Microbial Testing of Water: New Rapid Methods

Counting bacterial growth in an agar plate.

Introduction

Microbial testing of water is important in a variety of industries, particularly the food and pharmaceutical industries. Knowing the number of colony forming units (CFU) in water is crucial in determining if it is acceptable for use in a particular application as well as if the bacteria and chemicals in the water must be neutralized before disposal.

There are three types of water on which this type of testing is conducted. Process water is used in manufacturing, the paper industry, and the food and beverage industry for purposes such as rinsing pipes and working surfaces, rinsing products during manufacturing, and as a component of the products they produce. This type of water has a highly variable CFU and often contains a high concentration of chemicals that need to be neutralized before disposal.

Drinking water is typically low in chemicals and has a low CFU, but in some cases it may be necessary to neutralize any chemicals that are present prior to testing. Filtration is required if the client specification is listed at 100mL or 10mL, or if the limit is low enough that plating 1mL of the sample would be insufficient.

Sterile water is commonly used in the pharmaceutical industry, and should be void of CFU and chemicals. For this reason, it almost always requires filtration in order to obtain meaningful test results.

Test Methods

Standard Method 9215 provides detailed heterotrophic plate count methods for pour plate, spread plate, and membrane filtration and the specific test parameters involved in testing drinking or waste water. It is a good measure of water treatment plant efficiency, aftergrowth in transmission lines, and the general bacterial composition of the source water.

The pour plate method involves pouring ~1mL of the sample into a plate and pouring an agar solution over the top. The sample is allowed to incubate after the agar has set. This is an effective method for quantifying organisms, and also allows more space for the colonies to grow.

The spread plate method involves spreading ~0.1mL of the sample over a premade agar plate and allowing it to incubate. This method gives more 3-dimensional colonies than the pour plate method. The pour plate or spread plate methods are typically used for process water and drinking water.

Membrane filtration involves filtering a particular volume of a sample to get a higher concentration of bacteria, then rinsing the filter with a sterile buffer before placing it on an agar plate. This is generally the only method used to test sterile water. It can also be used to test drinking water depending on the application. This method provides good colony morphology, making it a good method for identifying the bacteria present in a sample. However, spreader colonies can cause a too numerous to count (TNTC) result due to the small test area. A macroscope is usually required to get an accurate CFU count.

Drawbacks to standard methods

Standard Method 9215 provides stringent rules for when to count the bacterial growth after incubation, which can be an issue in a busy lab with many different tests running at once since it may be difficult to count the CFU right away. Once the incubation period is over, the samples can be refrigerated for up to 24 hours before being counted, but this should not be done routinely.

In all of these standard test methods there is a significant amount of time and labor required, as well as a high chance of manual error. The costs associated with these methods can be high, not only the labor costs but also the cost of the necessary materials to conduct the testing.

It can take between 48-72 hours to get results, and if the sample is improperly diluted so the results are TNTC or not enough to count, the sample must be retested. Once testing is completed, the process of counting the number of bacterial colonies is complicated and time-consuming, and it is easy to make mistakes during this process. It takes additional time to calculate the CFU and to review the calculations to ensure there are no mistakes. This process is highly operator-dependent, and as a result the CFU can vary widely when calculated by different operators and different labs.

GreenLight® Rapid Microbial Screening

An alternative to these testing methods is the GreenLight system, a rapid microbial testing instrument that uses a fluorescence-based sensor to determine the bacterial growth inside the specialized APCheck® vial.

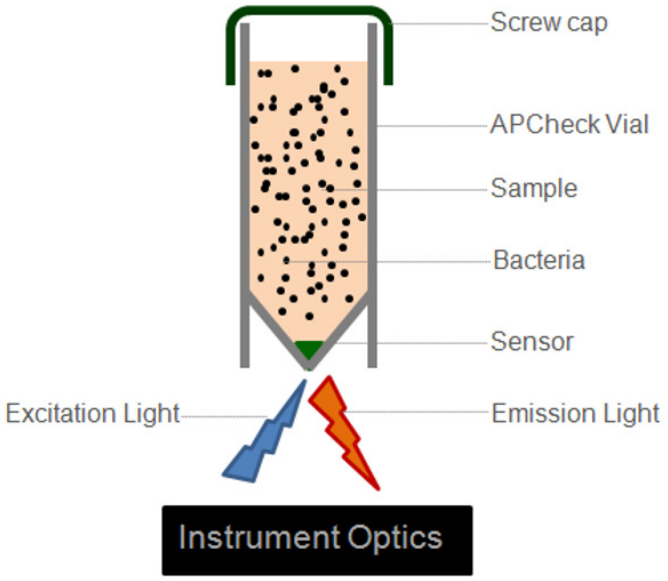


Figure 1. The APCheck vial.

As bacteria grow inside the vial, they consume oxygen. When the sensor at the bottom of the APCheck vial is excited by light from the dedicated reader inside the instrument, it returns information about the oxygen content that can be directly related to the microbial load inside the vial. The time to result depends on the incubation temperature and the growth medium used. The greater the initial microbial load, the faster the time to result.

