MICROBIOLOGICAL WATER QUALITY ASSESSMENT (CATCHMENT TO TAP)

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Keywords: Potable, drinking water, regulation, *E. coli*, *Cryptosporidium*, *Giardia*, bacteria, protozoa, monitoring, cholera, indicator organisms, public health

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Summary

The anthropogenic water cycle begins and ends in the water resources from where water is taken to be processed and turned into tap water. From the source, the catchment, the water is moved through a collection structure such as a pipe, to the water treatment plant where it is cleaned and disinfected, then to the consumer via a distribution pipe. The used water that the consumer discards is collected and channeled to a wastewater treatment plant where it is again cleaned and then returned to the river system it was taken from. Micro-organisms, some of which may be pathogenic (harmful to human health) are introduced into the water by the consumers and also by animals and birds in the natural environment. These micro-organisms can live in the water resources and they might not always be removed by the water treatment plant, so monitoring the microbiological water quality is necessary to assess whether the water quality is good or poor before the water is treated, when the water is sent to the consumer and again before the water is returned to the environment. The reasons for this are environmental and public health. Many micro-organisms cause disease in humans and in other animals, such as cholera, typhoid and shigellosis. Providing clean, safe water for people to drink and for protection of the aquatic environment includes ensuring that dangerous micro-organisms are being removed by the water treatment processes.

1. Introduction

Contaminated water sources are vehicles for the transmission of waterborne diseases such as cholera, shigellosis, and Campylobacteriosis (see *Classification of water-related disease*). The World Health Organization (WHO) recently estimated that about 1.1 billion people globally drink unsafe water and the vast majority of diarrheal diseases in the world (88%) are attributable to unsafe water, sanitation and hygiene. Approximately 3.1% of annual deaths (1.7 million) and 3.7% of the annual health burden (disability adjusted life years [DALYs]) world-wide (54.2 million) are attributable to unsafe water, sanitation and hygiene (see *Burden of disease: current situation and trends*). In order to prevent waterborne diseases, water is treated to eliminate pathogens and provide water safety. Effective water treatment is largely accepted as a necessity, but that the need for effective monitoring and management of water quality from raw water supply to point of use is less recognized and often very superficially performed.

Untreated water sources such as surface waters (streams, rivers, lakes, etc.) or unprotected open wells are vehicles for waterborne bacterial diseases such as cholera and typhoid fevers. Untreated waters may also play a role in the transmission of water-washed viral enteric diseases such as hepatitis (hepatitis A and E viruses), gastroenteritis (rotaviruses, noroviruses and sapoviruses), as well as an unknown number of ill-defined diseases caused by other enteric viruses (adenoviruses, astroviruses, coxsackieviruses and echoviruses). The fecal-oral route is probably the major route for transmission of these bacterial and viral diseases as well as of many parasitic diseases in poor sanitary conditions. An improvement of water quality and water usage for improving sanitary conditions should result in a decrease of waterborne as well as water-washed diseases.

1.1. The Route from Catchment to Tap

A drinking water supply system includes some or all of: raw water storage, raw water pipework, water treatment (commonly comprising coagulation / flocculation, filtration, clarification and disinfection), treated water storage and treated water distribution. In a conventional treatment system, raw water is abstracted from the source and conveyed to the treatment plant where it is treated in different processes. After treatment, the water is stored and then distributed to individual users. The water source, treatment, and distribution are the three main components of the system.

Surface water sources are frequently rivers, lakes, ponds or man-made reservoirs. Purpose-built reservoirs provide storage for the raw (untreated) water that is abstracted from natural water bodies and act as balancing tanks, buffering the changes in water quality that occur in surface waters. For example, heavy rain can cause sudden increases in the turbidity of a river. If the river water is abstracted into a storage reservoir, however, the reservoir water quality changes much more slowly than in the river.

