# Guidance on the Use of Heterotrophic Plate Counts in Canadian Drinking Water Supplies

Canada

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Any questions or comments on this document may be directed to:

Water, Air and Climate Change Bureau
Healthy Environments and Consumer Safety Branch
Health Canada
269 Laurier Avenue West, Address Locator 4903D
Ottawa, Ontario
Canada K1A 0K9

Tel.: 613-948-2566
Fax: 613-952-2574
E-mail: water_eau@hc-sc.gc.ca

Other documents concerning Canadian drinking water quality can be found on the following website: www.healthcanada.gc.ca/waterquality

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## Background on Guidance Documents

The main role of the Federal-Provincial-Territorial Committee on Drinking Water is the development of the Guidelines for Canadian Drinking Water Quality. This role has evolved over the years, and new methodologies and approaches have led the Committee to develop a new type of document, Guidance documents, to provide advice and guidance on issues related to drinking water quality for parameters that do not require a formal Guideline for Canadian Drinking Water Quality.

There are two instances when the Federal-Provincial-Territorial Committee on Drinking Water may choose to develop guidance documents. The first would be to provide operational or management guidance related to specific drinking water related issues (such as boil water advisories), in which case the documents would provide only limited scientific information or health risk assessment.

The second instance would be to make risk assessment information available when a guideline is not deemed necessary. The Federal-Provincial-Territorial Committee on Drinking Water establishes the Guidelines for Canadian Drinking Water Quality specifically for contaminants that meet all of the following criteria:

1. exposure to the contaminant could lead to adverse health effects;
2. the contaminant is frequently detected or could be expected to be found in a large number of drinking water supplies throughout Canada; and
3. the contaminant is detected, or could be expected to be detected, at a level that is of possible health significance.

If a contaminant of interest does not meet all these criteria, the Federal-Provincial-Territorial Committee on Drinking Water may choose not to establish a numerical guideline or develop a Guideline Technical Document. In that case, a guidance document may be developed.

Guidance documents undergo a similar process as Guideline Technical Documents, including public consultations through the Health Canada web site. They are offered as information for drinking water authorities, and in some cases to help provide guidance in spill or other emergency situations.

# Part A. Guidance on the use of heterotrophic plate counts in Canadian drinking water supplies

Measuring the heterotrophic plate count (HPC) is an analytic method that is a useful operational tool for monitoring general bacteriological water quality throughout the treatment process and in the distribution system. HPC results are not an indicator of water safety and, as such, should not be used as an indicator of potential adverse human health effects. Each drinking water system will have a baseline range of HPC bacteria levels depending on the site-specific characteristics. Unexpected increases in the HPC baseline range could indicate a change in the treatment process, a disruption or contamination in the distribution system, or a change in the general bacteriological quality of the water. The HPC test can be used in conjunction with monitoring for *Escherichia coli*, total coliforms, turbidity, and disinfectant residuals as part of a multi-barrier approach to producing safe drinking water. For water systems that monitor HPC levels, use of the most sensitive HPC method is desirable to ensure that the results are relevant to operations. The usefulness of including HPC monitoring as part of a multi-barrier approach in a drinking water system should be determined by the responsible authority.

Increases in HPC bacteria concentrations can be sudden or can gradually increase over time. Although some variation in HPC levels is normal and can occur seasonally, these increases can indicate a change in raw water quality, problems with drinking water treatment, or problems in the distribution system or plumbing and should be investigated. In groundwaters, HPC levels are generally low and stable over time. In surface waters and groundwaters under the direct influence of surface water, HPC bacteria concentrations can be highly variable, but are minimized through effective treatment and disinfection. A disinfection residual and the production of biologically stable water (i.e., water with low levels of assimilable organic carbon, which is the as the fraction of biodegradable organic matter most readily used by bacteria for growth) can help control bacterial regrowth in the distribution system. The implementation of a maintenance program to clean and flush the distribution system pipes at regular intervals also helps to prevent excessive accumulation of sediment and debris, which can provide a habitat for bacterial regrowth.

Consistently low levels of HPC bacteria in the finished drinking water are an indicator that the treatment system is functioning properly. In the distribution system, HPC results outside of the normal range can provide some indication of stagnation, tuberculation, low or no residual disinfectant, and availability of nutrients for bacterial regrowth. For meaningful HPC results, it is important that samples be stored and transported at a temperature of 5(plus or minus 3)°C. Ideally, samples will be analyzed within 8 hours; if this is not possible, the maximum time between collection and analysis of a sample must not exceed 24 hours.

