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Environmental Technology Verification Report

Mycometer®-test Rapid Fungi Detection and Bactiquant®-test Rapid Bacteria Detection Technologies

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Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

Mycometer®-test Rapid Fungi Detection and Bactiquant®-test Rapid Bacteria Detection Technologies

by

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Notice

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Foreword

The EPA is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technology across all media and to report this objective information to permitters, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of six environmental technology centers. Information about each of these centers can be found on the Internet at http://www.epa.gov/etv/.

Effective verifications of monitoring technologies are needed to assess environmental quality and to supply cost and performance data to select the most appropriate technology for that assessment. Under a cooperative agreement, Battelle has received EPA funding to plan, coordinate, and conduct such verification tests for "Advanced Monitoring Systems for Air, Water, and Soil" and report the results to the community at large. Information concerning this specific environmental technology area can be found on the Internet at http://www.epa.gov/etv/centers/center1.html.

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List of Abbreviations

ADQ	audit of data quality
AMS	Advanced Monitoring Systems
ARCA	aerosol research and component assessment
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
BQ	Bactiquant value
CFU	colony forming unit
cm	centimeter(s)
COA	certificate of analysis
°C	degrees Celsius
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
fu	fluorescence units
HPC	heterotrophic plate counts
in	inch(es)
LPM	liters per minute
µL	microliter(s)
mL	milliliter(s)
min	minutes
NRMRL	National Risk Management Research Laboratory
NTU	nephelometric turbidity unit
QA	quality assurance
QAPP	Quality Assurance Project Plan
QC	quality control
QMP	Quality Management Plan
R^2	coefficient of determination
RPD	relative percent difference
RSD	relative standard deviation
rtPCR	real-time polymerase chain reaction
SM	Standard Methods
TSA	technical systems audit
UV	ultraviolet

Chapter 1
Background

The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The EPA's National Risk Management Research Laboratory (NRMRL) and its verification organization partner, Battelle, operate the Advanced Monitoring Systems (AMS) Center under ETV. The AMS Center recently evaluated the performance of Mycometer®-test rapid fungi detection and Bactiquant®-test rapid bacteria detection technologies that are commercially available from Mycometer A/S in Europe and Mycometer, Inc. in North America. These technologies are based on fluorogenic detection of enzyme activities found predominantly in fungal biomass for the Mycometer®-test and bacterial biomass for the Bactiquant®-test.

Chapter 2
Technology Description

The objective of the ETV AMS Center is to verify the performance characteristics of environmental monitoring technologies for air, water, and soil. This report provides results for the verification testing of the Mycometer®-test rapid fungi detection and BactiQuant®-test rapid bacteria detection technologies developed and patented by Mycometer A/S based in Copenhagen, Denmark and available for distribution in the U.S. through Mycometer, Inc. The following is a description of the Mycometer®-test and Bactiquant®-test, based on information provided by the vendor.

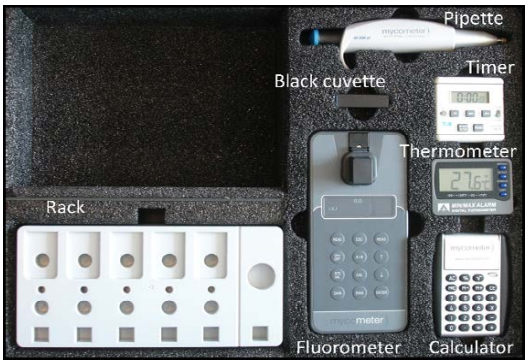

Figure 2-1. Mycometer®-test and Bactiquant®-test Fluorometer Kit Components

Both the Mycometer®-test rapid fungi detection and Bactiquant®-test rapid bacteria detection technologies (Figure 2-1) are based on fluorogenic detection of enzyme activities found predominantly in a taxonomic group of organisms. For the Mycometer®-test (correlating to fungal biomass) and the Bactiquant®-test (correlating to bacterial biomass), a sample (e.g., filter or swab) is contacted with a test solution containing a synthetic enzyme substrate. The enzyme present in the fungal cells or bacterial cells hydrolyzes the synthetic enzyme substrate as shown in Figure 2-2. When the synthetic substrate molecule is cleaved into two molecules by the enzyme, one of the molecules (as indicated by the yellow asterisk in Figure 2-2) can be made to fluoresce upon excitation with ultraviolet (UV) light at a wavelength of 365 nanometers. The amount of fluorescence is measured using a handheld fluorometer after processing for a reaction time based on the ambient temperature. This fluorescence correlates to the fungal or bacterial biomass. The same fluorometer may be used to measure fluorescence for both the Mycometer®-test and Bactiquant®-test. Fluorescence measurements can be captured electronically and downloaded to a computer, or can be transcribed by hand. Sample preparation and analysis can be performed on site in less than one hour.

According to the vendor, the Mycometer®-test for fungi is designed to measure both viable and non-viable spores, hyphae and fungal particles such as hyphal fragments in air, on surfaces, or in bulk materials to give a representation of the contamination in the environment. Although the Mycometer®-test cannot distinguish between fungal genera or viable/non-viable fungi, it provides a semi-quantitative measure of the total fungal biomass present. Air samples can be collected with traditional air sampling pumps onto filter media. Typically 300 liters of air are collected by sampling 20 liters per minute (LPM) for 15 minutes, or 15 LPM for 20 minutes. Surface samples are collected by swabbing a nine square centimeter area and bulk material samples are weighed. Enzyme substrate is added to the filter, swab, or bulk material and fungal

enzyme reacts with the substrate to release a fluorescent product. The amount of fungi in the sample is estimated by measuring the fluorescence produced.

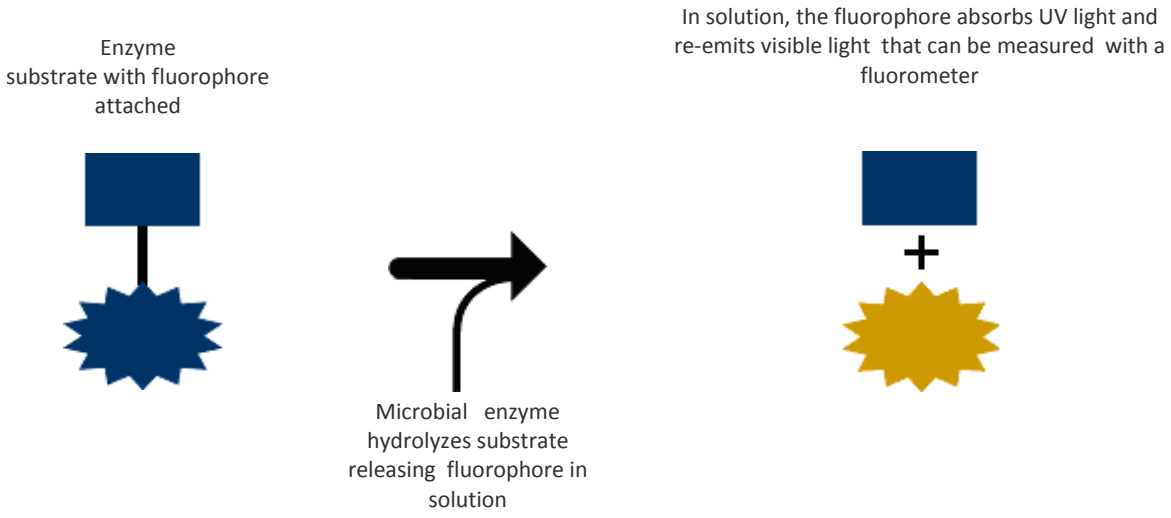

Figure 2-2. Principle of Fluorogenic Detection of Fungal or Bacterial Related Enzymatic Activity in an Environmental Sample

According to the vendor, the Bactiquant®-test is designed to provide a rapid method to estimate total bacteria in water samples. With the Bactiquant®-test, bacteria are concentrated from water samples by passing the sample (typically 250 milliliters (mL)) through a membrane filter. Enzyme substrate is added to the filter unit and left to react over a period of time based on temperature. Bacterial enzyme reacts with the substrate, releasing a fluorescent product. The amount of bacteria in the water sample correlates to the amount of the fluorescent product released into the solution during the reaction period. According to the vendor, this technology is designed for application to a range of liquid samples including: potable water, processed water, CIP (cleaning in place), wastewater, and recreational water. Bactiquant®-test can also be applied to surface and air samples.

The fluorometer used for both Mycometer®-test and Bactiquant®-test is provided in a hard-cover carrying case. The carrying case has dimensions of 45 centimeters (cm) wide × 15 cm deep × 32 cm high (17.5 inches (in) wide ×6 in deep × 12.5 in high) and weighs approximately 7.2 kilograms (16 pounds). The fluorometer is provided with the components shown in Figure 2-1. The Mycometer®-test and Bactiquant®-test reagents are sold separately in lots of 5-20 tests depending on the type and includes the sampling media (swab, filter or other), enzyme substrate, developer and calibration standard. Stationary vacuum manifolds for filtering up to five water samples simultaneously or portable manifolds for up to two samples simultaneously are sold separately through Mycometer, Inc. The recommended air sampling pumps (Gast 3-30 LPM IAQ Pump w/Tubing & Rotameter) are commercially available. For both the Mycometer®-test and Bactiquant®-test, the filter material and the type and material of the air sampling filter cartridge are critical for both sampling and the enzyme reaction that takes place directly on the filter. Therefore, it is important to use the filters provided by the vendor. The vendor provides a proficiency certification training program that is included with the fluorometer kit (on a flash drive) and is mandatory for use of their technology to document understanding and proper training.

Chapter 3
Test Design and Procedures

3.1 Introduction

The ETV AMS Center Water and Air Stakeholder Committees identified the use of rapid fungal and bacterial detection technologies as an area of interest for technology verification. Rapid technologies (results available same day of testing) to detect fungi and bacteria from matrices such as surfaces, bulk material, air, or water are of interest to improve the efficiency of delineating and documenting microbial contamination in buildings or water systems, and for monitoring progress during cleanup or remediation processes. Microbial contamination has the potential to cause health problems. Fungi are known to produce allergens, irritants, and potentially toxic substances[1] resulting in asthma, respiratory infections and a variety of allergic reactions[2], and bacteria ingested through the water supply can cause illnesses.[3]

Traditional methods of analysis for bacteria in drinking water include plate count and microscopy for total counts (e.g., heterotrophic plate count or direct total microbial counts) and specialized analysis for indicator organisms such as *Escherichia coli (E. coli)*. Plating methods are time consuming and can take up to seven days for results using traditional methods such as heterotrophic plate counting. Microscopic techniques such as direct microbial counts using epifluorescence are faster, but do not estimate microbial biomass or viability, and require an experienced analyst for differentiation of cells from other water constituents. Detection of fungi in air is tenuous using methods such as spore trap air sampling and analysis for identifying fungi present, quantifying spores, and assessing background debris (such as pollen). This technique is subject to variation due to concentrations of airborne particles (spores, hyphae, and debris) and analyst-to-analyst variability associated with microscopic techniques.

Technologies such as real-time polymerase chain reaction (rtPCR) can provide same day results, typically within a few hours or overnight and have increased accuracy and sensitivity. However, cost and time are the trade-offs to be considered with this type of technology. Screening technologies to monitor changes in water or air quality that are fast and affordable would help to control microbial outbreaks, expedite remediation efforts, and protect public health.

It should be noted that U.S. ETV verification does not represent an approval of methods for regulatory compliance.

3.2 Test Overview

This verification test was conducted according to procedures specified in the Quality Assurance Project Plan for Verification of Mycometer®-test Rapid Fungi Detection and Bactiquant®-test Rapid Bacteria Detection Technologies[4] (QAPP), and adhered to the quality system defined in the ETV AMS Center Quality Management Plan (QMP).[5] As indicated in the QAPP, the testing conducted satisfied EPA QA Category III requirements. The QAPP and this verification report were reviewed by:

- Dr. Timothy Dean, U.S. EPA Air Pollution Prevention and Control Division
- Dr. Connie Schreppel, Director of Water Quality of the Mohawk Valley Water Authority
- Dr. Nancy Clark Burton, Industrial Hygiene Team Leader for the Centers for Disease

In addition, the QAPP in general was reviewed with the broader AMS Center Stakeholder Committee as a presentation during regular stakeholder teleconferences, including the September 11, 2008 and January 22, 2009 meetings. Input from the water and air committees was also solicited during email updates of AMS Center activities in June 2010.