Raw water pipework comprises the infrastructure used to transfer water from the source to the treatment plant. These facilities may include a storage reservoir, screens to exclude large solids, an intake, pumps and valves and pipework or channels to convey the water to the plant. A flow meter downstream of the screens is used to monitor instantaneous and cumulative flow to enable correct operation of the treatment process.

Microbial pathogens, which include bacteria, viruses and protozoan parasites, can be physically removed as particles in treatment steps such as coagulation / flocculation, clarification and filtration (also called unit processes, or unit operations), or they can be chemically deactivated by disinfection. Disinfection is a critical component of water treatment in most countries. It is important in maintaining the microbiological quality of water, because the physical removal processes do not remove all microorganisms from the water. The disinfectant residual that remains in the drinking water in the distribution system also controls the microbial quality of water, preventing bacterial proliferation after treatment has been completed (re-growth) and limiting the development of biofilms in the water pipes. Disinfection has substantially decreased the occurrence of water-related diseases (see *Goals of water treatment and disinfection: Reduction in morbidity and mortality*).

1.2. Water Treatment

Water treatment processes are designed to remove solids (including microbial material) and solutes from the raw water.

Coagulation / flocculation for removal of solids involves the rapid mixing of chemical coagulants / flocculants with the water. One of the most commonly used coagulants is alum ($Al_2(SO_4)_3.18H_2O$), which reacts with the alkalinity in the water to form an aluminum hydroxide floc:

$$Al_2(SO_4).18H_2O + 3Ca(HCO_3)_2 \rightarrow CaSO_4 + 2Al(OH)_3 + 6CO_2 + 18H_2O \qquad (1)$$

It may be necessary to dose soda ash (Na_2CO_3) or lime (CaO) in addition to alum to provide enough alkalinity. Silica is also sometimes added to the water to provide nuclei for flocculation. After rapid mixing, slow mixing is used to aggregate large flocs that will settle quickly during clarification.

Clarification (also called sedimentation or settling) allows suspended particles to be separated from the water by gravity. Most of the suspended matter is usually removed in the clarification tanks. Raw water storage reservoirs also serve as sedimentation tanks, removing solids from the water before treatment begins, but they are not designed as clarifiers. Tanks designed specifically for clarifying water are normally tall, circular tanks with a funnel-shaped base, with slow or no mixing.

Filters remove most of the suspended particles remaining in the water after settling. One of two kinds of filtration (rapid and slow sand filtration) may be used, the choice depending on several factors including the nature of the water. Coarse sand is used for rapid sand filtration and fine sand is used in slow sand filtration. In slow sand filtration, sand is drawn downward through the filter by gravity. Particles are physically removed by straining and adsorption and broken down by biological action.

Storage reservoirs for the treated water may be installed either after the final unit process at the treatment plant, or at intervals in the distribution system. Reservoirs allow the treatment plant to meet peak water demands and maintain the water supply during treatment plant maintenance. Unless the water can be distributed entirely by gravity from the storage reservoir, pumps are required to deliver the stored, treated water to the tap. The pumps draw water from storage and supply it to the system under pressure.

1.2.1. Disinfection of Water

Disinfection of water usually involves the use of oxidants, such as chlorine, ozone, and ultraviolet radiation. Chlorination is the most commonly used of all these methods. When chlorine is added to water it has an immediate and lethal effect on most micro-organisms. Two reactions take place during chlorination, hydrolysis:

$$Cl_2 + H_2O \rightarrow HOCl + Cl^- + H^+ \qquad (2)$$

Hypochlorous acid

and ionization:

$$HOCl \rightarrow OCl^- + H^+ \qquad (3)$$

Hypochlorite ion

The sum of the concentrations of the hypochlorite ion and hypochlorous acid total the *free available chlorine*. The amount of available chlorine required to disinfect water depends on the amount of reductants (inorganic and organic matter) present. Most waters are considered to be disinfected when a free chlorine residual of 0.2 mg/l remains in solution 10 minutes after chlorination. A higher amount of chlorine residual can cause undesirable odors and tastes; a lower residual does not guarantee the safety of the treated water.

