbreak of *Cryptosporidium* occurs, additional investigations shall also be required.

Table 4.2 reports the classification score of *Cryptosporidium* risk assessment.
<table>
<thead>
<tr>
<th>Factor affecting risks</th>
<th>Possible score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals on the catchment</td>
<td></td>
</tr>
<tr>
<td>Cattle/calves</td>
<td>6</td>
</tr>
<tr>
<td>Sheep/lambs</td>
<td>6</td>
</tr>
<tr>
<td>Deer</td>
<td>2</td>
</tr>
<tr>
<td>Pig farms</td>
<td>2</td>
</tr>
<tr>
<td>High number of birds</td>
<td>2</td>
</tr>
<tr>
<td>Any other farmed animal/bird</td>
<td>1</td>
</tr>
<tr>
<td>Agricultural practices on the catchment</td>
<td></td>
</tr>
<tr>
<td>Slurry spraying, dung spreading, slurry or dung storage</td>
<td>6</td>
</tr>
<tr>
<td>Discarge into the catchment/water source</td>
<td></td>
</tr>
<tr>
<td>Septic tanks</td>
<td>1</td>
</tr>
<tr>
<td>Sewage works – Population served: less than 500</td>
<td>2</td>
</tr>
<tr>
<td>Sewage works – Population served: 501-5,000</td>
<td>3</td>
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<tr>
<td>Sewage works – Population served: 5,001-20,000</td>
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<td>Sewage works – Population served: 20,001-50,000</td>
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</tr>
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<td>Sewage works – Population served: greater than 50,001</td>
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<td>Abattoir/livestock market</td>
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<td>Water source type</td>
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<td>Boreholes and deep wells – Vulnerable soil type/geology</td>
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</tr>
<tr>
<td>Boreholes and deep wells – Non-vulnerable soil type/geology</td>
<td>1</td>
</tr>
<tr>
<td>Secure natural springs – Vulnerable soil type/geology</td>
<td>3</td>
</tr>
<tr>
<td>Secure Natural Springs – Non-vulnerable soil type/geology</td>
<td>1</td>
</tr>
<tr>
<td>Other shallow underground sources – Vulnerable soil type/geology</td>
<td>6</td>
</tr>
<tr>
<td>Other shallow underground sources – Non-vulnerable soil type/geology</td>
<td>4</td>
</tr>
<tr>
<td>Upland reservoir</td>
<td>4</td>
</tr>
<tr>
<td>Lowland reservoir</td>
<td>6</td>
</tr>
<tr>
<td>Upland river or burn – Direct abstraction</td>
<td>6</td>
</tr>
<tr>
<td>Lowland river or burn – Direct abstraction</td>
<td>8</td>
</tr>
<tr>
<td>River and burn intake management</td>
<td></td>
</tr>
<tr>
<td>Quality monitors on the intake</td>
<td>-1</td>
</tr>
<tr>
<td>No quality monitors on the intake</td>
<td>3</td>
</tr>
<tr>
<td>Quality monitors alarmed</td>
<td>-2</td>
</tr>
<tr>
<td>Quality monitors cause intakes to shut automatically</td>
<td>-4</td>
</tr>
<tr>
<td>Intakes shut manually under poor water quality conditions</td>
<td>-1</td>
</tr>
<tr>
<td>Intakes not shut under poor water quality conditions</td>
<td>3</td>
</tr>
<tr>
<td>Water treatment</td>
<td></td>
</tr>
<tr>
<td>Simple disinfection only</td>
<td>10</td>
</tr>
<tr>
<td>Microstraining</td>
<td>10</td>
</tr>
<tr>
<td>Simple sand filtration (not slow sand); sand depth: minimum 90 cm</td>
<td>8</td>
</tr>
<tr>
<td>Coagulation followed by DAF/sedimentation and filtration</td>
<td>-10</td>
</tr>
<tr>
<td>Coagulation followed by rapid gravity or pressure filtration only</td>
<td>-7</td>
</tr>
<tr>
<td>Slow-sand filtration – musthave a minimum sand depth of 30cm</td>
<td>-9</td>
</tr>
<tr>
<td>Roughing filters as pretreatment for slow-sand filtration</td>
<td>-4</td>
</tr>
<tr>
<td>For slow-sand filtration – at least 30 cm of sand</td>
<td>-2</td>
</tr>
<tr>
<td>For slow-sand filtration – at least 45 cm of sand</td>
<td>-4</td>
</tr>
<tr>
<td>Membrane filtration</td>
<td>-16</td>
</tr>
<tr>
<td>Ozone treatment</td>
<td>-8</td>
</tr>
<tr>
<td>Cartridge/kalsep filtration</td>
<td>-2</td>
</tr>
<tr>
<td>Filtamat or equivalent</td>
<td>-2</td>
</tr>
</tbody>
</table>