The Mycometer®-test and Bactiquant®-test technologies were verified for repeatability and inter-assay reproducibility by detecting fungi in air samples and bacteria in water samples, respectively. Linearity was assessed for both technologies using dilutions of stock cultures in tap water. The linearity test for fungi was a modification of test procedures in place for air and surface samples. In addition, sustainable operational factors are reported such as ease of use, required reagents, analysis time, laboratory space, and utilities required.

3.3 Experimental Design

3.3.1 Mycometer®-test for Fungi

3.3.1.1 Mycometer®-test Linearity. Mycometer®-test linearity was demonstrated using two fungal cultures from American Type Culture Collection (ATCC), *Cladosporium herbarum* ATCC 58927 and *Aspergillus flavus* ATCC 58870. These two fungal cultures were chosen based on their presence in indoor fungal isolates as reported in the literature.[6,7,8,9] The specific strains (species designation and ATCC number) were selected based on their being isolated from air samples as indicated by ATCC. The ATCC cultures were confirmed based on a Certificate of Analysis (COA) provided by ATCC. A dilution series for each of the fungal cultures (based on the spore counts) was performed using dechlorinated tap water. Tap water used for dilution was collected and dechlorinated with sodium thiosulfate as detailed in the QAPP Section B2.2.[4] The tap water was characterized for pH, free chlorine, and total chlorine. It was also characterized by Pace Analytical (Columbus, OH) for turbidity, total organic carbon, specific conductivity, alkalinity, hardness, and dissolved oxygen. These characterization results were not used in evaluating the technologies, but were included in the appendix for informational purposes since tap water characteristics can vary from location to location.

The dilution series originally targeted a test range of approximately 500 to 50,000 spores/mL of enzyme substrate based on vendor communication that these concentrations should be expected to generate fluorescence in the range typically encountered by a user. However, during the training session with the vendor, it became apparent that these concentrations did not provide sufficient fluorescence response. As a result, a deviation (Deviation Number 2) was prepared to change the target test concentration to approximately 2.4×10^5 to 4.8×10^6 spores/mL of enzyme substrate. Test solutions were prepared by diluting a stock solution with dechlorinated tap water to target a range of 5.0×10^6 to 1.0×10^8 spores/mL. The neat stock solution, along with three dilutions of the stock solution (1:5, 1:10, and 1:20) were used for linearity testing. Preliminary testing conducted during training confirmed that the stock solution generated sufficient fluorescence and that the dilutions were likely to be detectable. The actual stock solution concentration in spores/mL for each fungal culture was evaluated using a hemocytometer following procedures in American Society for Testing and Materials (ASTM) D4300-01 Annex[10] and was based on five replicate analyses for each fungal stock. Table 3-1 shows the actual concentrations used in testing. The stock solution and each of the three dilution concentrations were sub-sampled in five separate iterations. Each iteration involved processing one sample per

concentration level plus a blank. All samples were processed identically by adding 100 microliters (µL) of test sample (dechlorinated tap water for the blank) to 2 mL of enzyme substrate and incubating the solution for approximately 32 minutes based on temperature. The contents of the Mycometer®-test kit developer vial were then added to the test sample/enzyme substrate mix and the fluorescence was measured.

Table 3-1. Solutions Used to Generate Mycometer®-test Linearity Data

Actual Concentration of Stock Dilutions* (spores/mL)	Volume (mL) of Stock Dilution added to 2 mL Enzyme Substrate to create Test Solution	Total Spores Tested	Final volume of Test Solution (mL)	Actual Test Solution Concentration (spores/mL of Enzyme Substrate)	Number of Aliquots Processed	Equivalent Concentration in Air ** (spores/m^3)
***Aspergillus flavus* ATCC 58870**						
NEAT: 6.2×10^7	0.10	6.2×10^6	2.1	3.0×10^6	5	2.0×10^7
1:5: 1.2×10^7	0.10	1.2×10^6	2.1	5.9×10^5	5	3.9×10^6
1:10: 6.2×10^6	0.10	6.2×10^5	2.1	3.0×10^5	5	2.0×10^6
1:20: 3.1×10^6	0.10	3.1×10^5	2.1	1.5×10^5	5	9.8×10^5
***Cladosporium herbarum* ATCC 58927**						
NEAT: 9.6×10^7	0.10	9.6×10^6	2.1	4.6×10^6	5	3.0×10^7
1:5: 1.9×10^7	0.10	1.9×10^6	2.1	9.1×10^5	5	6.1×10^6
1:10: 9.6×10^6	0.10	9.6×10^5	2.1	4.6×10^5	5	3.0×10^6
1:20: 4.8×10^6	0.10	4.8×10^5	2.1	2.3×10^5	5	1.5×10^6

*NEAT solution concentration measured from hemocytometer counts. Dilutions calculated by dividing the NEAT solution concentration by the dilution factor.
**Calculated as the air concentration necessary to generate a test solution concentration the same as the actual test solution when a 300 L (0.30 m^3) air sample is collected and processed with 2 mL enzyme substrate (e.g. actual test solution concentration in spores/mL of enzyme substrate * 2.0 mL enzyme substrate/0.30 m^3).

According to the vendor, data can be transferred from the fluorometer to a computer. Originally, this was the intended method of data transfer for further data reduction; however, training for the data transfer software was not included as part of the vendor-provided training to use the technology. All fluorescence readings were recorded by hand onto data sheets, and transcribed into spreadsheets provided by Mycometer for further calculation. This change in the data recording procedure was documented as Deviation Number 7. The Mycometer®-test adjusted fluorescence values (fluorescence unit reading for the test solution – fluorescence unit reading for the blank) were plotted against the concentration of spores in each test solution displayed as the total number of spores tested to generate linearity data.

3.3.1.2. Mycometer®-test Repeatability and Inter-Assay Reproducibility. Mycometer®-test repeatability and inter-assay reproducibility were evaluated by producing controlled air samples in Battelle's Aerosol Research and Component Assessment (ARCA) chamber (Figure 3-1) and sampling and analyzing the air using the Mycometer®-test technology. A total of eight air pumps (GAST Model 1532 Pumps with IAQ option) supplied by the vendor was used. These pumps were placed outside of the chamber and were connected by tubing to the Mycometer®-test air sampling cartridges inside the chamber. The eight air sampling cartridges were arranged in close proximity inside the chamber on two tripod stands holding four Mycometer®-test sampling cartridges each (Figure 3-2). The flow rate for each pump was adjusted using a calibrated flow meter (Sierra Instruments, Model 821 or 822, Monterey, CA). Originally, samples were to be collected using a sampling flow rate of 20 LPM for 15 minutes to provide a total air volume of 300 L. This was based on guidance in the Mycometer 2008 air sampling protocol.[11] During

repeatability and inter-assay reproducibility tests, a flow rate of 20 LPM could not be established in all eight vendor-supplied pumps. This resulted in a deviation for the sampling flow rate and collection time (Deviation Number 4). Sampling was conducted using a flow rate of 15 LPM for 20 minutes, providing a total air volume of 300 L following an option in an updated air sampling protocol provided by Mycometer.[12] One fungal stock, *A. flavus*, was used to determine repeatability and inter-assay reproducibility. *A. flavus* was selected based on the fluorescence response observed during linearity testing and the consistency of linearity data.

Figure 3-1. ARCA Chamber

Figure 3-2. Sampling Cartridges Inside the ARCA Chamber with Tubing Leading to Pumps Outside the ARCA Chamber

In order to produce an aerosol sample resulting in a fluorescence signal approximately 300 fluorescence units (fu) above the level of a blank, an initial characterization run was performed. The starting spore stock concentration for generation of the aerosol used in the initial characterization (1.24×10^7 spore/mL) was estimated based on the fluorescence responses

observed during the *A. flavus* linearity test and calculations outlined in the QAPP.[4] Analysis of eight samples generated with this stock solution generated fluorescence ranging from 38 to 47 fu, below the goal of 300 fu above the blank; therefore, for testing, a spore stock concentration of 6.2×10^7 spores/mL was used and the chamber flow rate was lowered in attempt to increase the air sample fluorescence readings.

For testing, the 6.2×10^7 spores/mL of *A. flavus* solution was released into the chamber using a generation rate of 0.5 mL/minute (min) through a Collison nebulizer aerosol generator and a chamber velocity of 5,000 L/min for an expected chamber aerosol concentration of 6.2×10^3 spores/L of air. Once the system reached steady state as determined by checking aerodynamic particle sizer concentration readings, sampling commenced. A total of 300 L of air was pumped through each of the eight sampling filters simultaneously for an approximate expected sample concentration of 1.9×10^6 spores/sample. During the inter-assay reproducibility chamber run, the chamber air flow between the beginning and middle of the run dropped by 18%, falling below the ± 10% measurement quality objective. The flow was not adjusted to avoid further fluctuation in air flow and remained steady from the middle to the end of the run. At this lower flow rate, the *A. flavus* spores were still uniformly collected on all of the filters. This slight change to flow rate was considered to have no impact on the test since a specific concentration on the filters was not targeted.

Repeatability was determined by having one vendor-trained analyst process samples from cartridges connected to all eight pumps from one chamber test using one fluorometer. For inter-assay reproducibility, two vendor-trained analysts each processed samples on cartridges connected to four pumps during one chamber test. Each analyst used a separate fluorometer. The repeatability and inter-assay reproducibility test scheme is described further in Table 3-2. The QAPP[4] originally stated that one analyst would perform both sampling and analysis from all eight pumps for repeatability testing and two analysts would each perform sampling and analysis for the inter-assay reproducibility testing. Because of ARCA chamber access restrictions, a deviation to the sampling scheme was required (Deviation Number 5). Only one analyst, serving the role of Analyst 2 in Table 3-2, physically assembled and removed the sample filter for all air samples collected in the ARCA chamber. However, all sample processing was carried out as intended and as outlined in Table 3-2. Eight background air samples were collected in the chamber prior to release of the fungal culture. Each repeatability and inter-assay reproducibility air sample set (test sample and background) was processed with a blank. Blanks consisted of an air sampling filter through which no air passed. Blanks were handled, processed, and analyzed in the same manner as the air samples.

Table 3-2. Repeatability and Inter-assay Reproducibility Test Scheme for Mycometer®-test

Analyst	Fluorometer Unit	Number of Repeatability Samples	Number of Inter-assay Reproducibility Samples
Analyst 1	A	8	4
Analyst 2	B	None	4

The chamber schedule used for generation of repeatability and inter-assay reproducibility samples is listed below:

- Decontaminate the ARCA test chamber (prior to test),
- Set up,
- Characterization run to check test parameters and stock concentration,
- Perform an air wash of the chamber,

- Collect eight background samples (Mycometer®-test analysis),
- Run one test run with *A. flavus* to collect eight repeatability samples (Mycometer®-test analysis),
- Perform an air wash of the chamber,
- Surface decontaminate the tripod stands,
- Collect eight background samples - split between two analysts (Mycometer®-test analysis),
- Run one test with an aerosolized fungal stock to collect eight inter-assay reproducibility samples - split between two analysts (Mycometer®-test analysis),
- Perform an air wash of the chamber,
- Remove tubing and surface decontaminate the tripod stands, and
- Decontaminate the chamber (after test day).

3.3.2 Bactiquant®-test for Bacteria

3.3.2.1 Bactiquant®-test Linearity. Bactiquant®-test linearity was determined using two types of bacterial stocks: a quality control (QC) strain consisting of *Pseudomonas aeruginosa* ATCC 27853 obtained as a QuantiCult® culture from Remel, Inc. and a consortium of indigenous flora in water from a local lake. Both bacterial stocks were diluted with dechlorinated Columbus, Ohio tap water to prepare solutions for linearity testing. Tap water was collected and dechlorinated as described in the QAPP Section B2.2[4] and as noted above for the Mycometer®-test. The *P. aeruginosa* QC strain was prepared following the manufacturer's directions. The working stock for spiking into tap water was initially planned to be grown on a low nutrient R2A agar. Prior to testing, the vendor expressed concern that the content of hydrolyzed milk protein in R2A agar might have an effect on the Bactiquant®-test analysis as the vendor had never used this medium to generate bacteria for testing with their technology. The vendor did have considerable experience using yeast extract and, therefore, a deviation was prepared to use yeast extract agar to grow the working stocks of *P. aeruginosa* (Deviation Number 1).