Post-chlorination—the application of chlorine after filtration—is the most common method. Pre-chlorination, the application of chlorine before filtration, improves coagulation, reduces the organic load on the filters, and prevents the growth of algae. However, pre-chlorination can not be used if organic compounds are present in the water and the formation of trihalomethanes (THMs) from the reaction of the chlorine with the organics is a problem. Because of possible formation of THMs, ultraviolet (UV) radiation and ozone are sometimes used as alternatives. Both are powerful oxidizing agents, but they lack a persistent residual. If ozone or UV radiation is used, a secondary disinfectant such as chlorine may still be required for the protection of water quality in the distribution system.

Membrane processes can also be used for disinfection. Membrane processes include microfiltration, ultrafiltration, nanofiltration and reverse osmosis. Some membranes (e.g. reverse osmosis) disinfect water by physical removal of viruses and bacteria. Membrane systems have small land requirements, use fewer chemicals and are not as complicated to build and operate as conventional treatment systems with several unit processes. However, membranes do become fouled and block over time despite the ongoing cross-flow cleaning process. Pretreatment of the water by conventional means is often used to prolong the life of the membranes. A secondary disinfectant is almost always required to provide a chemical residual to protect treated water against recontamination.

2. Microbiological Water Quality Indicators

Waterborne diseases are usually caused by enteric pathogens transmitted by the fecal-oral route. They are generally excreted in feces by infected people, carried in fecally contaminated food or water and ingested by other individuals. Water can also play a role in the transmission of pathogens which are not fecally excreted, such as opportunistic pathogens that are normal external body flora. Some of these pathogens are natural inhabitants of the aquatic or soil environment. Most waterborne pathogens are distributed globally, but some, for example cholera and hepatitis E, tend to be regional.

In order to ensure that potable water is microbiologically safe to drink, there must be no pathogens in the water at its point of use. Since some pathogens are extremely resistant to certain water treatment processes, the microbiological quality of the raw water is linked to the quality of the treated water; hence both types of water should be monitored. Raw water quality is used to inform treatment process selection, and treated water quality has a direct impact on public health.

Three basic mechanisms govern the occurrence of pathogenic microorganisms in treated drinking water:

i) the microbes break through the treatment process from the raw water supply,
ii) the microbes re-grow after treatment from very low levels, typically in biofilms and
iii) the organisms result from recontamination of the treated water within the

distribution system.

To protect the end user from pathogens arising in the water supply at the tap, microbiological water quality is therefore monitored in samples of water taken from four stages in the pathway from catchment to tap, namely the water source, the raw water entering the treatment plant, the treated water leaving the plant and the water at the tap.

The detection of some waterborne and water-related pathogens requires expensive, complex and time consuming techniques, while others are not detectable by conventional methods at all. Water quality monitoring programs are, therefore, usually based on the test of indicator microorganisms. These organisms, such as certain coliform groups, are members of the normal microbial flora of the human gastrointestinal tract and are easy to detect. Their presence in treated water is taken as an indication that drinking water was not adequately treated or disinfected, even though the organisms themselves are not pathogenic. An ideal indicator microorganism should fulfill the following criteria:

i) it should always be present when the pathogen is present and should be absent in uncontaminated water,
ii) it should be present in numbers greater than the pathogen it indicates,
iii) its survival in the environment and resistance to the treatment processes should be comparable to that of pathogens,
iv) it should not be harmful to human health,
v) it should be easy to identify and to isolate and
vi) it should be suitable in all types of water.

Some other properties are also desirable, such as counts which are directly related to those of pathogens. However, the most important requirement is that the indicators used should be absent or inactivated whenever pathogens are absent or inactivated.

