Heterotrophic Plate Count (High School) Revised May 14th, 2010

Purpose: To determine if the water sample meets Canadian Drinking Water Guideline for bacterial growth by assessing the number of bacterial colonies that grow using the Heterotrophic Plate Count Method.
Testing will be done on 4 different water sources:

- Urban treated water
- Rural (Aboriginal and/or non-Aboriginal community) treated water
- Untreated raw source water
- Local community treated water
- Control

Currently, the Canadian guidelines recommend that HPC levels should not exceed 500 CFU/ml. Although there are no laws enforcing this recommendation, if this level is exceeded in municipal drinking water, an inspection is often conducted to determine the cause of the increase. Often, abnormal changes in HPC can be an indication of other problems in the water system.

Materials:

- 5 - Sterile pipettes
- 5 - Plastic spreaders
- 5 - Agar Plates
- 4 - Heterotrophic Sampling Tubes

(Contains 10% Sodium Thiosulphate to remove chlorine from the Water Sample)

Method:

1. Label the 5 Agar Plates: *Control*, and the names of the four water samples to be tested. (ie. Urban Treated, Rural Treated, Untreated Raw, Local Treated)
2. Label the 5 sterile pipettes: Control (CW), and the appropriate water sample names (ie. Urban Treated, Rural Treated, Untreated Raw, Local Treated)
3. Label the 4 Heterotrophic Sampling Tubes as follows: Urban Treated, Rural Treated, Untreated Raw, Local Treated. Fill the tubes to the 5 mL mark with the corresponding water sample using the corresponding pipette (shake to mix). The Heterotrophic Sampling tube contains 10% Sodium Thiosulphate which removes chlorine from the water sample – the chlorine is what helps keep bacteria from

forming in our water. By removing the chlorine from the water sample, colonies should form on the plate.
4. Using the pipette labeled Urban Treated Water, drip 2 drops of the Urban Treated water directly out of the labeled water bottle onto the agar in the *Control* Agar Plate.
5. With a spreader, use a back and forth motion to spread the Urban Treated water sample over the plate. Turn the Agar Plate a quarter turn and continue the back and forth motion to spread the sample. Continue turning and spreading until all the liquid has been absorbed into the plate. Be sure to spread the sample on the surface of the agar, and not to "dig into" the agar and make the agar all chopped up.
6. Repeat steps 4 & 5 using the water samples from the remaining Heterotrophic Sampling Tubes to put on the corresponding agar plates. Remember to use like labeled pipettes with like labeled water samples.
7. Replace the lid, and let them sit for 10 minutes.
8. Flip the plates over, Agar side up, and store plates at room temperature for 7 days.
9. After 7 days the colonies of bacteria can be counted on the Sample Agar Plate. Each colony looks like a small round dot on top of the agar and will vary in size, shape and color. The *Control* Agar Plate should not have any colonies.
10. Record the number of colonies counted.

Results

If the total number of bacteria colonies counted on the Water Sample Agar Plate is under 500, then it meets the Canadian Drinking Water Guidelines.
There should be no bacteria colony growth of the Control Agar Plate. If there are, this means that the plate was contaminated by possibly the air or something else.

Safe Handling of Materials

Caution must be taken at all times when handling any chemicals. Although this test is safe to use in any area, please be cautious with the materials supplied.

Visit the Safe Drinking Water Foundation Website www.safewater.org to learn more about issues affecting safe drinking water.

Heterotrophic Plate Count:

What is a heterotroph?

A heterotroph is any organism that cannot make its own food and is, therefore dependent on other substances for nutrition. All animals and people, for example, are heterotrophs because we need to eat other plants and animals in order to survive. Bacteria, yeast, and moulds are also heterotrophs, as they are unable to make all the nutrients they need to live and grow. Plants, on the other hand, use the sun's energy to make their own food; therefore, they are called autotrophs.

What is the Heterotrophic Plate Count?

The Heterotrophic Plate Count (HPC) is a procedure used to estimate the number of live heterotrophic bacteria that are present in a water sample. A sample of water is put on a plate that contains nutrients that the bacteria need to survive and grow. The nutrient media that is most often used for this test is called R2A Agar, which is a gelatine-like substance that is best suited to the needs of water bacteria. After 5-7 days, the number of small spots on the plate, called colonies, is counted, and a measure of how many bacteria are present in each millilitre of water can be determined. The HPC results are generally reported as CFU/ml or Colony Forming Units per millilitre. Each colony-forming unit represents an initial single, live bacterium that was capable of multiplying until it could be observed on the plate. It is important to understand that the colony count, alone, does not allow one to draw conclusions about the risks to public health. However, it currently serves as a relatively easy way to measure filtration and disinfection efficiency, as well as the estimated numbers of bacteria in areas that have the potential for increased contamination.

What do the different colours of the bacteria mean?

Mycobacterium avim is tan, Aeromonas hydrophila is yellow or green with a darker point at the centre, E. coli is white, Salmonella is grey, Campylobacter is blue and Pseudomonas aeruginosa can be blue, green, yellow, red and brown in a combination described as pearlescent.

What is the current Canadian limit for HPC?

Currently, the Canadian guidelines recommend that HPC levels should not exceed 500 CFU/ml. Although there are no laws enforcing this recommendation, if this level is exceeded in municipal drinking water, an inspection is often conducted to determine the cause of the increase. Often, abnormal changes in HPC can be an indication of other problems in the water system.

What are the health risks associated with high heterotrophic plate counts?

Different bacteria pose different risks to public health. Micro organisms recovered through HPC tests generally include those that are part of the natural (typically non-hazardous) microbial flora found in water. Some predominant bacterial species detected in drinking water, as well as the doses that would need to be ingested to cause an infection are outlined in the table below.

Bacterial Species	Infectious Dose (Ingested)
Pseudomonas aeruginosa	10^8-10^9 CFU
Aeromonas hydrophila	>10^{10} CFU
Mycobacterium avium	10^4-10^7 CFU
Xanthomonas maltophila	10^6-10^9 CFU

Common bacterial species detected in drinking water
Source: http://www.who.int/water_sanitation_health/dwq/en/HPC2.pdf

A higher risk exists for those people with a depleted immune system, such as the elderly and infants, or those with HIV. Those people on antibiotics also seem to be at an increased risk for infection following ingestion. Some of the HPC bacteria found in drinking water can cause other problems such as skin and wound infections in both, the community, but more often in hospitals. For example, certain bacteria are able to grow in large numbers on catheters used in hospitals and are capable of causing an infection at the point where the catheter is inserted.

It is important to state that not all bacterial species fall under the HPC category and several bacteria that are not routinely found in drinking water can present serious public health risks. See the waterborne pathogens fact sheet for a complete list of those bacteria that can cause illness in humans.

What do I do if my water exceeds HPC limits?

Growth of bacteria following drinking water treatment is normally referred to as re-growth. This type of growth is typically reflected in higher HPC values measured in water samples. Re-growth generally occurs in areas of distribution or plumbing systems where the water may remain stationary for a longer amount of time, in bottled water, water softeners or carbon filters. In order to ensure that re-growth of bacteria is kept to a minimum, general water safety practices such as maintenance protocols, regular cleaning, temperature management and maintenance of a disinfectant residual (e.g. Chlorine) should be in place. If the HPC points exceed recommendations, one should consider looking at whether the system has been adequately cleaned, whether the disinfectant residual is effective, and the efficiency of temperature management. A failing in any of these areas could lead to elevated HPC levels.