The original target concentration for the bacterial stocks ranged from approximately 50 to 50,000 colony forming units per milliliter (CFU/mL). However, during the technology training session with the vendor, it was apparent that these concentrations would not provide sufficient fluorescence for the *P. aeruginosa* QC strain using the reaction time and sample volumes agreed to for verification testing. Therefore, adjustments were made to the concentrations for both the indigenous flora from lake water (Deviation Number 3) and the *P. aeruginosa* QC strain (Deviation Number 6).

Indigenous Bacteria from Lake Water. The lake water was first analyzed neat (no dilution with dechlorinated tap water) to determine the water fluorescence reading. Following the Bactiquant®-test processing procedures, 250 mL of lake water were filtered and processed using the Bactiquant®-test reagents. The fluorescence response of the neat water was 48,384 fu. Based on this, the four sample concentrations selected for testing were a 1:5, 1:10, 1:20, and 1:100 dilution of the neat lake water using dechlorinated tap water. Each of the four test concentrations were sub-sampled five times and processed using the Bactiquant®-test reagents. The samples (250 mL) were filtered as shown in Figure 3-3. Each filter was processed by flushing the filter with 2.5 mL of enzyme substrate and incubating the filter for 30 minutes at room temperature. This reaction was terminated by flushing the filter with developer solution provided in the kit. The fluorescence of the resulting solution was then measured. The actual bacterial concentration of each solution used for testing was determined using heterotrophic plate counts (SM 9215[13])

conducted in triplicate. For the 1:100 dilution, the heterotrophic plate counts (HPC) were too low for reliable count; therefore, the concentration of this solution was determined by taking the neat water HPC- determined concentration and dividing by the dilution factor of 100. The HPC results for the lake water solutions used in testing are shown in Table 3-3. Linearity data was generated by plotting the Bactiquant®-test fluorescence results against the HPC-determined concentration of bacteria in each testing solution (CFU/mL).

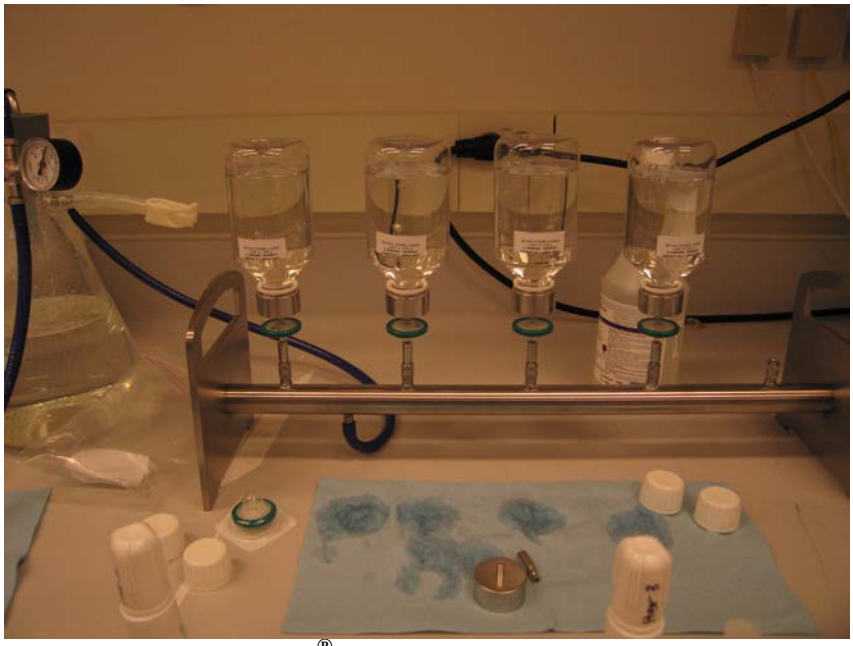

Figure 3-3. Bactiquant®-test Water Sample Filtration System

***Pseudomonas aeruginosa* ATCC 27853**. Following the training session, the vendor provided additional information from the vendor's experiments with a different strain of *P. aeruginosa.* In these experiments, solution concentrations of 5.0 x 10^3 to 5.0 x 10^6 CFU/mL resulted in Bactiquant (BQ) values ranging from 50 to 55,000. BQ values are calculated values adjusting the fu results to the standard reaction time (30 minutes), temperature (23 °C), and sample volume (250 mL) and are lower than actual fluorescence readings. The BQ value is calculated as:

$$BQ = (Fs - Fb) \times 0.59 \times \frac{250}{V} \times \frac{30}{R} \times 3.3344e^{(-0.0522 \times T)}$$

Where Fs is the sample fluorescence, Fb is the blank fluorescence, V is the volume of the water sample in milliliters, R is the reaction time in minutes, and T is the room temperature in degrees Celsius.

In this equation, 0.59 is a transformation constant that adjusts the results to an earlier Bactiquant protocol. The exponential function adjusts for the influence of temperature on the reaction rate.

The vendor also noted in separate communication that high fluorescence readings (> 20,000 fu) may generate results that are not linear because the enzyme substrate concentration will have decreased significantly and the enzyme reaction will slow down. Therefore, to generate detectable fluorescence that would not exceed 20,000 fu, a *P. aeruginosa* solution containing approximately 5.0 x 10^5 CFU/mL was prepared from a working stock with turbidity equivalent to a 0.5 McFarland standard (estimated concentration ranging from 1 x 10^7 to 1 x 10^8 CFU/mL).

This working stock was diluted by a factor of 100 with dechlorinated tap water to make the starting solution with a target concentration of approximately 5.0 x 10^5 CFU/mL.

A single 250 mL sample of this starting solution was filtered and processed. If the fluorescence reading for this starting solution was between 1,000 and 20,000 fu, testing was to proceed using the starting solution and dilutions of the starting solution with dechlorinated tap water by factors of 1:2, 1:5, and 1:10 (filtering 250 mL of each dilution). The actual fluorescence reading of the starting solution was 12,784 fu and so the starting solution, 1:2, 1:5 and 1:10 dilutions were used for testing. The actual bacterial concentration of each solution used for testing was determined using HPC conducted in triplicate. The HPC results for the *P. aeruginosa* solutions used in testing are shown in Table 3-3. The concentration of the *P. aeurignosa* starting solution was approximately two logs lower than the target concentration. The working stock and all dilutions prepared in dechlorinated tap water may have had reduced viability or stressed the organisms as a result of the change in osmotic pressure when the culture was introduced into the water resulting in lower counts. Linearity data was generated by plotting the Bactiquant®-test fluorescence results against the HPC-determined testing solution concentrations (CFU/mL).

3.3.2.2. Bactiquant®-test Repeatability and Inter-Assay Reproducibility. Inter-assay reproducibility and repeatability were determined by having two vendor-trained analysts each perform sampling and analysis of four sub-samples taken from one concentration of tap water spiked with indigenous flora (3.7 x 10^2 CFU/mL) and four sub-samples from one concentration of tap water spiked with *P. aeruginosa* (4.7 x 10^3 CFU/mL). Each analyst used a separate fluorometer. This testing scheme is further described in Table 3-4. Repeatability evaluated variability of the performance of the technology by each analyst and inter-assay reproducibility evaluated variability of the performance of the technology between analysts and fluorometers.

Table 3-3. Solutions Used to Generate Bactiquant®-test Linearity Data

Actual Concentration of Bacteria in Testing Solution* (CFU/mL)	Volume (mL) of Testing Solution Filtered	Total CFU Tested	Number of Aliquots Processed
Indigenous Bacteria from Lake Water			
NEAT: 3.7 x 10^4			
1:5: 6.0 x 10^3	250	1.5 x 10^6	5
1:10: 3.0 x 10^3	250	7.5 x 10^5	5
1:20: 1.3 x 10^3	250	3.3 x 10^5	5
1:100: 3.7 x 10^2	250	9.3 x 10^4	5
***Pseudomonas aeruginosa* ATCC 27853**			
Starting solution: 8.0 x 10^3	250	2.0 x 10^6	5
1:2: 4.7 x 10^3	250	1.2 x 10^6	5
1:5: 2.1 x 10^3	250	5.3 x 10^5	5
1:10: 8.7 x 10^2	250	2.2 x 10^5	5

*Each testing solution concentration was determined from heterotrophic plate count measurements conducted in triplicate. Plates with counts outside of the 30-300 target were estimated.

Table 3-4. Repeatability and Inter-assay Reproducibility Test Scheme for Bactiquant®-test

Analyst	Fluorometer Unit	Number of Repeatability* Samples-Indigenous Flora	Number of Repeatability* Samples-*P. aeruginosa*
Analyst 1	A	4	4
Analyst 2	B	4	4

*Repeatability sample results were also used to generate inter-assay reproducibility data.

3.3.3 Data Completeness and Operational Performance Parameters.

For both technologies, data completeness was determined from a review of the valid data (i.e., data that met all measurement quality objectives [MQO]) collected during the verification testing period against the expected amount of total data to be generated. Operational performance parameters such as maintenance requirements, ease of use, sustainability factors, and portability were determined from observations by the Battelle testing staff.

Chapter 4
Quality Assurance/Quality Control

QA/QC procedures were performed according to the QAPP for this verification test[4] and the QMP for the AMS Center[5]. QA/QC procedures and results are described in the following subchapters.

During testing, there were eight deviations from the QAPP. Deviations are described in Section 3.3.2.1 (Deviation 1), 3.3.1 (Deviation 2), 3.3.2.1 (Deviation 3), 3.3.1.2 (Deviations 4 and 5), 3.3.2.1 (Deviation 6), 3.3.1.1 (Deviation 7) and 6.1 (Deviation 8) and discussed in Section 4.4. These deviations were judged by the Battelle Verification Test Coordinator to not result in any adverse impacts on the quality of the data generated. The deviations were reviewed by the EPA ETV AMS Center Project Officer and EPA ETV AMS Center Quality Manager.

4.1 Quality Control Samples

A blank was processed with every sample set. For the Mycometer®-test linearity testing, the blank consisted of 100 µL of dechlorinated tap water processed with the kit reagents and procedures as a sample. For Mycometer®-test repeatability and inter-assay reproducibility, the blank consisted of an air sampling filter through which no air passed, processed with the kit reagents and procedures as a sample. For the Bactiquant®-test, the blank was prepared using the blank reagents provided in the Bactiquant®-test kit by adding 0.35 mL of enzyme substrate to a cuvette containing the developer, and processing it as a sample. All blanks had fluorescence readings below the measurement quality objective specified for this verification test of 300 fu.

For the HPC tests used to determine bacterial concentrations of test solutions, a positive media control, negative media control, and diluent blank controls were prepared each day that test solutions were plated. All positive media controls exhibited growth, and the negative media controls and diluent blanks exhibited no growth each day plating was conducted.

4.2 Audits

Two types of audits were performed during the verification test; a technical systems audit (TSA) of the verification test procedures, and an audit of data quality (ADQ). Audit procedures for the TSAs and ADQs are described further below.

4.2.1 Technical Systems Audits

The Battelle AMS Center Quality Manager or designee performed two TSAs throughout testing. The first TSA for Mycometer®-test was conducted in two phases on May 19 and June 9-10, 2011 at Battelle's microbiology laboratory in Columbus, OH. The EPA AMS Center Project Officer

participated in the audit on May 19. The second TSA for Bactiquant®-test was conducted on June 2, 2011. The TSAs consisted of interviews with Battelle personnel, observations of test sample preparation and observation of sample analysis during testing at Battelle. The purpose of these audits was to verify that:

- Sample preparation procedures were performed by Battelle according to the QAPP requirements;
- Reference methods for analyzing test samples conformed to the QAPP and reference method requirements;
- Technology testing was performed according to the QAPP and vendor instructions;
- Test documentation provided a complete and traceable record of sample preparation and analysis; and
- Equipment used in the test was calibrated and monitored according to QAPP requirements and standard laboratory procedures.

Zero (0) Findings, two (2) Observations, and one (1) Comment were identified during the first TSA. Zero (0) Findings, three (3) Observations, and zero (0) Comments were identified during the second TSA. It was determined by Battelle that none of these had an adverse impact on the test results and all Observations and Comments have received a satisfactory response.

