Colony Forming Units (CFU) vs. Most Probable Number (MPN) Test Results for Microbial Water Quality Testing

Introduction

When choosing microbial water quality testing methods, there are multiple factors to consider, such as what type of water sources you are testing, what you are trying to accomplish by water testing, and what resources are available for testing.

Another important topic to decide is what type of testing methodology to use in terms of test results, namely Presence/Absence or Quantified testing methods. For a summary of these testing methodologies, see the Aquagenx technical brief “Microbiological Water Quality Testing Methods for *E. coli* and Total Coliform Bacteria.”

This technical brief will explain the differences in terms of test results between the two quantified testing methodologies, CFU vs. MPN.

Equivalencies Between CFU and MPN

CFU tests are based on counting target bacteria colonies on or in a solidified culture medium. An example of this method is the Aquagenx GEL EC CFU Kit, which is a field test that does not require membrane filtration equipment or the use of agar media.

MPN tests are statistical methods that estimate bacteria concentrations in liquid cultures, such as the Aquagenx CBT EC+TC MPN Kit or tests done in multiple cultures tubes held in racks or multiple wells in a tray. The Aquagenx test is self-contained and does not require laboratories, electricity or extra accessories or equipment. Multiple tube and well tests require additional materials such as culture tubes, pipets and tube racks for multiple tube tests or a plate sealer or a sample volume dispenser such as pipets for the multiple well test in a tray.

CFU and MPN test results are not exactly equivalent, but are very similar when bacteria concentrations are low, as would be expected in drinking water. However, this statement requires important clarifications for drinking water quality testing as will be further explained below.

In terms of analyzing drinking water, there is a slight positive bias in MPN values, that is, MPN values are a little higher than CFU values based on parallel analysis of the same samples by both methods. The positive bias of MPN is greater at higher concentrations of bacteria in water samples, such as surface and wastewater samples, in which concentrations are often in excess of 100 organisms per 100 mL.

The CBT EC+TC MPN Kit measures lower concentrations of bacteria found in most groundwater sources such as boreholes and wells, having concentrations ranging from 0 (none detected) to 100 per 100mL of water. The uncensored, upper detection limit is 48.3 MPN/100 mL. The censored upper detection limit when all five compartments in the Compartment Bag are positive (blue) is ≥100 MPN/100 mL, which is about twice the value of the maximum uncensored concentration.

Because the CBT EC+TC MPN Kit for drinking water only measures lower concentrations of bacteria for drinking water testing, the positive bias effect is relatively small because the MPN values are small. Equally important, test result differences between the CBT MPN Kit and CFU tests do not change the order of magnitude classification or category of bacteria concentrations, which define the different World Health Organization (WHO) health risk levels for drinking water quality.

Aquagenx has found there are some misperceptions about MPN vs. CFU test results. Now we will explain how we know this is true.

Comparisons of Methodologies

Competent statisticians and other independent parties have conducted side by side comparisons of the CBT MPN Kit to membrane filtration (CFU) methods. The bacteria concentration results were statistically indistinguishable and did not change the order of magnitude of WHO health risk levels for drinking water.

For example, a paper published in *Science of The Total Environment* on deep tubewell microbial water quality and access in arsenic mitigation programs in rural Bangladesh, describes a project that used both the CBT MPN Kit for *E. coli* and a standard membrane filter test. The bacteria concentration results of the two tests are statistically the same. This is a set of field drinking water quality data showing the CBT is a quantitative *E. coli* test that give results equivalent to other *E. coli* tests like membrane filtration followed by colony counting on solid culture media.

Another example is a paper published in the *American Journal of Tropical Medicine and Hygiene, entitled* Evaluating Human Sensory Perceptions and the Compartment Bag Test Assays as Proxies for the Presence and Concentration of Escherichia coli in Drinking Water in Western Kenya. This study compared the CBT MPN Kit to membrane filtration and plating on media resulting in colonies (US EPA Method 1604) and IDEXX Colilert, a multiwell MPN test in trays.

A third example was published by *Journal of Microbiological Methods,* Evaluation of the Compartment Bag Test for the Detection of Escherichia coli in Water.

In all three of these studies, differences between the Aquagenx CBT and CFU methods found them essentially indistinguishable in their predictability of concentration and the level of variability in results. The bottom line is the differences between our MPN values and the anticipated, somewhat lower CFU values would not change the classification of the health risk level of the water. The net change is negligible.

The use of MPN tests like the CBT EC+TC Kit for quantitative analysis of water quality have been used for more than 100 years, are widely accepted globally and can be performed in the lab and in the field.

Which Method Do You Choose?

There is no one-size-fits-all answer to this question. Practitioners should decide on the method that is best suited to their application, setting and resources. The choice of test method, along with their water quality testing protocol and sampling requirements, defines the water quality testing program, with special consideration of national water quality standards.

Both testing methods, MPN and CFU, are reliable and give accurate assessments of potential microbial health risks of drinking water sources. An assortment of reliable testing methods, especially tools that are easy to use in the field, is available to the water, sanitation and hygiene sector. This array of microbial water quality testing products allows practitioners to make informed choices for decisions on monitoring water safety that are best suited to their needs.