If an unusual, rapid, or unexpected increase in HPC bacteria concentrations does occur, the system should be inspected and the cause determined. Boil water advisories should not be issued based solely on HPC results. Corrective actions should be taken, if deemed necessary and may include:

- Verify the integrity of the treatment process and distribution system.
- Verify that the required disinfectant residual is present throughout the distribution system.
- Increase the chlorine dosage. If dealing with a well, shock chlorinate the well and plumbing system, and then flush the system.
- Clean treated water storage tanks and domestic cisterns, if applicable, and check for the presence of cross-connections and/or the occurrence of pressure losses.

- Clean and flush distribution system pipes to remove accumulated sediment and debris.
- Look for dead zones (stagnant water) in water mains and storage reservoirs.
- Conduct an investigation to identify the problem and prevent its recurrence; this may include assessing raw water quality (e.g., bacteriological profile, colour, turbidity, conductivity) and variability.
- Continue selected sampling and testing of all identified sites during the investigative phase to confirm the extent of the problem and verify the success of the corrective actions.

The responsible authority may recommend or require the use of accredited laboratories for HPC analysis. In some cases, non-accredited laboratories or field kits may be used in conjunction with quality control programs.

## Part B.  Supporting information

## B.1    Description, sources, and exposure

### B.1.1   Description and sources

Bacteria, moulds, and yeasts that require organic carbon for growth are known as heterotrophs. Most bacteria, including many of the bacteria associated with drinking water systems, are heterotrophs. Heterotrophic plate counts (HPCs) are derived from a number of standard methods that are recognized internationally for measuring heterotrophic bacteria in drinking water (Reasoner, 2004). HPC methods use colony formation on culture media to approximate the levels of heterotrophs in a drinking water sample, with bacteria being more common than moulds and yeasts. HPC methods do not provide an indication of the specific heterotrophic bacteria present or their sources.

The genera of bacteria recovered using HPC methods, and their concentrations, will vary depending on many factors including the cultivation conditions, type of medium, incubation temperature, incubation time, type of sample (e.g., surface water reservoir, treated and disinfected drinking water), season, and the time between collection and analysis of the water sample (Payment, 1999; Allen et al., 2004). There can also be variation in the concentration and types of bacteria recovered at the same sampling location over time. Some of the species of heterotrophic bacteria that have been recovered include those naturally found in the water environment and others from diverse pollutant sources. These include species within the genera *Pseudomonas*, *Aeromonas*, *Alcaligenes*, *Acinetobacter*, *Chryseobacterium* (*Flavobacterium*), *Klebsiella*, *Xanthomonas*, and many others (Allen et al., 2004; Edberg and Allen, 2004).

HPC bacteria are present in all water types. In groundwater, the biologically available organic carbon and thus heterotrophic metabolism, are often limited (Foulquier et al., 2011; Griebler and Lueders, 2009). Thus, HPC levels in groundwater are generally low and stable over time. In surface water and groundwater under the direct influence of surface water, HPC counts vary and can be minimized through effective treatment. Drinking water treatment does not remove or inactivate all heterotrophic organisms. Some heterotrophs are resistant to disinfection because they are (1) in a spore form, (2) in a vegetative form with an impervious membrane, or (3) have been protected from disinfection as part of an aggregate. As a result, these organisms pass through the treatment system into the distribution and/or plumbing systems. HPC bacteria can also enter the distribution system in open finished water reservoirs, during line repairs, in backflows from pipelining projects, or new pipe network additions (Geldreich, 1996).

High levels of HPC bacteria in a distribution or plumbing system are usually the result of bacterial regrowth. Bacterial regrowth refers to the increase in concentration of the viable bacteria present in the water after drinking water treatment, including the recovery and growth of organisms that were previously injured during the water treatment process (Robertson and Brooks, 2003). The concentration of heterotrophic bacteria in the distribution system can be influenced by the bacteriological quality of the finished water entering the system, as well as water temperature, residence time, levels of disinfectant residual, pipe materials, surface area-to-volume ratio, flow conditions, and the availability of nutrients for growth (Reasoner, 1990; Prévost et al., 1997; Payment, 1999; Carter et al., 2000; Clement et al., 2004). Bacterial regrowth can promote or cause corrosion of pipes, be responsible for foul-tasting or discoloured water, and promote slime growth. HPCs can be used as a marker for the underlying cause of some aesthetic problems in drinking water (WHO, 2002).