2.1. Bacteria

Bacteria that are typically transmitted by the fecal-oral route include *Salmonella* spp., *Shigella* spp., pathogenic *Escherichia coli*, *Campylobacter* spp., *Vibrio cholerae* and *Yersinia enterocolitica*.

The recognized bacterial indicators most commonly used for assessing water quality are bacteria of the *Enterobacteriaceae* family defined as the total coliform bacteria and the fecal coliform bacteria. The coliform bacteria are Gram-negative rods, aerobic or facultative anaerobic, non spore-forming, rapid-lactose-fermenting with gas formation within 48 h at 35°C. Some bacteria from this group are indigenous to soil and waters. This necessitates the use of a more precise indicator for fecal contamination in many situations. The fecal coliform bacteria are members of the coliform group of bacteria usually (but not always) found in the feces of warm-blooded animals; they have the characteristics of the coliform group, but will also produce gas within 24 h at 44.5°C. The presence of fecal coliform bacteria in water is indicative of contamination by fecal material and is therefore considered indicative of a health risk, because many enteric pathogens (bacterial, viral and parasitic) are present in feces. Furthermore, the significance of the coliform group density has long been established as an indication of the degree of pollution and thus the microbiological quality of water.

The preferred methods for assessing water quality, using as indicators the members of the coliform group and the fecal coliform group, are the membrane filtration technique and the multiple-tube fermentation techniques. Both are quantitative methods with high levels of sensitivity and they can be used to evaluate all types of waters including treated drinking waters, recreational waters and untreated surface waters. The membrane filtration technique is usually more expensive and requires more equipment than the multiple-tube fermentation technique. Table 1 summarizes the number and type of tests investigated.

Indicator Organism	**Use in Water Quality Management**
Standard plate count / HPC organisms	**Refers** to all micro-organisms which produce visible colonies on non-selective medium after incubation at 35°C for 48 hours. Indicator of the general microbiological quality of water. **Used** for monitoring of efficiency of water treatment and disinfection processes, or after growth in water distribution systems.
Total coliform bacteria (total coliforms)	**Refers** to all bacteria which produce colonies with a typical metallic sheen within 20-24 hours of incubation at 35°C on m-Endo agar. Gives an indication of the general sanitary quality of water, since this group includes bacteria of fecal origin. However, many of the bacteria in this group may originate from growth in the aquatic environment. **Used** to evaluate quality of drinking water and related waters.
Fecal coliform bacteria (fecal coliforms)	**Refers** to all bacteria which produce typical blue colonies on m-FC agar with 20-24 hour of incubation at 44.5°C, and comprises members of the total coliform group which are capable of growth at elevated temperature. Indicator of probable fecal pollution of water since this group is much more closely associated with fecal pollution than the broader total coliform group. Some fecal coliforms may not be of fecal origin. **Used** to evaluate the quality of wastewater effluents, river water, sea water at bathing beaches, raw water for drinking water supply and recreational waters.
Escherichia coli (*E. coli*)	**Refers** to fecal coliforms which are indole-positive at 44.5°C, and generally consists only of *E. coli* which is almost definitely of fecal origin from warm-blooded animals. **Used** to evaluate the possible fecal origin of total and fecal coliforms, usually when these are isolated from drinking water.
Fecal streptococci (enterococci)	**Refers** to bacteria which produce typical reddish colonies on m-Enterococcus agar after 48 hours incubation at 35°C. These bacteria often appear in human and animal feces, but in lower numbers than total or fecal coliforms, and are more resistant than coliform bacteria. Enterococci comprise a sub-group of fecal streptococci, being considered to include predominantly fecal streptococci of proven fecal origin. **Used** in evaluation of treatment processes and recreational waters.
Clostridia (presumptive *Clostridium perfringens*)	**Refers** to bacteria which produce typical black colonies on TSC (yolk-free tryptose-sulfate-cycloserine) agar when incubated anaerobically at 45°C for 20 hours. The group includes *C. perfringens*, but is likely to also include other species of *Clostridium*. *Clostridium perfringens* is almost conclusive

