

THE HETEROTROPHIC PLATE COUNT TEST

By Dan Kroll

What is a heterotroph?

Heterotrophs are a group of microorganisms including yeasts, molds, and bacteria that use organic carbon as their sole carbon and energy source. This classification is in contrast to autotrophic organisms such as algae that can utilise inorganic carbon and sunlight for their needs. The overwhelming number of known species of bacteria, both aerobic and anaerobic, are heterotrophs. Many heterotrophic organisms consume carbon compounds such as sugars, alcohol, and organic acids as their food source. However, there are some specialised organisms capable of decomposing cellulose, lignin, chitin, keratin, complex hydrocarbons, phenol and other substances. Heterotrophic organisms are widely found in soil, water, food, and the bed soils of bodies of water.

Why measure heterotrophs?

Microorganisms will normally grow in water and as biofilms on surfaces that are in contact with water. Growth of these organisms after water treatment is typically referred to as regrowth. The occurrence of regrowth is typically characterised by increased Heterotrophic Plate Count (HPC) values in water samples. Elevated HPC values can commonly be found in stagnant parts of distribution systems, in domestic plumbing, in bottled water, and associated with plumbed in devices such as softeners, carbon filters and beverage dispensing vending machines.

HPC testing has been used for a long time to verify the quality of water supplies. HPC tests were applied early on as a check on water treatment processes, particularly sand filtration, and as such were an indicator of water safety. As monitoring techniques for more specific indicators such as *E. coli* and total coliforms became more readily available, the use of HPC for these purposes declined. HPC still plays a role in many regulations and guidelines and also as a valuable process control tool.



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HPC measurements are used:

- to indicate the effectiveness of water treatment processes as an indirect indicator of pathogen removal;
- as a check on the occurrence and numbers of regrowth organisms that may or may not have sanitary significance;
- as a check on the potential to interfere with lactose based coliform measurements;
- as health based targets based upon public health protection and disease prevention as part of water safety plans;
- to assess the ability of the entire water supply chain to deliver water of a quality that meets defined targets;
- to monitor the steps in the supply chain that are of particular importance in securing safe drinking water;
- to ensure that premise plumbing including storage tanks are cleaned and maintained in such a manner so as to reduce public health risk;
- to measure the performance of filtration and disinfection processes;
- to keep a check on distribution systems where HPC measurements are assumed to respond primarily to distribution system conditions that may arise from stagnation, loss of residual disinfectant, high levels of assimilable organic carbon, higher water temperatures and the availability of nutrients;
- to monitor chloraminated systems and in systems that contain ammonia in the source water as a possible indicator of the onset of nitrification;
- to monitor as a potential indicator of the onset of taste and odor problems and other aesthetic problems.

The Heterotrophic Plate Count test (HPC):

As the definition of heterotrophs above implies, heterotrophs are a broadly defined and diverse group of organisms that require organic carbon for growth. Over the years, a variety of simple culture-based tests intended to recover a wide range of waterborne microorganisms have been introduced. These types of test are referred to as "heterotrophic plate count" or "HPC" test procedures.