Heterotrophic bacteria growth will promote more rapid chloramine decomposition within the distribution system. Thus in systems that chloraminate or that have naturally occurring ammonia, an increase in HPC bacteria counts is one of the indicators of a nitrification episode (Noguera et al., 2008).

Bacterial biofilms that result from regrowth may also harbour opportunistic pathogens, such as *Legionella* species or non-tuberculous mycobacteria species, as well as increase the demand for disinfectant. An opportunistic pathogen is an organism that is considered non-pathogenic to immunologically competent individuals, but can cause disease in individuals who are immunologically compromised because of existing illness or are immunosuppressed because of medical treatment, old age, or very young age (Symons et al., 2000). A well-designed bacteriological sampling plan can help correctly identify microbiological contamination and/or regrowth in the distribution system and provide information on how to mitigate the problem (Narasimhan and Brereton, 2004). For example, monitoring of HPC and other indicators (e.g. nitrate, nitrite), at the entry point of the distribution system and throughout the system where chloramine is used (or where there is naturally-occurring ammonia), would help identify a nitrification episode. This information can then be used to select the most appropriate type of mitigation measures (Kirmeyer et al., 1995, 2004; Odell et al., 1996; Wilczak et al., 1996).

### B.1.2   Exposure

Heterotrophic bacteria, moulds, and yeast are naturally present in the environment; exposure can occur through many routes including drinking water, recreational activities, objects (e.g., doorknobs), foodstuffs, and through the air. The number of heterotrophs found in drinking water samples has been reported to range from less than 1 to more than 10 000 CFU/mL (Payment, 1999; Pepper et al., 2004; Stine et al., 2005). A study conducted in the United States showed that only 0.048–4.5% of the total bacteria ingested by an individual comes from household drinking water (Stine et al., 2005). Exposure to heterotrophic organisms is much higher through foodstuffs (Wadhwa et al., 2002; WHO, 2002; Stine et al., 2005).

## B.2   Health effects

The heterotrophic population in potable water includes a broad range of genera. Although the majority of these organisms do not present a health risk to immunocompetent individuals, some of the bacteria present may be opportunistic pathogens.

Heterotrophic bacteria belonging to the following genera have been associated with opportunistic infections: *Acinetobacter*, *Aeromonas*, *Chryseobacterium* (*Flavobacterium*), *Klebsiella*, *Legionella*, *Moraxella*, *Mycobacterium*, *Serratia*, *Pseudomonas*, and *Xanthomonas* (Rusin et al., 1997; Pavlov et al 2004). These organisms have been mainly associated with nosocomial (hospital acquired) infections, including wound infections, urinary tract infections, post-operative infections, respiratory infections, and infections in burn patients (Hunter and Ensign, 1947; Wilson et al., 1961; Herman and Himelsback, 1965; Lowbury et al., 1970; Rusin et al., 1997; Hoque et al., 2001; Glasmacher et al., 2003; Allen et al., 2004; Tan et al., 2004). *Pseudomonas aeruginosa* has been identified as one of the main etiological agent in skin and ear infections resulting from swimming and using whirlpools and hot tubs, and in eye infections from contaminated contact lens solution (Rusin et al., 1997; WHO, 2006). *Aeromonas* spp. have also been associated with community-acquired illness, which can cause diarrhea mainly in children and the elderly (Deodar et al., 1991; Chopra et al., 2009), but it has been noted that *Aeromonas* strains identified in clinical cases often have different genetic patterns from the strains found in

drinking water (Havalaar et al., 1992) Other opportunistic heterotrophs have received attention based on their ability to grow within drinking water distribution systems, including *Legionella* spp. and *Mycobacterium* spp. (van der Kooij, 2003). Aerosols from showers or hot tubs can be a route of infection from *Legionella* and *Mycobacterium* species, particularly in a hospital setting or in the case of individuals with compromised immune systems (Payment and Robertson, 2004). However, there is no evidence that HPC levels in treated water have a direct relationship with the presence of these organisms (WHO, 2002). Although these bacteria may be present in drinking water and have been associated with opportunistic infections, consumption of treated drinking water has not been reported as the route of exposure leading to infection. Health Canada (2006) provides further information on *Aeromonas*, *Legionella*, and non-tuberculous mycobacteria.