	proof of fecal pollution. These bacteria produce spores which are resistant to treatment processes and other unfavorable conditions. **Used** to indicate remote fecal pollution and to assess efficacy of treatment and disinfection processes.
Coliphages	**Refers** to viruses which have bacterial hosts (bacteriophages) and which produce a visible plaque in a double-layer-agar plaque assay using *E. coli* strain as host and incubation at 35°C for 16 hours. **Used** as indicators of the incidence and behavior of human enteric viruses in the evaluation of drinking water. Also serves as an indicator of the presence of host bacteria.

Table 1. Established indicator microorganisms, typical methods and uses in water quality management.

Enterococci, sometimes referred to as fecal streptococci, are bacteria more closely related to fecal pollution than total coliforms, because most enterococci do not replicate readily in water environments. These Gram-positive bacteria are more resistant to water treatment than fecal coliforms (Gram-negative), and are detectable by practical techniques, such as membrane filtration using m-enterococcus agar and incubation at 44.5º or 37ºC for 48 hours.

The enterococci include *Enterococcus faecalis*, *E. faecium*, *E. durans* and *E. hirae*. Enterococci are identified by the ability to hydrolyze 4-methyl-umbelliferyl-ß-D-glucoside (MUD) in the presence of thallium acetate, nalidixic acid and 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) resulting in release of a fluorogen which in liquid media is readily detectable under ultraviolet light.

The spores of sulfite-reducing clostridia (Gram-positive, anaerobic bacteria) are more resistant to water treatment and disinfection than most pathogens. Clostridia are sometimes considered as too resistant, and their inclusion in water quality guidelines as too stringent. One of the members of the group, *Clostridium perfringens*, is highly specific for fecal pollution. Clostridia generally occur in lower numbers in wastewater than coliform bacteria and their detection is relatively expensive and time-consuming.

The heterotrophic plate count (HPC) test is also known as the total or standard plate count. The test detects a wide variety of organisms, primarily bacteria, which give an indication of the general microbiological quality of water.

The test is simple and inexpensive, yields results in a relatively short time, and has proved one of the most reliable and sensitive indicators of treatment or disinfection failure. The generally used test method is pour plates using a rich growth medium such as yeast extract agar and incubation for 48 hours at 37ºC.

The methods of detection of pathogenic and indicator bacteria are mainly well-established, classical cell culture techniques designed to international standards which are then adopted at national level.

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Biography Sketches

Brett Pletschke is currently the Head of Biochemistry in the Department of Biochemistry, Microbiology and Biotechnology at Rhodes University. Dr. Pletschke received his PhD at the University of Port Elizabeth in South Africa in 1996 and was a Postdoctoral fellow/Chief Technical Officer at the University of Cape Town (UCT) from 1996-1999. Dr. Pletschke has been a member of WISA, IWA, AAAS and SASBMB. Currently, Dr Pletschke is the Vice-President of SASBMB Council (Council of the South African Society for Biochemistry and Molecular Biology). Dr. Pletschke's research interest is focused on the use of enzymes for the rapid identification of toxicants and indicator micro-organisms in the environment and bioremediation, and has published in journals such as "Enzyme and Microbial Technology", "Chemosphere" and "Water Research". In his spare time, Dr. Pletschke (tries to) play(s) golf and also enjoys surf and rock angling.

Jo Burgess works as a research manager at the WRC in Pretoria, South Africa. She manages programs of research in drinking water treatment technology and mine water treatment and management. Her background is in environmental biology; from a BSc (Hons) in the subject she progressed to an MSc in Water Pollution Control Technology and a PhD in Environmental Biotechnology, both at Cranfield University in England, and then went to Rhodes University in South Africa. Jo was Head of Biotechnology at Rhodes until 2008, when she left to join the WRC and now is engaged in funding, facilitating and coordinating projects to interdigitate South African water research.