*to be continued*
<table>
<thead>
<tr>
<th>Factor affecting risks</th>
<th>Possible score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment works performance</strong></td>
<td></td>
</tr>
<tr>
<td>No major quality deviation from normal background levels for filtered or treated water</td>
<td>-4</td>
</tr>
<tr>
<td>Process known to be bypassed</td>
<td>4</td>
</tr>
<tr>
<td>Coliform exceedences considered trivial by DWI</td>
<td>2</td>
</tr>
<tr>
<td>Coliform exceedences considered not trivial by DWI</td>
<td>4</td>
</tr>
<tr>
<td>Significant increase in turbidity before and after filter wash</td>
<td>4</td>
</tr>
<tr>
<td>No significant increase in turbidity before and after filter wash</td>
<td>0</td>
</tr>
<tr>
<td>Signs of significant media loss from filters/severe cracking of filter bed</td>
<td>2</td>
</tr>
<tr>
<td>Filters in good conditions</td>
<td>-2</td>
</tr>
<tr>
<td><strong>Treatment works monitoring</strong></td>
<td></td>
</tr>
<tr>
<td>Each filter has a turbidity meter with a trend or an alarm setting</td>
<td>-3</td>
</tr>
<tr>
<td>Each filter has a turbidity meter without a trend or an alarm setting</td>
<td>0</td>
</tr>
<tr>
<td>One turbidity meter is shared over several filters</td>
<td>2</td>
</tr>
<tr>
<td>No turbidity meter on filters</td>
<td>6</td>
</tr>
<tr>
<td>Alarmed continuous residual coagulant monitor on works outlet</td>
<td>-2</td>
</tr>
<tr>
<td>Continuous residual coagulant monitor on works outlet (but not alarmed)</td>
<td>0</td>
</tr>
<tr>
<td>No continuous residual coagulant monitor on works outlet</td>
<td>2</td>
</tr>
<tr>
<td>Routine sampling and analysis of the water quality process carried out at the WTW for turbidity/residual coagulant</td>
<td>-1</td>
</tr>
<tr>
<td>No routine water quality process sampling and analysis undertaken</td>
<td>4</td>
</tr>
<tr>
<td><strong>Treatment works operation</strong></td>
<td></td>
</tr>
<tr>
<td>Treatment works process control manuals available</td>
<td>-1</td>
</tr>
<tr>
<td>Treatment works process control manuals not available</td>
<td>1</td>
</tr>
<tr>
<td>Action plans for dealing with deviations in quality available</td>
<td>-1</td>
</tr>
<tr>
<td>Action plans for dealing with deviations in quality not available</td>
<td>1</td>
</tr>
<tr>
<td>Record of actions/audit trails available</td>
<td>-1</td>
</tr>
<tr>
<td>Record of actions/audit trails not available</td>
<td>1</td>
</tr>
<tr>
<td>Slow start on filters</td>
<td>-2</td>
</tr>
<tr>
<td>No slow start on filters</td>
<td>2</td>
</tr>
<tr>
<td>Filters run to waste or head of works for a period after a backwash</td>
<td>-4</td>
</tr>
<tr>
<td>Water flow through plant changes suddenly</td>
<td>2</td>
</tr>
<tr>
<td>Water flow through plant stable</td>
<td>-2</td>
</tr>
<tr>
<td>Backwash and/or sludge supernatant has to be recycled</td>
<td>2</td>
</tr>
<tr>
<td>Alternative disposal route available for backwash and sludge supernatant</td>
<td>-2</td>
</tr>
<tr>
<td>Slow-sand filters matured before going into service</td>
<td>-3</td>
</tr>
<tr>
<td>Slow-sand filters not matured before going into service</td>
<td>3</td>
</tr>
<tr>
<td>Plant frequently run above capacity</td>
<td>4</td>
</tr>
<tr>
<td>Turbidity of backwash supernatant monitored when recycled</td>
<td>-2</td>
</tr>
<tr>
<td>Turbidity of backwash supernatant not monitored when recycled</td>
<td>2</td>
</tr>
<tr>
<td><strong>Cryptosporidium monitoring</strong></td>
<td></td>
</tr>
<tr>
<td>No Cryptosporidium data are available for the raw water</td>
<td>3</td>
</tr>
<tr>
<td>Raw water concentrations all less than 1 oocyst/L (treatment score negative)</td>
<td>-2</td>
</tr>
<tr>
<td>Treated water concentrations over 1 oocyst/L (treatment score negative)</td>
<td>4</td>
</tr>
<tr>
<td>No Cryptosporidium data are available for the treated water</td>
<td>4</td>
</tr>
<tr>
<td>Treated water concentrations all less than 1 oocyst/10 L</td>
<td>-5</td>
</tr>
<tr>
<td>Treated water concentrations over 1 oocyst/10 L</td>
<td>5</td>
</tr>
<tr>
<td>Continuous monitoring of treated water</td>
<td>-5</td>
</tr>
<tr>
<td>Facility for continuous monitoring if required</td>
<td>-1</td>
</tr>
<tr>
<td><strong>Epidemiology</strong></td>
<td></td>
</tr>
<tr>
<td>No known cases of cryptosporidiosis in the area served</td>
<td>-1</td>
</tr>
<tr>
<td>No past history of suspected, possible or probable outbreaks</td>
<td>-2</td>
</tr>
<tr>
<td>Past history of suspected outbreaks associated with supply</td>
<td>15</td>
</tr>
<tr>
<td>Cause of any suspected outbreak or cases has been removed, or suitable treatment added</td>
<td>0</td>
</tr>
<tr>
<td><strong>Population weighting:</strong> 0.4 x log10 (population served by supply)</td>
<td></td>
</tr>
<tr>
<td><strong>Final risk assessment score:</strong> sum of scores for each factor x population weighting</td>
<td></td>
</tr>
</tbody>
</table>
### Table 4.2. Cryptosporidium risk assessment. Classification score