### B.2.1 Risk assessment

Although some heterotrophic bacteria in drinking water are opportunistic pathogens, numerous studies indicate that heterotrophic bacteria isolated from water using HPC methods possess very few virulence factors (Lye and Dufour, 1991; Payment et al., 1994; Edberg et al., 1996, 1997; Stelma et al., 2004). In addition, two large epidemiological studies have shown no association between the ingestion of drinking water containing heterotrophic bacteria and the incidence of gastroenteritis in participants (Calderon et al., 1988; Calderon and Mood, 1991). Additional epidemiological studies have shown no significant differences in the rate of gastroenteritis between individuals receiving municipal tap water with and without additional treatment (Hellard et al., 2001; Colford et al., 2002). While the occurrence of HPC bacteria is universal in soil, food, air and all types of water, including treated drinking water, the clinical and epidemiological evidence is insufficient to conclude that HPC bacteria in drinking water can pose increased health risks.

## B.3 Analytical methods

Standard HPC tests are simple culture-based methods designed to estimate the number of viable heterotrophic bacteria in drinking water (Lillis and Bissonette, 2001; Reasoner, 2004; APHA et al., 2005). Standard HPC tests do not provide information on the genus or species of organisms that are detected. The population of viable bacteria recovered will differ according to the method used, inter-specific competition, and the selected incubation temperature (Allen et al., 2004). Possible explanations for differences include the presence of some bacteria in a viable but non-culturable state and the fact that HPC media do not provide the complex nutritional requirements necessary for the growth of all heterotrophs. Only a small percentage, approximately 1% of the total bacteria found using direct microscopy, are enumerated with HPC procedures (Wagner et al., 1993).

Although no single growth medium, temperature, or incubation time will ensure the recovery of all organisms present in water, the 21st edition of *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 2005) does specify requirements that will permit a meaningful estimate of selected members of the culturable population. There are currently three methods—pour plates, spread plates, and membrane filtration—that are routinely used for HPC determinations (APHA et al., 2005). In addition to the above methods, enzyme-based methods have been developed and are commercially available (Jackson et al., 2000; Allen et al., 2004). Regardless of which method is used for HPC analysis, it is important that a quality control program be in place to demonstrate confidence in the results. An additional method that has been investigated for determining the bacterial concentration in a drinking water sample is

the adenosine triphosphate (ATP) assay (Deininger and Lee, 2001); however, this method has not been routinely used. It should be noted that to make meaningful comparisons between HPC results, the same method, growth medium, and incubation procedures should be used. Samples collected for HPC analysis should be analysed within 8 hours of collection where possible. However, when transit to the laboratory requires a longer storage time, the sample should be held at 5 (plus or minus 3°)C  to minimise bacterial regrowth; samples should not be frozen, and should be analyzed within 24 hours (Narasimhan and Brereton, 2004; APHA et al., 2005; ISO, 2006).

### B.3.1   Traditional methods

*B.3.1.1 Pour plate method*

The pour plate method involves adding a small volume of sample (0.1–2.0 mL) to melted agar (44–46°C) and then pouring the mixture into plates and allowing it to solidify. The plates are then inverted to prevent condensation on the covers and incubated for the required time. As detailed in *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 2005), two incubation procedures are generally used. Plates can be stored either at 35°C for 48 hours or at 20–28°C for 5–7 days. Using the pour plate method, colonies are generally small and compact and therefore easier to count, but may have few morphological characteristics that could assist in identification. The colonies are embedded or submerged in the agar medium, and are often slower growing and difficult to transfer. The bacteria embedded in the agar are subjected to a micro-aerobic environment that may not be optimal for growth. Finally, the bacterial sample is being added to tempered agar at between 43 and 46°C, which could result in secondary stress due to heat shock to the bacteria that would reduce viability. As such, the pour plate method is the least desirable of the three methods for enumerating bacteria in water (Reasoner, 2004; APHA et al., 2005).

*B.3.1.2 Spread plate method*

The spread plate method has the advantage of using solidified agar, thereby eliminating the possibility of heat shock. The sample is spread on the surface of the agar with a clean sterile spreading rod and the plates are incubated as described in the protocols used for the pour plate method. The resultant colonies are on the surface and can be easily transferred, and their morphology can be distinguished. Since the sample has to be absorbed into the agar surface, only a small sample volume (0.1–0.5 mL) can be used. The spread plate method generally yields higher bacterial counts than the pour plate method, although it is limited to a smaller sample volume (Allen et al., 2004; Reasoner, 2004; APHA et al., 2005).