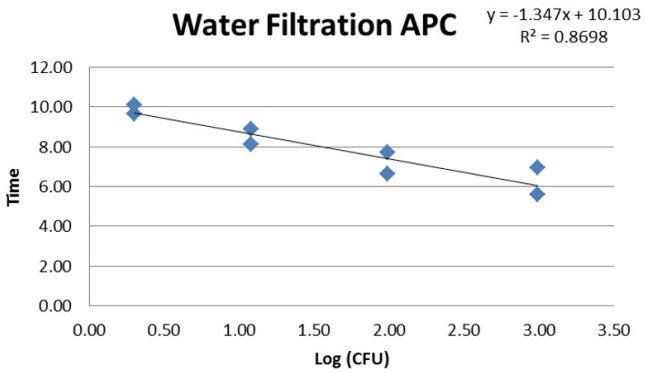
CFU/g	$\geq 10^8$	$\geq 10^7$	$\geq 10^6$	$\geq 10^5$	$\geq 10^4$	$\geq 10^3$	$\geq 10^2$	≥ 10
Time (h)	≥ 1	≥ 3.0	≥ 5.0	≥ 7.1	≥ 9.2	≥ 11.3	≥ 14	≥ 16

Figure 2. The time to result based on CFU using the GreenLight system.

No dilution of the test samples is necessary due to the wide measurement range of the instrument; the sample and growth medium can be added directly to the AP-Check vial, as can a filter if filtration is necessary. The size of the vials (either 2mL or 15mL) allows for the testing of a greater sample volume than the standard agar plate techniques. The time to result and CFU are calculated automatically, eliminating the need for manual counting and calculation as well as the operator-dependence that is present in the standard methods.

Test Data

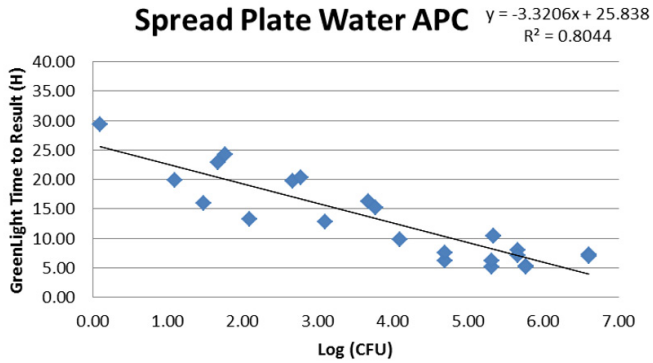
The following tests were conducted to determine the correlation of the CFU obtained using standard test methods and the time to test using the GreenLight system. Test samples were created in duplicate, with one filter being placed inside an APCheck vial for testing and the other placed onto an agar plate.



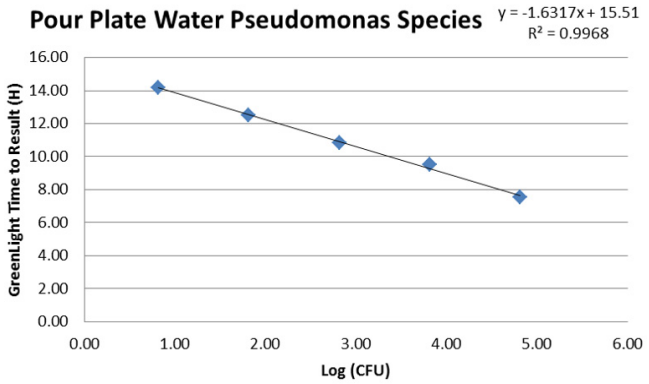
In the first test, in-house testing was conducted for water filtration aerobic plate count (APC) using drinking water that contained *Pseudomonas aeruginosa* and *E. coli*.

Based on the y-intercept of the slope of the line associated with this test data, the time needed to conduct this type

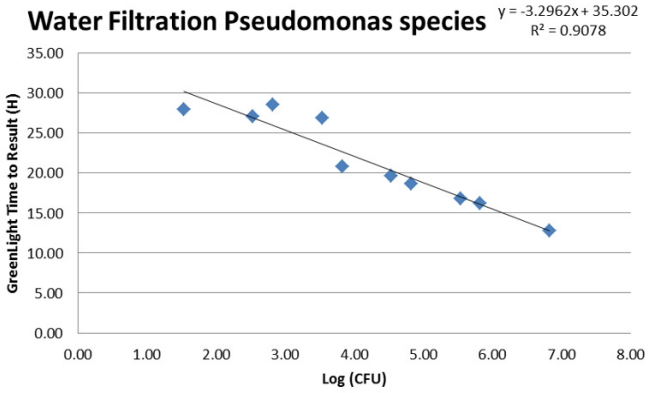
of testing using the GreenLight system is just over 10 hours, and the correlation with the plate count (R^2) is high.



The second test was conducted on process water from an industrial site. The spread plate method was used with dilutions depending on the expected CFU. The estimated time for this test using the GreenLight system was just under 26 hours.



In the next two tests, a *Pseudomonas*-selective growth medium was used. In the third test 9mL of this medium was inoculated with 1mL of bacteria, with duplicate samples incubated using an APCheck vial and pour plates. The estimated time of this test was about 15.5 hours, and the correlation with the pour plate method was nearly perfect.



In the fourth test, the process water was filtered before being analyzed. The estimated time for this type of testing was 35 hours.

Conclusion

The time to result is faster using the GreenLight system than it is with the standard test methods, and there is far less labor and cost involved. The results of testing are also less operator-dependent, allowing for greater accuracy and reproducibility than the standard test methods.

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