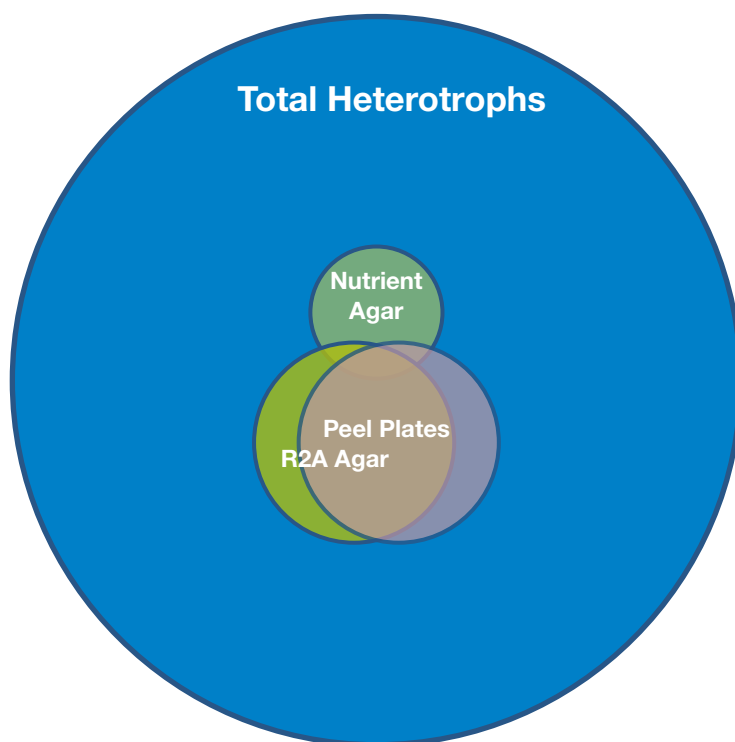
There is no universal test for HPC measurement. Generally, due to the wide variety of test methods available and the diversity of organisms that may be present, a method that works for the location being sampled should be selected, and then more emphasis put on trending than actual counts. Different methods may perform better (i.e. recover more organisms) in a given sample. This does not mean that one method is correct and the other incorrect, each method simply selects for different subpopulations that may or may not be present in the given sample. Heterotrophic plate count analyses are a prime example of a method-defined parameter.

Although portions of HPC methods are standardised, such as the utilization of nutrient agar or R2A agar, HPC test methods involve a wide variety of user defined test conditions that lead to a wide range of quantitative and qualitative results. Using newer methods (Reasoner and Geldreich 1985 a.k.a. R2A) it is possible to significantly increase the proportion of bacteria that can be cultured from drinking water samples compared to older methods such as nutrient agar. The use of media with low nutrient levels (e.g. R2A), which are better suited to the needs of the microorganisms found in drinking water when compared to classic nutrient agar, allows an increase in the proportion of waterborne microorganisms that can be determined by the cultivation method. A disadvantage of the method is the longer cultivation time (5-7 days at 280°C).

No single set of nutrient growth factors, temperature, light, and time is conducive to facilitating the growth of all of the wide variety of bacterial species and strains that may be found in a given water sample. This has resulted in a number of different methods for measurement, each with different culture media, incubation times, and temperatures. Some typical incubation temperatures employed range from around 20°C to 40°C, incubation times from a few hours to seven days or even a few weeks, and nutrient conditions can vary widely from the rich conditions found in nutrient agar to the nutrient poor conditions prevalent in R2A media. Culture methods may also differ and can include spread plate techniques, pour plate techniques, and membrane filtration methods.

The HPC test itself does not specify the organisms that are detected. In reality, only a small proportion of the metabolically active microorganisms present in a water sample may grow and be detected under any given set of conditions, and the population recovered will differ significantly according to the method used. Approximately 0.01% of waterborne microorganisms are thought to belong to the group of culturable heterotrophic bacteria, and approximately 1% of the viable bacteria are not culturable. Correlations of the results from different samples using the same method and the same sample using different methods or even slight modifications of the same method can be difficult as they can yield dramatically different

results. The actual organisms recovered in HPC testing can also vary widely between locations, between seasons and even between consecutive samples at a single location. Microorganisms recovered through HPC tests usually include those that are part of the natural (typically non-hazardous) microbiota of water: in some instances, they may also include organisms derived from pollutant sources and/or pathogens.



Different Methods Detect Different Subpopulations of Total Heterotrophs.

Conclusion

Heterotrophic plate count analysis is a useful tool for evaluating drinking water samples. Proper application of HPC monitoring provides insight into the effectiveness of water treatment processes, verification of ongoing supply chain quality, and indication of potential system failures. Elevated counts of heterotrophic bacteria can represent a process upset or even a public health hazard. Selection and application of a suitable HPC test can provide valuable understanding of the condition of drinking water throughout the production and distribution system.

Source: Heterotrophic Plate Counts and Drinking-water Safety. The Significance of HPCs for Water Quality and Human Health. Edited by J. Bartam, J. Cotruvo, M. Exner, C Fricker and A. Glasmacher. Published on behalf of the World Health Organization by IWA Publishing, Alliance House, 12 Caxton Street, London SW1H 0QS, UK. 2003