<table>
<thead>
<tr>
<th>Supply classification</th>
<th>Risk assessment score</th>
<th>Action to be taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>High risk</td>
<td>&gt; 100</td>
<td>Implement continuous monitoring of treated water for <em>Cryptosporidium</em> as soon as practicable, and provide the Department, within 3 months, with measures intended to be taken to reduce the risk assessment score to less than 100.</td>
</tr>
<tr>
<td>Significant risk</td>
<td>50-100</td>
<td>Implement continuous monitoring of treated water for <em>Cryptosporidium</em> as soon as practicable, and provide the Department, within 3 months, with intended plan of action to be taken to reduce the risk assessment score to less than 50.</td>
</tr>
<tr>
<td>Low risk</td>
<td>&lt; 50</td>
<td>Monitoring of treated water is not required but shall be carried out if there is a significant deterioration of raw water or a disturbance at a water treatment plant. If an outbreak of <em>Cryptosporidium</em> occurs, additional investigations shall also be required.</td>
</tr>
</tbody>
</table>

### 4.12. Conclusions

Many waterborne outbreaks of cryptosporidiosis have been reported in industrialised countries.

The first reported human outbreak of cryptosporidiosis due to contaminated water supplies occurred in Texas in 1984 in conjunction with a Norwalk virus outbreak. The water source was an artesian well and was suspected of being contaminated with sewage. Disinfection by chlorination was the only treatment and although adequate to control coliform bacteria, it was apparently insufficient in controlling Norwalk virus and *Cryptosporidium*. A second outbreak in Carrollton, Georgia (USA) occurred in January 1987, where over 13,000 people were affected.

The Carrollton drinking water supply underwent conventional treatment, including coagulation, sedimentation, rapid sand filtration, and disinfection. Subsequent investigations revealed no violations for coliform or turbidity levels. At the same time, an outbreak in the UK was reported that was caused by contamination of the distribution network through contamination of breakpressure tank, which was enhanced during rainfall. Again, the water complied with the coliform standard. These first waterborne outbreaks learned that:

- *Cryptosporidium* could be transmitted by municipal drinking water systems and cause large outbreaks;
- systems with chlorination only without filtration were especially vulnerable;
- outbreaks could occur through drinking water that met the coliform and turbidity standards.