In response to these audit reports, the following actions were taken:

- Deviation 5 was prepared to correctly describe the collection of samples generated in the ARCA chamber;
- Deviation 7 was prepared to describe that data was recorded by hand, not electronically;
- Deviation 8 was prepared to describe the tap water fluorescence measurements made;
- Data records were updated where clarification was needed to facilitate understanding of procedures.

Two separate TSA reports were prepared and distributed to EPA.

4.2.2 Data Quality Audit

Records generated in the verification test received a one-over-one review before these records were used to calculate, evaluate, or report verification results. Data were reviewed by a Battelle technical staff member involved in the verification test. The person performing the review added his/her initials and the date to a hard copy of the record being reviewed.

In addition, ADQs were conducted for Day 1 Mycometer test results on May 19; for Day 1 Bactiquant test results on July 7; and for Days 3 and 4 test results and the final report on August 4 - 11, 2011. During the audits, laboratory data generated at Battelle using the Mycometer®-test and Bactiquant®-test were reviewed and verified for completeness, accuracy and traceability. The EPA quality system utilizes a "graded approach" for establishing the appropriate level of QA/QC for various types of research activities based on the intended use of the data and the visibility of the research effort dictate the required level of quality. The verification of rapid fungi and bacteria detection technologies was determined by the EPA AMS Center Project Officer to be a Category III test. “Category III” establishes the QA/QC requirements for projects involving applied research or technology evaluations. In addition to preparation of the QAPP, Category III projects require a technical systems audit and maintenance of project data for 20 years. The ETV program further requires an audit of data quality for each project. Accordingly, at least 10% of

the results for each of the testing scenarios were verified versus the raw data, and 100% of the QC sample results were verified. The data were traced from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. Data verification included re-calculation of intermediary and final test results from the raw data files.

In all, four (4) Findings and seven (7) Observations were identified during the ADQs. The findings involved sample custody, missing test data, missing documentation, and failed quality control (QC) objectives. Battelle believes that none of these had an adverse impact on the test results and all have received a satisfactory response.

In response to these audit reports, the following actions were conducted:

- Testing forms were updated to prompt for documentation of calibration readings;
- The excel spreadsheet for Bactiquant®-test repeatability was corrected for a cell marked as text that was not being included in calculations;
- Data records were updated where clarification was needed to facilitate understanding of procedures; and
- The report was revised to discuss the impact of the failed QC.

Three separate ADQ reports were prepared and distributed to EPA.

4.3 Deviations

Eight deviations were documented during testing:

Deviation 1 (4-15-11): The QAPP stated that the working stock of *P. aeruginosa* would be grown on the low nutrient medium, R2A agar. However, the vendor expressed concern after QAPP approval that the content of hydrolyzed milk protein in R2A agar might have an effect on the Bactiquant®-test analysis and recommended that yeast extract agar be used to grow the working stocks of *P. aeruginosa*. Yeast extract agar was used to grow the *P. aeruginosa* working stocks. Impact: None; the yeast extract agar was a suitable growth medium that eliminated the vendor's concern with R2A agar.

Deviation 2 (5-18-2011): The QAPP stated that fungal linearity testing would target a test concentration range of 500-50,000 spores/mL of enzyme substrate; however, during the technology training session with the vendor, it became apparent that these concentrations did not provide sufficient fluorescence response. The target test solution concentration range was changed to approximately 240,000 to 4,800,000 spores/mL of enzyme substrate based on range finding experiments conducted during the training session. Impact: None, the revised concentration range provided test solutions that had a response with the vendor's technology in a range that will be useful to the user.

Deviation 3 (5-27-2011): The QAPP stated that bacterial linearity testing would target using stock solutions of 50-50,000 CFU/mL; however, during the technology training session with the vendor using the *P. aeruginosa* strain, it became apparent that these concentrations did not provide sufficient fluorescence response. For the indigenous bacteria in lake water, in order to determine solution concentrations that would provide sufficient fluorescence response, the lake water was processed neat to obtain a base fluorescence measurement (48,384 fu). Based on this result, the four sample concentrations selected for testing were a 1:5, 1:10, 1:20, and 1:100 dilution of the neat lake water. The actual bacteria concentrations of each solution were

determined using HPC. Impact: None, the revised approach to obtaining concentrations of indigenous bacteria that would provide a fluorescence response were obtained in a manner that more closely simulated collection and analysis of a real-world water sample, as opposed to targeting a specific bacteria count that might not have provided sufficient fluorescence.

Deviation 4 (6/9/2011): The QAPP stated that air sampling for the Mycometer®-test repeatability and inter-assay reproducibility tests would be conducted using a sampling flow rate of 20 LPM for 15 minutes to collect a total air volume of 300 L. This was based on guidance in the Mycometer protocol "Quantification of mold in air – Protocol for quantification of fungal propagules in air samples using the Mycometer-test" (2008). For the training session Mycometer provided an updated protocol, "Mycometer®-air Sampling and analysis," that included the option of collecting the 300 L volume by either 20 LPM for 15 minutes, or 15 LPM for 20 minutes. During set up for the repeatability and inter-assay reproducibility tests, a flow rate of 20 LPM could not be established in all 8 pumps that would be used for testing; therefore, the sampling was conducted using 15 LPM for 20 minutes for all samples so that all pumps were operating under identical flow rate and collection time. Impact: None; a 300 L air sample was collected uniformly with all eight pumps provided for testing.

Deviation 5 (6/9/2011): The QAPP stated that one analyst would perform sampling and analysis of air samples collected from all eight pumps for the Mycometer®-test repeatability test and two analysts would each perform sampling and analysis for the Mycometer®-test inter-assay reproducibility test. Because of limitations on who can be in the ARCA chamber for collection of samples, only one analyst physically hooked up and removed the sample filter for all air samples collected in the ARCA for both the repeatability and inter-assay reproducibility tests. The filter samples were then distributed for sample preparation and analysis as described in QAPP. Impact: None; the sample processing and analysis were carried out as intended to show repeatability and inter-assay reproducibility with the Mycometer®-test reagents and fluorometers.

Deviation 6 (6/21/2011): The QAPP stated that bacterial linearity testing would target using stock solutions of 50-50,000 CFU/mL; however, during the technology training session with the vendor using the *P. aeruginosa* strain, it became apparent that these concentrations did not provide sufficient fluorescence response. Therefore, to attempt to generate detectable fluorescence a *P. aeruginosa* solution of approximately 5.0×10^5 CFU/mL was prepared by creating a working stock with turbidity equivalent to a 0.5 McFarland standard. This working stock was diluted by a factor of 100 to make a starting solution with a concentration of approximately 5.0×10^5 CFU/mL (based on the turbidity resulting in an estimated concentration ranging from 1×10^7 to 1×10^8 CFU/mL). A single 250 mL sample of the starting solution containing approximately 5.0×10^5 CFU/mL was filtered and processed. The fluorescence reading of this starting solution was 12,784 fu and so the starting solution, 1:2, 1:5 and 1:10 dilutions were used for testing. The actual bacterial concentration of each solution used for testing was determined using HPC. Impact: None, the revised concentration range provided test solutions that had a response with the vendor's technology in a range that will be useful to the user.

Deviation 7 (7/7/2011): The QAPP stated that Bactiquant®-test and Mycometer®-test fluorescence readings, and calculated Mycometer®-test air fungal concentration values would be recorded electronically by each technology unit and then downloaded to a computer daily. However, electronic transfer of data was not part of the technology training and so all fluorescence reading values were recorded by hand onto data sheets and then hand-typed into

Mycometer spreadsheets for further calculation. Impact: Battelle believes that this did not affect the data generated, only the means in which data was recorded.

Deviation 8 (7/21/2011): The QAPP stated that tap water blanks would be run during sample analysis to determine the fluorescence associated with the water. This was to be conducted as part of the tap water characterization and was performed during the days of Mycometer®-test mold linearity testing and the Bactiquant®-test lake water indigenous bacteria testing, but was inadvertently omitted from the day of Bactiquant®-test *P. aeruginosa* testing. Impact: None; a measure of the tap water fluorescence was not required to generate the verification test data and was only included to provide information to help characterize the tap water. Tap water was evaluated at least once with the Mycometer®-test and once with the Bactiquant®-test during verification testing.

Chapter 5
Statistical Methods

The statistical methods used to evaluate the quantitative performance factors are presented in this chapter. Qualitative observations were also used to evaluate verification test data.

5.1 Linearity

Linearity with respect to concentration (determined as heterotrophic plate counts for bacteria and as spore counts for fungi) was assessed by a linear regression analysis of the Mycometer®-test and Bactiquant®-test fluorescence units using the spore counts or heterotrophic plate counts as appropriate as the independent variable and the Mycometer®-test and Bactiquant®-test results as the dependent variable. The results were plotted and linearity expressed in terms of slope, intercept, and coefficient of determination (R^2).

5.2 Repeatability

Repeatability was determined as percent relative standard deviation (%RSD) of the replicate measurements of fungal and bacterial cultures taken with the Mycometer®-test and Bactiquant®-test, respectively. Equations 1 and 2 were used to calculate repeatability:

$$S = \left[\frac{1}{n-1} \sum_{k=1}^{n} \left(M_k - \overline{M} \right)^2 \right]^{1/2} \quad (1)$$

Where S is the standard deviation, n is the number of replicate samples, M_k is the technology fluorescence measurement for the k^{th} sample, and M is the average technology fluorescence measurement of the replicate samples.

$$RSD(\%) = \left| \frac{S}{\overline{M}} \right| \times 100 \quad (2)$$

5.3 Inter-Assay Reproducibility

Inter-assay reproducibility was evaluated from four measurements of one concentration of each bacterial or fungal culture by two separate analysts using two separate fluorometer units. The average and %RSD of each analyst's measurements were calculated. Inter-assay reproducibility was determined as relative percent difference (RPD) of the average measurements as noted in Equation 3:

$$RPD(\%) = \frac{\left| M_1 - M_2 \right|}{M_1 + M_2} \times 200 \quad (3)$$

Where M_1 is the average of replicate measurements made by the first unit of the technology and analyst 1 and M_2 is the average of replicate measurement made by the second unit of the technology and analyst 2.

5.4 Data Completeness

Data completeness was assessed based on the overall data return achieved by each Mycometer®-test and Bactiquant®-test analysis during the testing period. For each technology, this calculation used the total number of valid data points divided by the total number of data points potentially available from all testing.

Chapter 6
Test Results

As mentioned previously, this verification test included both quantitative and qualitative evaluations. The quantitative evaluation was conducted to assess the linearity, repeatability, and inter-assay reproducibility of the Mycometer®-test and Bactiquant®-test. The qualitative evaluation was performed to document the operational aspects of the Mycometer®-test and Bactiquant®-test during verification testing. The following sections provide the results of the quantitative and qualitative evaluations.

6.1 Characterization of Columbus, Ohio Tap Water Used for Testing

The dechlorinated tap water used to prepare Mycometer®-test linearity solutions and Bactiquant®-test linearity, repeatability, and inter-assay reproducibility solutions was characterized for pH, free chlorine, and total chlorine. The water was also characterized by Pace Analytical (Columbus, OH) for turbidity, total organic carbon, specific conductivity, alkalinity, hardness, and dissolved oxygen. Additionally, fluorescence of the tap water was to be measured as part of the characterization. Tap water fluorescence was measured with the Mycometer®-test on the day of fungal culture linearity testing and with the Bactiquant®-test on the day of testing linearity, repeatability, and inter-assay reproducibility with the indigenous bacteria from lake water. A separate measurement of the tap water used during Bactiquant®-test *P. aeruginosa* testing was inadvertently omitted and is described in Deviation Number 8. These characterization measurements were not used in evaluating the technologies, but are included for informational purposes since tap water can vary from location to location. Results for these characterization parameters are shown in the appendix.

6.2 Mycometer®-test for Fungi

6.2.1 Linearity

Tables 6-1 and 6-2 summarize the data obtained for linearity testing with *A. flavus*. Measurements were made using 3.1×10^5 to 6.2×10^6 total *A. flavus* spores. Within this range, replicate measurements of each testing solution had RSDs between 3.2 and 6.7%. Figure 6-1 shows the plot of total *A. flavus* spore counts as the independent variable and Mycometer®-test results as the dependent variable. Mycometer®-test results are expressed as adjusted fluorescence which is the fluorescence reading of the sample minus the fluorescence reading of the blank. The blank consisted of 100 µL of dechlorinated tap water processed prepared with the same reagents as the test samples. The relationship between total *A. flavus* spores in the concentration range

tested and adjusted fluorescence was linear with a slope of 0.0007, a y-intercept of 20.637, and R^2 equal to 0.9979.