*B.3.1.3 Membrane filtration method*

This method of HPC determination is more flexible than the pour plate method or the spread plate method because it permits the analysis of sample volumes from less than 1.0 mL to as much as 10 L depending on the quality of the water being tested. By means of the membrane filtration method a water sample is passed through a 0.45-µm filter, and the heterotrophic organisms are retained on the filter surface. The filter is then placed onto the culture media and incubated as described in the protocols used for the pour plate method. This method also eliminates the possibility of heat shock. However, because the colonies form only within the confines of the filter used, it limits the size of the viewing surface. This may require analyzing several different sample volumes if the expected HPC level of the water is not known, as colonies

can be too numerous to count in some samples. The use of contrast stains is helpful for the detection of colourless colonies . Other considerations when using this method include possible damage to cells by excessive filtration pressures, and variations in membrane filter quality. However,because high volumes of low-turbidity water can be filtered, the membrane filtration method is ideal for waters where bacteria levels are expected to be low (HPCs less than 10 CFU/mL) (Allen et al., 2004; Reasoner, 2004; APHA et al., 2005).

### B.3.2   Other methods

In addition to the traditional methods for monitoring HPC bacteria, there are other methods that have been used to determine HPC concentrations in drinking water. These methods have been used in research settings, but are not currently used for routine monitoring.

#### B.3.2.1 *Enzyme-based methods*

Enzyme-based methods use HPC media that contain substrates that are hydrolyzed by microbial enzymes to release products that can be detected visually, for example, by releasing a product that fluoresces. This technique has been used to produce a commercially available HPC detection method. The performance of the commercially available enzyme-based method was compared with the standard HPC pour plate method and a strong positive correlation was found between both methods, suggesting that it may be an alternative test method for HPC in drinking water. Advantages of the commercially available enzyme-based method are that it is easy to use and does not require preparation of media or sterilization. Also, the counting of positive fluorescent wells is easy and takes less time than does counting colonies on the standard HPC plate (Jackson et al., 2000).

#### B.3.2.2 *ATP assay method*

The ATP bioluminescence assay is described in *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 2005). In this test the somatic-cell ATP is separated from the bacterial-cell ATP. A somatic-cell releasing agent is used to lyse all non-bacterial cells and release their ATP. The remaining bacterial ATP is mixed with luciferin-luciferase to develop light; the light emission is measured in a luminometer. The activity of the luciferin-luciferase is checked with an ATP standard. The relative light units (RLU) emitted are proportional to the amount of ATP present, and the amount of ATP is proportional to the number of viable bacteria.

With this method, the bacterial water quality can be determined in a much shorter time frame, e.g., in less than 5 minutes in comparison with a minimum of 48 hours with the various HPC methods (Deininger and Lee, 2001). There is also a small portable device available that allows testing to be carried out on site with only a small amount of sample.

## B.4   Treatment technology

The purpose of treating drinking water is to provide a product that is microbiologically and chemically safe for consumption. Unlike *E. coli* and total coliforms, it is not feasible or necessary to reduce the level of heterotrophic organisms to zero in drinking water. However, water utilities should aim to minimize HPC levels by implementing appropriate and effective treatment.

Heterotrophic bacterial counts in source water vary widely, from less than 1 to 10 CFU/mL in groundwater to greater than $1 \times 10^7$ CFU/mL in highly polluted surface water. Overall, physical removal methods (including coagulation, flocculation, sedimentation, slow or

rapid sand filtration, and direct filtration with or without filtration aid) can remove 1–3 logs of heterotrophic bacteria. Disinfection can achieve an additional 2–4 logs removal, to give a total removal of 5–7 logs. With this level of removal, it is possible to achieve HPC bacteria concentrations of 10 CFU/mL or less in finished water (Fox and Reasoner, 2006).

HPC levels can be minimized in a distribution system by supplying water containing low levels of assimilable organic carbon and a disinfectant residual, and exercising best practices to ensure proper distribution system maintenance. Assimilable organic carbon can be removed by various treatment processes such as conventional treatment (coagulation, flocculation, sedimentation, and filtration), reverse osmosis, and biological filtration (van der Kooj, 2003).