In 1991 an outbreak occurred in the Swindon/Oxfordshire area of the UK through conventionally treated (coagulation/filtration and disinfection) drinking water. In this case, recirculation of filter backwash water caused an increased challenge of the treatment systems with *Cryptosporidium* oocysts that could break through the filters (Richardson *et al.*, 1991).

In April 1993, the largest North American outbreak of cryptosporidiosis was described as apparently affecting some 403,000 people in Milwaukee, Wisconsin and as being caused by cattle faeces passing through the conventional treatment plant (Mac Kenzie *et al.*, 1994).

Since that time both the true size and sources of the outbreak have been questioned, with molecular epidemiological evidence pointing to a human rather than a cattle source and the true
size of the outbreak possibly being exaggerated by orders of magnitude through over-reporting bias.

These and many other outbreaks learned that also conventional treatment systems can be vulnerable to outbreaks when the coagulation and filtrations systems are not carefully operated and maintained.

In 1998 Sydney experienced a succession of Cryptosporidium contamination events. A combination of early detection in samples by the monitoring laboratory, subsequent boil water alerts issued to consumers, and the possibility that oocysts in the supply system were not viable or counts overestimated may have been the reason that no cases of cryptosporidiosis were traced to drinking water during these three events. The incidents did result in a large body of research into the origin of the contamination events. This has lead to the understanding of the transport of Cryptosporidium in reservoirs following rainfall events. In this case, the heavy rainfall after a period of draught caused relatively cold floodwater to enter the reservoir (Lake Burrangorang) and fill the reservoir. The thermal stratification of the water in the reservoir caused the colder floodwater to flow along the lake bottom and reached the dam with the off take within days in stead of months. This flow caused an internal wave in the reservoir that hit the off take on several subsequent days, leading to relatively high Cryptosporidium counts in the water that entered the treatment.

In the summer of 2002, increased counts of Cryptosporidium in treated water (found in the statutory monitoring) led to a boil water alert for Glasgow and Edinburgh. Also here, no increase in the number of gastro-enteritis cases was observed.

Several authors have reviewed the causes of outbreaks through drinking water and have made recommendations for optimising water treatment practice. In a significant number of these outbreaks, the drinking water that was implicated as the cause of the outbreak complied with the WHO-guidelines for Escherichia coli levels and turbidity. In most outbreaks, deviations from normal raw water quality or treatment operation could be identified. However, in a drinking waterborne outbreak in Las Vegas, no abnormalities in operation or water quality (raw or treated) were detected.

The information on the events/failures that led to these outbreaks can be used for the identification of hazards. A common thread of many of the reported outbreaks and contamination events is that the disinfection and filtration systems were thought to have been inadequate to prevent contamination, at least in their operational state at the time of contamination.

More researches are needed notwithstanding a large amount of data have been collected on the removal of Cryptosporidium during filtration or the inactivation upon exposure to disinfectants.

Most of these studies are done in laboratory experiments or pilot scale treatment systems. This information can be and is used in QMRA-studies in the form of generic log-credits.

This is very useful in screening-level QMRAs to set risk management priorities. As the screening-level QMRA has indicated that a water supply system may be at risk, a more site-specific QMRA is needed. In the vast majority of water supplies, no site-specific data on the removal of Cryptosporidium are present. To extrapolate the body of available scientific knowledge and water treatment experience to a treatment process at a specific site, performance models for the removal of Cryptosporidium by the treatment process are very useful. This is already used in ozone disinfection for instance, using the disinfectant concentration, contact time, reactor hydraulics and temperature as model parameters.

The performance of a treatment process is not constant. As a result of variation in feed water quality, temperature, water flow and process control, the removal of pathogens varies over time. Of particular importance for QMRA are the moments of poor performance. In a well-operated
treatment process, these moments are scarce, but when they occur, they result in a relatively high health risk. Many of the outbreaks are related to treatment deficiencies. Data on the occurrence of such events are available in the scientific literature, but other data may be deduced from treatment performance monitoring data (i.e. turbidity, particle counts, disinfectant residual) or the microbiological monitoring programs (*E. coli* and other microbial indicators) of water utilities.
REFERENCES


LeChevallier MW, Norton WD, Lee RG. Occurrence of *Giardia* and *Cryptosporidium* spp. in surface water supplies. *Appl Environ Microbiol* 1991;57(9):2610-16