Table 6-1. Mycometer®-test Linearity Data for *Aspergillus flavus* ATCC 58870

Test Iteration	Test Solution	Test Solution Concentration * (spores/mL)	Total Spores Tested**	Fluorescence Reading (fu)	Adjusted Fluorescence*** (fu)
1	Blank	Tap water	N/A	36	0
	NEAT	6.2×10^7	6.2×10^6	4280	4245
	1:5	1.2×10^7	1.2×10^6	904	868
	1:10	6.2×10^6	6.2×10^5	506	471
	1:20	3.1×10^6	3.1×10^5	260	224
2	Blank	Tap water	N/A	43	0
	NEAT	6.2×10^7	6.2×10^6	4158	4115
	1:5	1.2×10^7	1.2×10^6	944	901
	1:10	6.2×10^6	6.2×10^5	506	463
	1:20	3.1×10^6	3.1×10^5	238	196
3	Blank	Tap water	N/A	43	0
	NEAT	6.2×10^7	6.2×10^6	4413	4370
	1:5	1.2×10^7	1.2×10^6	876	833
	1:10	6.2×10^6	6.2×10^5	506	463
	1:20	3.1×10^6	3.1×10^5	254	212
4	Blank	Tap water	N/A	42	0
	NEAT	6.2×10^7	6.2×10^6	4370	4328
	1:5	1.2×10^7	1.2×10^6	905	863
	1:10	6.2×10^6	6.2×10^5	536	493
	1:20	3.1×10^6	3.1×10^5	264	222
5	Blank	Tap water	N/A	38	0
	NEAT	6.2×10^7	6.2×10^6	4590	4552
	1:5	1.2×10^7	1.2×10^6	940	902
	1:10	6.2×10^6	6.2×10^5	531	492
	1:20	3.1×10^6	3.1×10^5	274	235

*NEAT solution concentration measured from hemocytometer counts. Dilutions calculated by dividing the NEAT solution concentration by the dilution factor.
**Based on adding 0.10 mL of test solution to the enzyme substrate.
***Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading and is expressed in fluorescence units.
Fluorescence readings in the table are rounded to the nearest whole number, adjusted fluorescence calculations were based on actual measured results.

Table 6-2. Summary of Replicate Measurements for *A. flavus* ATCC 58870 Mycometer®-test Linearity Data

Test Iteration	Adjusted Fluorescence (fu)			
	6.2×10^6 spores tested	1.2×10^6 spores tested	6.2×10^5 spores tested	3.1×10^5 spores tested
1	4245	868	471	224
2	4115	901	463	196
3	4370	833	463	212
4	4328	863	493	222
5	4552	902	492	235
Average	4322	873	476	218
Standard Deviation	161	29	15	15
RSD (%)	3.7	3.3	3.2	6.7

Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading.

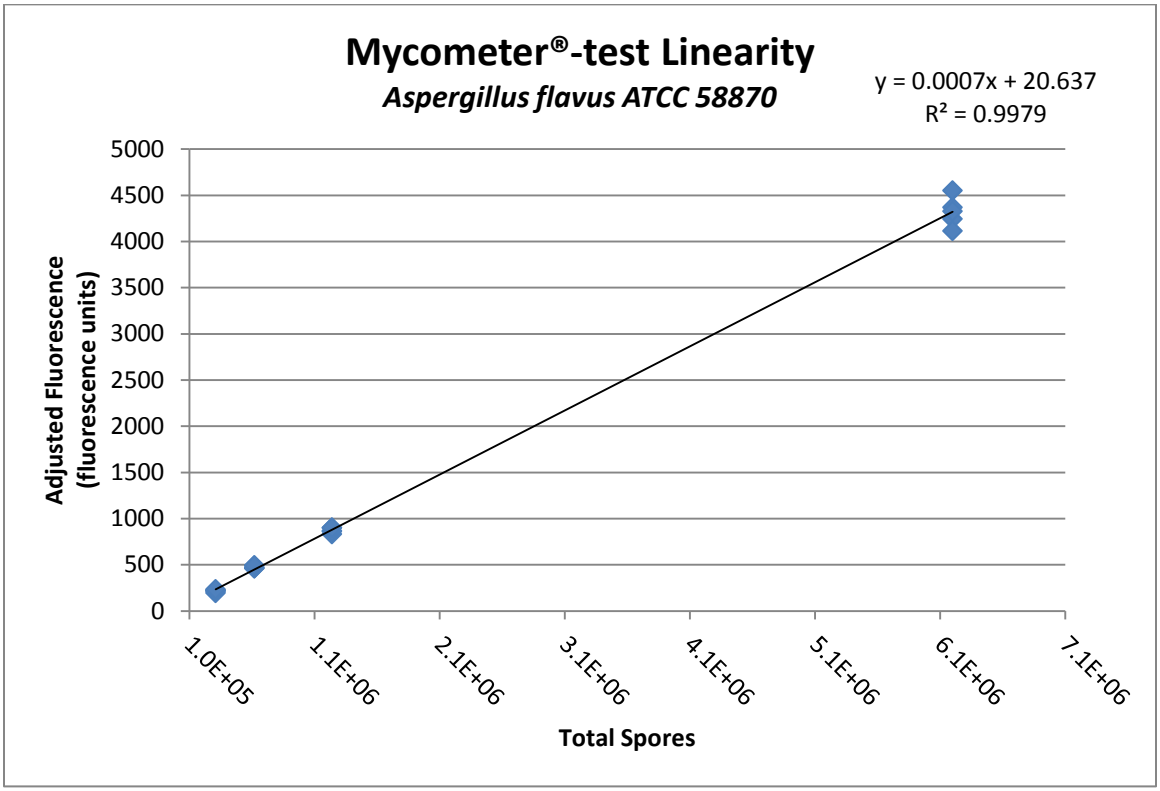

Figure 6-1. Plot of Mycometer®-test fluorescence response vs. *A. flavus* spore counts

Tables 6-3 and 6-4 summarize the data obtained for linearity testing with *C. herbarum*. Measurements were made using 4.8×10^5 to 9.6×10^6 total *C. herbarum* spores. Within this range, replicate measurements of each testing solution had RSDs between 1.6 and 11.4%. Figure 6-2 shows the plot of total *C. herbarum* spore counts as the independent variable and Mycometer®-test adjusted fluorescence results as the dependent variable. The relationship between total *C. herbarum* spores in the concentration range tested and adjusted fluorescence was linear with a slope of 0.0004, a y-intercept of -135.25 and R^2 of 0.9976.

Table 6-3. Mycometer®-test Linearity Data for *Cladosporium herbarum* ATCC 58927

Test Iteration	Dilution	Test Solution Concentration * (spores/mL)	Total Spores Tested**	Fluorescence Reading (fu)	Adjusted Fluorescence*** (fu)
1	Blank	Tap water	N/A	32	0
	NEAT	9.6×10^7	9.6×10^6	3454	3422
	1:5	1.9×10^7	1.9×10^6	598	566
	1:10	9.6×10^6	9.6×10^5	260	228
	1:20	4.8×10^6	4.8×10^5	174	142
2	Blank	Tap water	N/A	38	0
	NEAT	9.6×10^7	9.6×10^6	3490	3452
	1:5	1.9×10^7	1.9×10^6	526	488
	1:10	9.6×10^6	9.6×10^5	238	201
	1:20	4.8×10^6	4.8×10^5	165	127
3	Blank	Tap water	N/A	38	0
	NEAT	9.6×10^7	9.6×10^6	3375	3337
	1:5	1.9×10^7	1.9×10^6	513	476
	1:10	9.6×10^6	9.6×10^5	228	190
	1:20	4.8×10^6	4.8×10^5	154	116
4	Blank	Tap water	N/A	39	0
	NEAT	9.6×10^7	9.6×10^6	3371	3332
	1:5	1.9×10^7	1.9×10^6	523	484
	1:10	9.6×10^6	9.6×10^5	219	180
	1:20	4.8×10^6	4.8×10^5	165	126
5	Blank	Tap water	N/A	40	0
	NEAT	9.6×10^7	9.6×10^6	3442	3402
	1:5	1.9×10^7	1.9×10^6	481	441
	1:10	9.6×10^6	9.6×10^5	211	171
	1:20	4.8×10^6	4.8×10^5	154	114

*NEAT solution concentration measured from hemocytometer counts. Dilutions calculated by dividing the NEAT solution concentration by the dilution factor.
**Based on adding 0.10 mL of test solution to the enzyme substrate.
***Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading and is expressed in fluorescence units.
Fluorescence readings in the table are rounded to the nearest whole number, adjusted fluorescence calculations were based on actual measured results.

Table 6-4. Summary of Replicate Measurements for *C. herbarum* ATCC 58927 Mycometer®-test Linearity Data

Test Iteration	Adjusted Fluorescence (fu)			
	9.6 x 10^6 spores tested	1.9x 10^6 spores tested	9.6 x 10^5 spores tested	4.8 x 10^5 spores tested
1	3422	566	228	142
2	3452	488	201	127
3	3337	476	190	116
4	3332	484	180	126
5	3402	441	171	114
Average	3389	491	194	125
Standard Deviation	53	46	22	11
RSD (%)	1.6	9.3	11.4	8.9

Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading.

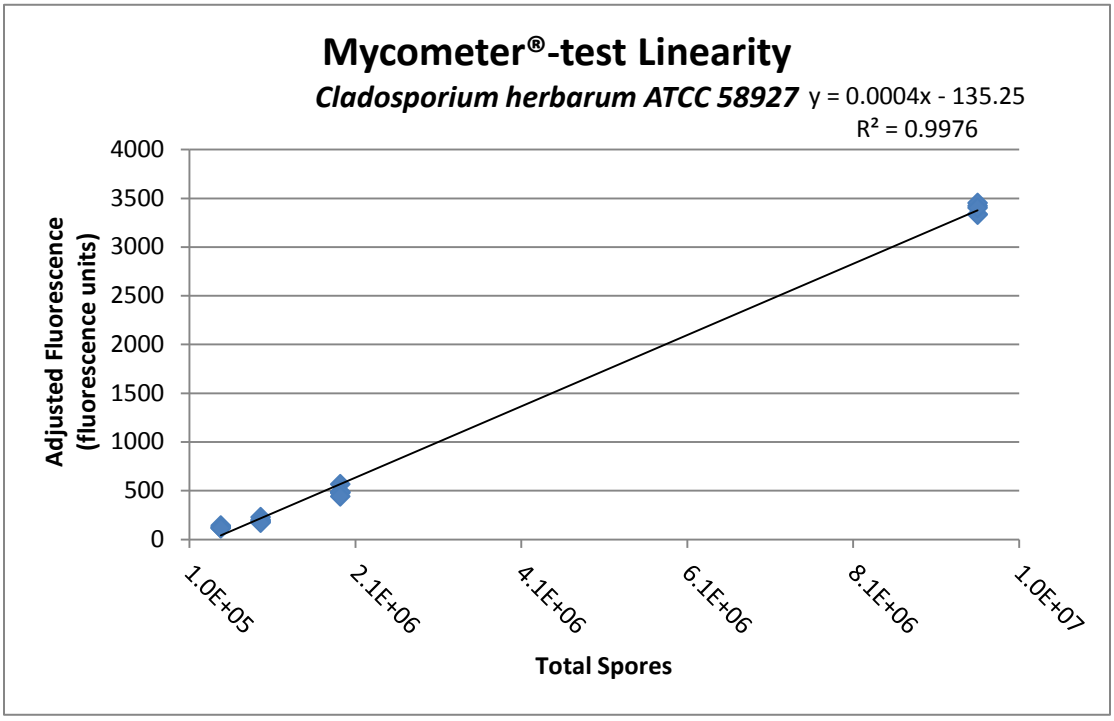

Figure 6-2. Plot of Mycometer®-test fluorescence response vs. *C. herbarum* spore counts

6.2.2 Repeatability

Table 6-5 shows the results of repeatability testing for eight *A. flavus* air samples collected in the ARCA chamber and processed using the Mycometer®-test by one analyst using one fluorometer. The approximate concentration of *A. flavus* in the air was 6.2 x 10^3 spores/L. The RSD for eight measurements was 8.0%. Eight background air samples before addition of *A. flavus* to the air are also included for reference.