A disinfectant residual will limit the growth of organisms within the distribution system and may afford some protection against contamination from intrusion. The disappearance of the residual may provide an immediate indication of the entry of oxidizable matter into the system or a malfunction of the treatment process. The type of disinfectant residual can also be a contributing factor in limiting bacterial growth. Chloramines may be more effective than free chlorine for controlling biofilm regrowth in distribution pipes (LeChevallier, 2003). In addition, high HPC in chloraminated systems can be an indication of an inappropriate chlorine to ammonia ratio, resulting in nitrification within the distribution system (Noguera, et al., 2008). Nitrification in the distribution system can have adverse impacts on water quality such as increased nitrite and nitrate levels, reduced chloramine residuals and increased bacterial regrowth (Kirmeyer et al., 1995, 2004; Odell et al., 1996; Wilczak et al., 1996; Bremer et al., 2001; U.S. EPA, 2002; Lytle et al., 2007; Muylwyk, 2009; Zhang et al., 2009).

Corrosion of iron pipes can influence the effectiveness of chlorinated disinfectant residual for the inactivation of the biofilm bacteria. In full-scale studies, systems that used a phosphate-based corrosion inhibitor had lower coliform levels than systems that did not practice corrosion control (LeChevallier, 2003). But, the addition of phosphate in waters where phosphate is limited may significantly increase the growth of bacteria, especially when the biodegradable organic carbon level is elevated (Park et al., 2008).

It is possible to maintain low levels of HPC bacteria in a distribution system in the absence of a disinfectant residual, if the water being distributed and the materials being used are biologically stable, and the distribution system is optimized to prevent stagnation, sediment accumulation, and pressure fluctuations (Smeets et al., 2009). Cleaning and flushing of accumulated sediments and debris in distribution pipes can help reduce the habitats where bacteria grow. However, these procedures must be performed routinely, as they do not address the underlying reasons for the bacterial growth (LeChevallier, 2003). High HPC levels can also occur during a contamination event. As such, repair and maintenance operations need to follow rigorous procedures to prevent contamination of the water.

At the residential scale, HPC levels can also increase in water that has been treated by point-of-use or point-of-entry devices such as a carbon filter or water softener, and in water dispensing devices. A properly maintained and operated treatment device should not have water quality problems associated with regrowth bacteria (Robertson and Brooks, 2003).

## B.5    International considerations

An international meeting of experts in Geneva, Switzerland, concluded that heterotrophic bacteria in drinking water are not a health concern to the general public (WHO, 2002; Bartram et al., 2004). However, there may be a concern for individuals with weakened immune systems (Rusin et al., 1997; Pavlov et al., 2004). The conclusions drawn from this meeting of experts

support the drinking water guidelines in various countries. For example, the Australian Drinking Water Guidelines do not set a numerical limit for HPC; rather, HPC is used as an indicator of operational performance in assessing the efficacy of drinking water treatment. It may also be used to assess the cleanliness and integrity of the distribution system, whereby a significant increase in HPC levels could be an early sign of contamination (NHMRC, 2004).

The National Primary Drinking Water Regulations established by the U.S. EPA indicate that HPC is not necessarily indicative of health effects, but that a lower concentration of heterotrophic bacteria in the drinking water is linked to a better maintenance of the treatment and distribution systems. These regulations state that for surface waters and groundwaters under the influence of surface waters, treatment techniques should be used to control the HPC concentration in drinking water to fewer than 500 CFU/mL, measured using Standard Methods Agar incubated at 35°C for 48 hours (U.S. EPA, 2009). This is not a health-based standard, but reflects the concern that at concentrations above 500 CFU/mL, heterotrophic bacteria can interfere with some total coliform and *E. coli* recovery methods (Geldreich et al., 1972, Allen et al., 2004).

The Drinking Water Inspectorate of England and Wales, based on the European Union Council Directive on the quality of water for human consumption (Council of the European Union, 1998), has not set a numerical limit for HPC levels in drinking water, but has specified that HPC levels should show no abnormal changes at the consumer's tap or within treatment works or service reservoirs (DWI, 2000).

# Part C.  References and acronyms

## C.1    References

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## C.2   List of acronyms

| | |
|---|---|
| APHA | American Public Health Association |
| ATP | adenosine triphosphate |
| AWWA | American Water Works Association |
| CFU | colony-forming unit |
| DWI | Drinking Water Inspectorate (United Kingdom) |
| EPA | Environmental Protection Agency (United States) |
| HPC | heterotrophic plate count |
| NHMRC | National Health and Medical Research Council (Australia) |
| RLU | relative light units |
| WHO | World Health Organization (UN) |
| WEF | Water Environment Federation |