Table 6-5. Mycometer®-test Repeatability: Air Samples Containing *A. flavus*

Test Iteration	Adjusted Fluorescence (fu)	
	A. Flavus	Background
1	316	1.9
2	313	-0.7
3	320	-1.8
4	309	2.7
5	343	4.0
6	390	2.9
7	348	4.6
8	334	3.1
Average	334	2.1
Standard Deviation	27	
RSD (%)	8.0	

Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading.

6.2.3 Inter-Assay Reproducibility

Table 6-6 shows the results of inter-assay reproducibility testing for eight *A. flavus* air samples collected in the ARCA chamber. The concentration of *A. flavus* in the air was approximately 6.2 x 10^3 spores/L. These eight samples were split into two sets of four for processing using the Mycometer®-test reagents by two analysts using two different fluorometers. The RSDs for four samples were 4.7 (Analyst 2) and 8.7% (Analyst 1); the RPD between analysts was 5.3%. Eight background air samples before the addition of *A. flavus* to the air were processed in the same way and are included for reference.

Table 6-6. Mycometer®-test Inter-Assay Reproducibility: Air Samples Containing *A. flavus*

Test Iteration	Adjusted Fluorescence (fu)			
	A. flavus		Background	
	Analyst 1	Analyst 2	Analyst 1	Analyst 2
1	298	314	-4.6	-1.4
2	320	294	-7.4	-2.8
3	288	325	-5.6	2.5
4	259	297	-7.4	-4.6
Average	291	307	-6.2	-1.6
Standard Deviation	25	15		
RSD (%)	8.7	4.7		
RPD (%)	5.3			

Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading.

6.2.4 Data Completeness

All of the Mycometer®-test data expected to be generated during testing was generated for a 100% data return.

6.2.5 Operational Factors

The verification staff found that the Mycometer®-test was easy to use. A Mycometer A/S representative came to Battelle to train the verification staff in the use of the Mycometer®-test and Bactiquant®-test reagents and operation of the fluorometer. This training lasted one day and staff felt it was more than sufficient to be comfortable using the reagent kits and fluorometer without assistance. This on-site training focused on the technology operating protocols for air and water matrices. While the operational aspects of this training were similar to the proficiency certification program noted in Chapter 2, the proficiency certification program also focuses on understanding the principles behind the technology as well as additional applications. The fluorometer is provided with the components listed in Table 6-7. The verification staff found the fluorometer and carrying case, described in Chapter 2, to be easy to transport. The fluorometer operates on four AAA batteries. The fluorometer was found to have push-button operation, a display that was easy to read, and surfaces that could be wiped clean. The fluorometer required a calibration check once daily with the black cuvette provided with the fluorometer and a calibration standard provided in the reagent kit. Both an instruction manual and a quick reference card were provided for the Mycometer®-test. Verification staff found that the instructions provided were not always consistent between the manual and the quick reference. For example, the manual indicated that the blank sample for air testing was to be a blank filter processed alongside the test filters, while the quick reference guide indicated that the blank was to be an aliquot of the substrate combined with the developer.

The Mycometer®-test reagents are sold in lots of 10 for air assays and lots of 20 for surface assays. Each reagent kit included the sampling media (filters for air samples), enzyme substrate, developer, and calibration standard, all of which were clearly labeled for identification and storage conditions. Syringes and cuvettes used for processing were also included. All containers and packaging were easy to open; however, verification staff found there was packaging waste involved with the different components, particularly if multiple kits were needed to analyze the required number of samples. All reagents were ready for use with the exception of the enzyme substrate that required re-hydration. Each sample resulted in approximately 5 mL of liquid waste from the substrate and developer used to process the sample. Based on the expiration date stamped on the kits, the shelf life of the kits received for testing was over one year from receipt date. Several kit components required refrigeration. Once rehydrated, the enzyme substrate could be stored in a refrigerator for up to one week or at -18 °C for up to 6 months. All components needed to prepare and analyze a sample were included either in the reagent kit or the fluorometer kit. Prices for the Mycometer®-test reagents and the fluorometer are available from the vendor. No other laboratory equipment was needed for processing air samples. For air sample collection, however, a sampling pump must be obtained. The recommended air sampling pumps (Gast 3-30 LPM IAQ Pump w/Tubing & Rotameter) are commercially available.

Verification staff found they were able to collect and analyze eight air samples in one hour given the availability of enough air sampling pumps to generate eight air samples simultaneously. For data reduction, a laptop or personal computer is needed. Mycometer provides an Excel spreadsheet for quantification of mold/fungi in air that converts fluorescence unit values into a "Mycometer-Air" value and provides suggested interpretation guidelines based on the resulting value obtained. The Mycometer-Air value calculation converts the fluorescence reading to fu per volume of air measured in cubic meters. The calculation is (sample fu – blank fu)/volume of air in cubic meters. This can be used to standardize the results for consistent comparison and interpretation if there are slight variations in the air volume sampled. Because all sample volumes used in verification testing were the same, conversion of results to a Mycometer-Air value were

not needed for verification testing. In addition, the interpretation guidelines associated with the Mycometer-Air values were not verified as part of this test.

Table 6-7. MYCOMETER™ Analysis Equipment Kit

Components	
1 Field fluorometer	1 Calculator
1 Black calibration cuvette	1 Thermometer – ambient air
1 Automatic pipette – 100 µL	1 Handbook
1 Field carrying case	1 Certification training flash drive
1 Timer	All batteries
2 Test racks	20 assays

6.3 Bactiquant®-test for Bacteria

6.3.1 Linearity

Tables 6-8 and 6-9 summarize the data obtained for Bactiquant®-test linearity testing with indigenous bacteria from lake water. Measurements were made by filtering 250 mL of solutions containing from 3.7×10^2 to 6.0×10^3 CFU/mL as measured by HPC and processing with the Bactiquant®-test reagents. Within this concentration range, replicate measurements of each testing solution had RSDs between 5.3 and 10.9%. Linearity was evaluated by plotting the lake water indigenous bacteria concentrations as the independent variable and Bactiquant®-test results expressed as adjusted fluorescence as the dependent variable. The adjusted fluorescence is the fluorescence reading of the sample minus the fluorescence reading of the blank. The blank was prepared using the blank reagents provided in the Bactiquant®-test kit by adding 0.35 mL of enzyme substrate to a cuvette containing the developer and processing it as a sample. The relationship between indigenous flora in the concentration range tested and adjusted fluorescence was linear with a slope of 3.72, a y-intercept of 3502 and R^2 of 0.9147.

Following this set of testing, the vendor provided information that a fluorescence reading greater than 20,000 fu may generate results that are not linear because the enzyme substrate concentration will have decreased significantly and the enzyme reaction will have slowed down. Therefore, linearity was also examined without the 1:5 dilution results since they were consistently greater than 20,000 fu. The relationship between concentration, using only the 1:10, 1:20, and 1:100 dilutions, and adjusted fluorescence was linear with a slope of 5.54, a y-intercept of 1153 and R^2 of 0.9692.

Since the vendor recommends reporting Bactiquant®-test results as BQ values, which standardize the fu results for reaction time, temperature, and sampling volume, the relationship between concentration and BQ value was also evaluated. Similar to the linearity results for adjusted fluorescence, the relationship between concentration and BQ value was linear in the concentration range tested with a slope of 2.38, a y-intercept of 2243 and R^2 of 0.9138 using all data points. Results using BQ values only from adjusted fluorescence responses less than 20,000 fu plotted against concentration are shown in Figure 6-3. Using only the 1:10, 1:20, and 1:100 dilutions, the relationship between concentration and BQ value was linear with a slope of 3.55, a y-intercept of 739 and R^2 of 0.9689.

Table 6-8. Bactiquant®-test Linearity Data for Lake Water Indigenous Bacteria

Test Iteration	Dilution	Test Solution Concentration * (CFU/mL)	Total CFU Tested**	Fluorescence Reading (fu)	Adjusted*** Fluorescence (fu)	BQ Value
1	Blank	N/A	N/A	109	0	N/A
	1:5	6.0×10^3	1.5×10^6	26240	26131	16647
	1:10	3.0×10^3	7.5×10^5	17676	17567	11191
	1:20	1.3×10^3	3.3×10^5	9768	9659	6153
	1:100	3.7×10^2	9.3×10^4	2546	2437	1552
2	Blank	N/A	N/A	116	0	N/A
	1:5	6.0×10^3	1.5×10^6	23550	23434	14852
	1:10	3.0×10^3	7.5×10^5	16894	16778	10633
	1:20	1.3×10^3	3.3×10^5	9463	9347	5924
	1:100	3.7×10^2	9.3×10^4	2707	2591	1642
3	Blank	N/A	N/A	112	0	N/A
	1:5	6.0×10^3	1.5×10^6	20267	20155	12908
	1:10	3.0×10^3	7.5×10^5	18537	18425	11800
	1:20	1.3×10^3	3.3×10^5	8621	8509	5449
	1:100	3.7×10^2	9.3×10^4	2447	2335	1495
4	Blank	N/A	N/A	113	0	N/A
	1:5	6.0×10^3	1.5×10^6	26693	26580	17111
	1:10	3.0×10^3	7.5×10^5	17981	17868	11503
	1:20	1.3×10^3	3.3×10^5	10492	10379	6682
	1:100	3.7×10^2	9.3×10^4	2151	2038	1312
5	Blank	N/A	N/A	107	0	N/A
	1:5	6.0×10^3	1.5×10^6	25632	25525	16518
	1:10	3.0×10^3	7.5×10^5	16184	16077	10404
	1:20	1.3×10^3	3.3×10^5	10147	10040	6497
	1:100	3.7×10^2	9.3×10^4	2747	2640	1708

*Solution concentrations measured from heterotrophic plate counts conducted in triplicate, except for 1:100 dilution. The 1:100 dilution concentration was based on the neat lake water heterotrophic plate count divided by the dilution factor of 100.
**Based on filtering 250 mL of test solution
***Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading and is expressed in fluorescence units.
N/A = not applicable
Fluorescence readings in the table are rounded to the nearest whole number, adjusted fluorescence calculations were based on actual measured results.

Table 6-9. Summary of Replicate Measurements for Lake Water Indigenous Bacteria Bactiquant®-test Linearity Data

Test Iteration	Adjusted Fluorescence (fu)			
	6.0 x 10^3 CFU/mL	3.0 x 10^3 CFU/mL	1.3 x 10^3 CFU/mL	3.7 x 10^2 CFU/mL
1	26131	17567	9659	2437
2	23434	16778	9347	2591
3	20155	18425	8509	2335
4	26580	17868	10379	2038
5	25525	16077	10040	2640
Average	24365	17343	9587	2408
Standard Deviation	2644	924	717	240
RSD (%)	10.9	5.3	7.5	10.0

Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading.

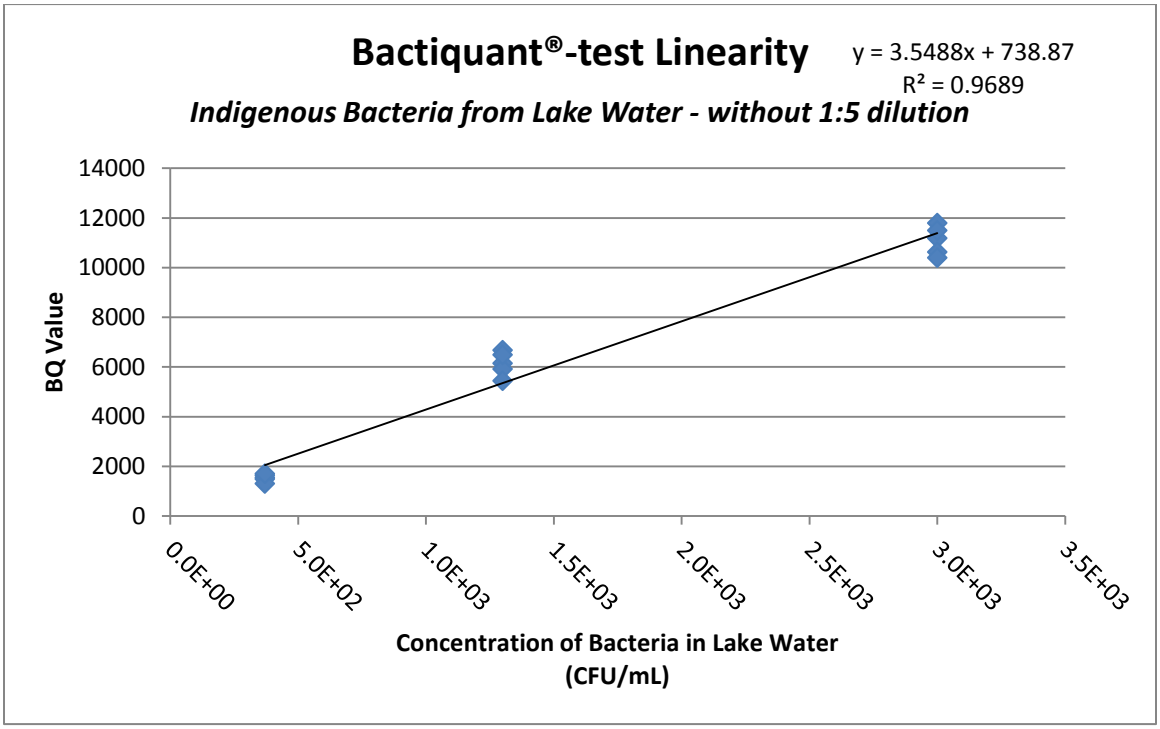

Figure 6-3. Plot of Bactiquant®-test BQ Values vs. Lake Water Indigenous Bacteria Concentration in CFU/mL – Responses less than 20,000 fu

Tables 6-10 and 6-11 summarize the data obtained for Bactiquant®-test linearity testing using the QC strain of *P. aeruginosa* ATCC 27853. Measurements were made by filtering 250 mL of solutions containing from 8.7 x 10^2 to 8.0 x 10^3 CFU/mL as measured by HPC and processing with the Bactiquant®-test reagents. Within this range, replicate measurements of each testing solution had RSDs between 3.2 and 6.3%. In the concentration range tested, the relationship between concentration and adjusted fluorescence was linear with a slope of 1.45, a y-intercept of -207 and R^2 of 0.9923.

Figure 6-4 shows the plot of *P. aeruginosa* bacteria concentrations as the independent variable and Bactiquant®-test results expressed as BQ values as the dependent variable. For the concentration range tested, the relationship between concentration and BQ value was linear with a slope of 0.95, a y-intercept of -136 and R^2 of 0.9923.

Table 6-10. Bactiquant®-test Linearity Data for *Pseudomonas aeruginosa* ATCC 27853

Test Iteration	Dilution	Test Solution Concentration * (CFU/mL)	Total CFU Tested**	Fluorescence Reading (fu)	Adjusted *** Fluorescence (fu)	BQ Value
1	Blank	N/A	N/A	126	0	N/A
	starting solution	8.0×10^3	2.0×10^6	11518	11392	7489
	1:2	4.7×10^3	1.2×10^6	6254	6128	4028
	1:5	2.1×10^3	5.3×10^5	2925	2799	1840
	1:10	8.7×10^2	2.2×10^5	1375	1249	821
2	Blank	N/A	N/A	109	0	N/A
	starting solution	8.0×10^3	2.0×10^6	12440	12332	8106
	1:2	4.7×10^3	1.2×10^6	6182	6074	3992
	1:5	2.1×10^3	5.3×10^5	2818	2710	1781
	1:10	8.7×10^2	2.2×10^5	1355	1247	819
3	Blank	N/A	N/A	110	0	N/A
	starting solution	8.0×10^3	2.0×10^6	11485	11376	7478
	1:2	4.7×10^3	1.2×10^6	6121	6012	3952
	1:5	2.1×10^3	5.3×10^5	2668	2559	1682
	1:10	8.7×10^2	2.2×10^5	1406	1297	852
4	Blank	N/A	N/A	111	0	N/A
	starting solution	8.0×10^3	2.0×10^6	11624	11513	7568
	1:2	4.7×10^3	1.2×10^6	6619	6508	4278
	1:5	2.1×10^3	5.3×10^5	2879	2768	1820
	1:10	8.7×10^2	2.2×10^5	1548	1437	945
5	Blank	N/A	N/A	118	0	N/A
	starting solution	8.0×10^3	2.0×10^6	11732	11614	7635
	1:2	4.7×10^3	1.2×10^6	6374	6256	4113
	1:5	2.1×10^3	5.3×10^5	3063	2945	1936
	1:10	8.7×10^2	2.2×10^5	1493	1375	904

*Solution concentrations measured from heterotrophic plate counts conducted in triplicate.
**Based on filtering 250 mL of test solution
***Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading and is expressed in fluorescence units.
N/A = not applicable
Fluorescence readings in the table are rounded to the nearest whole number, adjusted fluorescence calculations were based on actual measured results.

Table 6-11. Summary of Replicate Measurements for *P. aeruginosa* ATCC 27853 Linearity Data – Adjusted Fluorescence

Test Iteration	Adjusted Fluorescence (fu)			
	8.0 x 10^3 CFU/mL	4.7 x 10^3 CFU/mL	2.1 x 10^3 CFU/mL	8.7 x 10^2 CFU/mL
1	11392	6128	2799	1249
2	12332	6074	2710	1247
3	11376	6012	2559	1297
4	11513	6508	2768	1437
5	11614	6256	2945	1375
Average	11645	6196	2756	1321
Standard Deviation	396	196	140	83
RSD (%)	3.4	3.2	5.1	6.3

Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading.

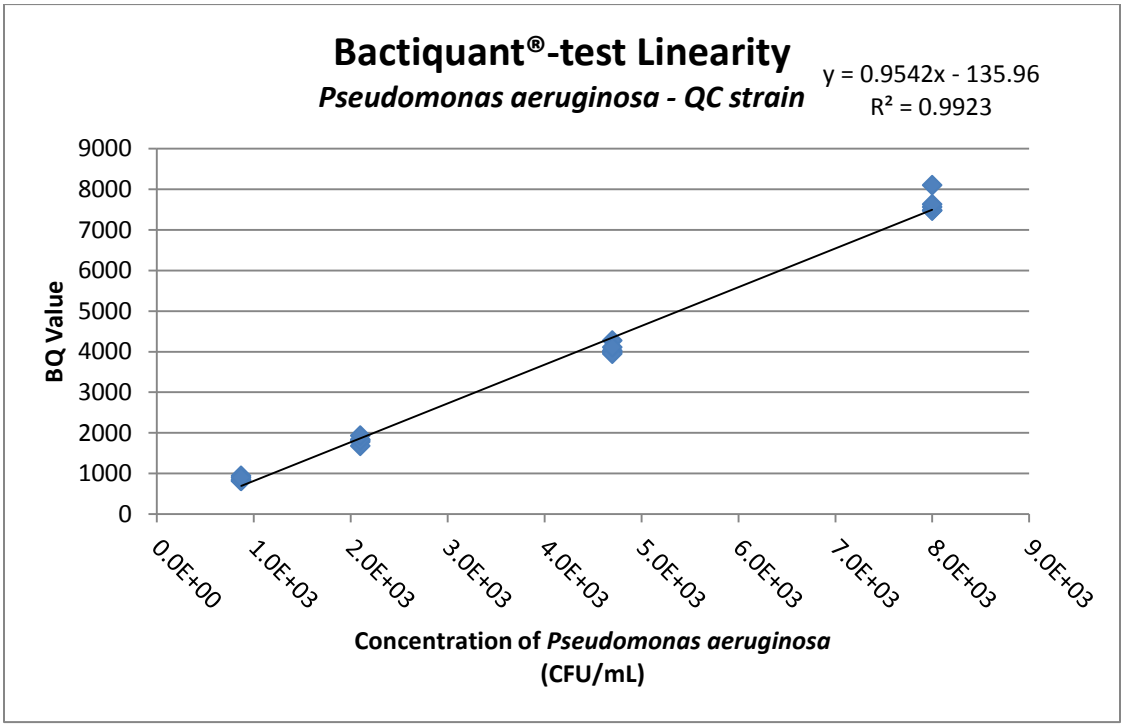

Figure 6-4. Plot of Bactiquant®-test BQ Values vs. *P. aeruginosa* Concentration in CFU/mL

6.3.2 Repeatability and Inter-Assay Reproducibility

Tables 6-12 and 6-13 show the results of repeatability and inter-assay reproducibility testing with indigenous bacteria from lake water and the QC strain of *P. aeruginosa*, respectively. For both tests, two analysts each processed four water samples containing the bacteria using the Bactiquant®-test reagents and separate fluorometers. The RSDs for four samples of indigenous bacteria from lake water were 2.6% (Analyst 2) and 6.4% (Analyst 1); the RPD between analysts was 6.0%. For *P. aeruginosa*, the RSDs for four samples were 1.4% (Analyst 2) and 4.8% (Analyst 1); the RPD between analysts was 2.9%.

Table 6-12. Bactiquant®-test Repeatability and Inter-Assay Reproducibility: Indigenous Bacteria from Lake Water

Test Iteration	Adjusted Fluorescence (fu)	
	Indigenous Bacteria from Lake Water (3.7×10^2 CFU/mL)	
	Analyst 1	Analyst 2
1	2520	2284
2	2397	2218
3	2156	2247
4	2379	2149
Average	2363	2225
Standard Deviation	152	57
RSD (%)	6.4	2.6
RPD (%)	6.0	

Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading.

Table 6-13. Bactiquant®-test Repeatability and Inter-Assay Reproducibility: P. aeruginosa ATCC 27853

Test Iteration	Adjusted Fluorescence (fu)	
	P. aeruginosa ATCC 27853 (4.7×10^3 CFU/mL)	
	Analyst 1	Analyst 2
1	6830	6689
2	6618	6567
3	7370	6791
4	6736	6717
Average	6888	6691
Standard Deviation	333	93
RSD (%)	4.8	1.4
RPD (%)	2.9	

Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading.

6.3.3 Data Completeness

All of the Bactiquant®-test data expected to be generated during testing was generated for a 100% data return.

6.3.4 Operational Factors

The verification staff found that the Bactiquant®-test was easy to use. The fluorometer used with Bactiquant®-test is identical to that used with Mycometer®-test and both the fluorometer and traning provided by the vendor is discussed in Section 6.2.5. For the Bactiquant®-test, an instruction manual, a photo manual, and a quick reference card were provided. Verification staff found that the instructions provided were not always consistent among all three references and would have been confusing on ocassion had they not had training. Per the vendor's instruction manual, the fluorometer required a calibration check with each series of measurement with the black cuvette provided with the fluorometer and a calibration standard provided in the reagent kit.

The Bactiquant®-test reagents are sold in lots of five for water assays. Each reagent kit included the sampling filter, enzyme substrate, developer, and calibration standard, all of which were clearly labeled for identification and storage conditions. Syringes and cuvettes used for processing were also included. All containers and packaging were easy to open; however, verification staff found there was packaging waste involved with the different components, particularly if multiple kits were needed to analyze the required number of samples. All reagents were ready for use. Each sample resulted in approximately 5 mL of liquid waste from the substrate and developer used to process the sample plus 250 mL of spent sample. Based on the expiration date stamped on the kits, the shelf life of the kits received for testing was over one year from receipt date. Several kit components required refrigeration. All components needed to prepare and analyze a sample were included either in the reagent kit or the fluorometer kit.

Prices for the Bactiquant®-test reagents and the fluorometer are available from the vendor. No other laboratory equipment was needed for processing samples; however, for collection of water samples a vacuum manifold and pump were needed for filtering the 250 mL samples. Both manual and automated filtration apparatus are available through the vendor. For verification testing, only the manual filtration apparatus was used. Verification staff found they were able to collect and analyze ten water samples in one hour using a five sample manifold to simultaneously filter five samples. For data reduction, a laptop or personal computer is needed. Mycometer provides an Excel spreadsheet for quantifying bacteria in water that converts fluorescence unit values into a BQ value as described in Section 3.3.2.1 and provides suggested interpretation guidelines based on the resulting BQ value obtained. These interpretation guidelines were not verified as part of this test.

Chapter 7
Performance Summary

Rapid technologies (results available same day of testing) to detect fungi and bacteria from matrices such as surfaces, bulk material, air, or water are of interest to improve the efficiency of delineating and documenting microbial contamination in buildings and water systems, and for monitoring progress during cleanup and remediation processes. Traditional methods of analysis can take up to seven days for results. Technologies providing same day or near "real-time" results indicating changes in water or air quality would help to control microbial outbreaks, expedite remediation efforts, and protect public health. Therefore, for the purpose of this verification, the Mycometer®-test and Bactiquant®-test technologies developed by Mycometer A/S were verified for repeatability and inter-assay reproducibility by detecting fungi in air samples and bacteria in water samples, respectively. Linearity was assessed for both technologies using dilutions of stock cultures in tap water. The linearity test for fungi was a modification of test procedures in place for air and surface samples. In addition, sustainable operational factors such as ease of use, required reagents, analysis time, laboratory space, and utilities required are reported. The results of the verification of the Mycometer®-test and Bactiquant®-test technologies are summarized below:

7.1 Results for Mycometer®-test

Table 7-1 summarizes the linearity results for Mycometer®-test using two fungal cultures in water, *Aspergillus flavus* ATCC 58870 and *Cladosporium herbarum* ATCC 58927.

Table 7-1. Linearity Results for Mycometer®-test Adjusted Fluorescence vs. Total Spores Tested

Test Organism	Total Spores Tested	Range of Average Adjusted Fluorescence (fu)	Slope	Y-intercept	Coefficient of Determination (R^2)
A. flavus ATCC 58870	3.1×10^5 to 6.2×10^6	218 to 4322	0.0007	20.637	0.9979
C. herbarum ATCC 58927	4.8×10^5 to 9.6×10^6	125 to 3389	0.0004	-135.25	0.9976

Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading.

Table 7-2 summarizes the repeatability results for Mycometer®-test using eight replicates of one fungal culture in air, all analyzed by one person.

Table 7-2. Mycometer®-test Repeatability: Air Containing *A. Flavus*

Test Iteration	Adjusted Fluorescence (fu)
	A. flavus (6.2×10^3 spores/L) n=8
Average	334
Standard Deviation	27
RSD (%)	8.0

Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading.

Table 7-3 summarizes the inter-assay reproducibility results for Mycometer®-test using eight replicates of one fungal culture in air split into four samples each for analysis by two people with two different fluorometers.

Table 7-3. Mycometer®-test Inter-Assay Reproducibility: Air Containing *A. Flavus*

Test Iteration	Adjusted Fluorescence (fu)	
	A. flavus (6.2×10^3 spores/L) n=4	
	Analyst 1	Analyst 2
Average	291	307
Standard Deviation	25	15
RSD (%)	8.7	4.7
RPD (%)	5.3	

Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading.

Operational Factors. The verification staff found that the Mycometer®-test was easy to use. A Mycometer A/S representative came to Battelle to train the verification staff in the use of the Mycometer®-test and Bactiquant®-test reagents and operation of the fluorometer. This training lasted one day and staff felt it was more than sufficient to be comfortable using the reagent kits and fluorometer without assistance. This on-site training focused on the technology operating protocols for air and water matrices. While the operational aspects of this training were similar to the proficiency certification program noted in Chapter 2, the proficiency certification program also focuses on understanding the principles behind the technology as well as additional applications.

The fluorometer is provided in a hard-cover carrying case. The carrying case has dimensions of 45 cm wide × 15 cm deep × 32 cm high (17.5 in wide × 6 in deep × 12.5 in high) and weighs approximately 7.2 kilograms (16 pounds). Included with the fluorometer is a black calibration cuvette, a 100 µL automatic pipette, a timer, two test racks, a calculator, a thermometer, and training materials. The fluorometer operates on four AAA batteries and has push-button operation. Testing staff found that the display was easy to read and surfaces were easy to wipe clean. The fluorometer required a calibration check once daily with the black cuvette provided with the fluorometer and a calibration standard provided in the reagent kit. Both an instruction manual and a quick reference card were provided for the Mycometer®-test. Verification staff found that the instructions provided were not always consistent between the manual and the quick reference. For example, the manual indicated that the blank sample for air testing was to be a

blank filter processed alongside the test filters, while the quick reference guide indicated that the blank was to be an aliquot of the substrate combined with the developer.

The Mycometer®-test reagents are sold in lots of 10 for air assays and lots of 20 for surface assays. Each reagent kit included the sampling media (filter for air samples), enzyme substrate, developer, and calibration standard, all of which were clearly labeled for identification and storage conditions. Syringes and cuvettes used for processing were also included. All containers and packaging were easy to open; however, verification staff found there was packaging waste involved with the different components, particularly if multiple kits were needed to analyze the required number of samples. All reagents were ready for use with the exception of the enzyme substrate that required re-hydration. Each sample resulted in approximately 5 mL of liquid waste from the substrate and developer used to process the sample. Based on the expiration date stamped on the kits, the shelf life of the kits received for testing was over one year from receipt date. Several kit components required refrigeration. Once rehydrated, the enzyme substrate could be stored in a refrigerator for up to one week or at -18 °C for up to 6 months. All components needed to prepare and analyze a sample were included either in the reagent kit or the fluorometer kit. No other laboratory equipment was needed for processing air samples. For air sample collection, however, a sampling pump must be obtained. The recommended air sampling pumps (Gast 3-30 LPM IAQ Pump w/Tubing & Rotameter) are commercially available. Verification testing staff found they were able to collect and analyze eight air samples in one hour given the availability of enough air sampling pumps to generate eight air samples simultaneously.

For data reduction, a laptop or personal computer is needed. Mycometer provides an Excel spreadsheet for quantification of mold/fugi in air that converts fluorescence unit values into a "Mycometer-Air" value and provides suggested interpretation guidelines based on the resulting value obtained. The Mycometer-Air value calculation converts the fluorescence reading to fu per volume of air measured in cubic meters. This can be used to standardize the results for consistent comparison and interpretation if there are slight variations in the air volume sampled. Because all sample volumes used in verification testing were the same, conversion of results to a Mycometer-Air value were not needed for verification testing. In addition, the interpretation guidelines associated with the Mycometer-Air values were not verified as part of this test.

7.2 Results for Bactiquant®-test

Table 7-4 summarizes the linearity results for Bactiquant®-test using two types of bacteria in water: indigenous bacteria from lake water and a QC strain of *Pseudomonas aeruginosa* ATCC 27853. In Table 7-4, linearity is evaluated for Bactiquant BQ values (fluorescence unit readings standardized for reaction time, temperature and sample volume) against concentration. During the lake water indigenous bacteria test, the vendor provided information that fluorescence readings above 20,000 fu may generate results that are no longer linear because the enzyme substrate concentration will have decreased significantly and the enzyme reaction will slow down. Therefore, lake water indigenous bacteria linearity data was examined both with and without the most concentrated test solution results since they were consistently greater than 20,000 fu.

Table 7-4. Bactiquant®-test Linearity: BQ Value vs. Concentration

Test Organism	Concentration Range (CFU/mL)	Range of Average Adjusted Fluorescence (fu)	Range of Average BQ values	Slope	Y-intercept	Coefficient of Determination (R^2)
Lake Water Indigenous Bacteria - with all test solutions	3.7×10^2 to 6.0×10^3	2408 to 24365	1542 to 15607	2.38	2243	0.9138
Lake Water Indigenous Bacteria- without the most concentrated test solution	3.7×10^2 to 3.0×10^3	2408 to 17343	1542 to 11106	3.55	739	0.9689
P. aeruginosa ATCC 27853	8.7×10^2 to 8.0×10^3	1321 to 11645	868 to 7655	0.95	-136	0.9923

Table 7-5 summarizes the repeatability and inter-assay reproducibility results for Bactiquant®-test using two bacterial cultures in water. Two different people analyzed four samples of each bacterial culture, using different fluorometers.

Table 7-5. Bactiquant®-test Repeatability and Inter-Assay Reproducibility

Test Iteration	Adjusted Fluorescence (fu)			
	Indigenous Bacteria from Lake Water (3.7×10^2 CFU/mL)		*P. aeruginosa* ATCC 27853 (4.7×10^3 CFU/mL)	
	Analyst 1	Analyst 2	Analyst 1	Analyst 2
Average	2363	2225	6888	6691
Standard Deviation	152	57	333	93
RSD (%)	6.4	2.6	4.8	1.4
RPD (%)	6.0		2.9	

Adjusted fluorescence = sample fluorescence reading – blank fluorescence reading.

Operational Factors. The verification staff found that the Bactiquant®-test was easy to use. The fluorometer used and training provided by the vendor are the same as that described in Section 7.1 for Mycometer®-test. For the Bactiquant®-test, an instruction manual, a photo manual, and a quick reference card were provided. Verification staff found that the instructions provided were not always consistent among all three references and would have been confusing on ocassion had they not had training. Per the vendor's instruction manual, the fluorometer required a calibration check with each series of measurements using the black cuvette provided with the fluorometer and a calibration standard provided in the reagent kit.

Bactiquant®-test reagents are sold in lots of five for water assays. Each reagent kit included the sampling filter, enzyme substrate, developer, and calibration standard, all of which were clearly labeled for identification and storage conditions. Syringes and cuvettes used for processing were also included. All containers and packaging were easy to open; however, verification staff found

there was packaging waste involved with the different components, particularly if multiple kits were needed to analyze the required number of samples. All reagents were ready for use. Each sample resulted in approximately 5 mL of liquid waste from the substrate and developer used to process the sample plus 250 mL of spent sample. Based on the expiration date stamped on the kits, the shelf life of the kits received for testing was over one year from receipt date. Several kit components required refrigeration. All components needed to prepare and analyze a sample were included either in the reagent kit or the fluorometer kit. No other laboratory equipment was needed for processing samples; however, for collection of water samples a vacuum manifold and pump were needed for filtering the 250 mL samples. Both manual and automated filtration apparatus are available through the vendor. For verification testing, only manual filtration apparatus was used. Verification testing staff found they were able to collect and analyze ten water samples in one hour using a five sample manifold to simultaneously filter five samples.

For data reduction, a laptop or personal computer is needed. Mycometer provides an Excel spreadsheet for quantifying bacteria in water that converts fluorescence unit values into a BQ value that standardizes the fluorescence unit readings for reaction time, temperature and sample volume. Mycometer also provides suggested interpretation guidelines based on the resulting BQ values obtained. These interpretation guidelines were not verified as part of this test.

Chapter 8
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Appendix
Data from Tap Water Analyses

Table A1-1. Water Quality Parameters for Characterizing Columbus, Ohio Tap Water Used for Testing

Analysis	Method/ (reporting unit)	May 19, 2011 Mycometer®-test Linearity	June 2, 2011 Bactiquant®-test Linearity, Repeatability, Inter-Assay Reproducibility - Indigenous Lake Water	June 27, 2011 Bactiquant®-test Linearity, Repeatability, Inter-Assay Reproducibility – QC Strain *P. aeruginosa*
Turbidity*	EPA Method 180.1[14]/ (NTU)	<1	<1	<1
Total Organic Carbon*	SM 5310-C[13]/ (mg/L)	2.6	2.8	2.1
Specific Conductivity*	SM 2510[13]/ µmhos/cm	450	340	470
Alkalinity*	SM 2320-B[13]/ (mg/L)	50.6	48.0	50.9
Hardness*	SM 2340-B[13]/ (mg/L)	116	111	112
Dissolved Oxygen*	SM 4500-O[13]/ (mg/L)	9.0	9.7	9.5
pH before/after dechlorination	Battelle SOP GEN.V-003**/ (pH units)	6.66/6.97	7.93/8.01	7.46/7.35
Free Chlorine before/after dechlorination	HACH Method 8021[15]/(mg/L)	1.16/0.15	1.19/0.15	1.42/0.17
Total Chlorine before/after dechlorination	HACH Method 8167[16] /(mg/L)	1.48/0.14	1.52/0.12	1.64/0.14
Heterotrophic Plate Counts	SM 9215[13]/ (CFU/mL)	20[1]	20[1]	30[1]
Fluorescence Reading	Mycometer®-test on 5/19 and Bactiquant®-test on 6/2 (fu)	38.8[2]	23.2[3]	N/A

*Analyses provided by Pace Analytical
**Battelle Standard Operating Procedure: GEN. V-003 Standard Operating Procedure for the Use of pH Meters to Measure pH
[1] Estimated value due to low number of counts
[2]Average of eight measurements made by processing 100 µL of dechlorinated tap water with the same reagents as the test samples
[3] Dechlorinated tap water (250 mL) filtered and processed with the same reagents as the test samples
N/A = not applicable