THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

Battelle
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ETV Verification Statement

TECHNOLOGY TYPE:	**RAPID BACTERIA DETECTION**	
APPLICATION:	**ANALYSIS OF BACTERIA IN WATER**	
TECHNOLOGY NAME:	**Bactiquant®-test**	
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The U.S. Environmental Protection Agency (EPA) has established the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved 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. Information and ETV documents are available at www.epa.gov/etv.

ETV works in partnership with recognized standards and testing organizations, with stakeholder groups (consisting of buyers, vendor organizations, and permitters), and with 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 and 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 Advanced Monitoring Systems (AMS) Center, one of six verification centers under ETV, is operated by Battelle in cooperation with EPA's National Risk Management Research Laboratory. The AMS Center evaluated the performance of a rapid bacteria detection technology. This verification statement provides a summary of the test results for the Bactiquant®-test developed by Mycometer A/S and distributed in the United States by Mycometer, Inc.

VERIFICATION TEST DESCRIPTION

Rapid technologies (results available same day of testing) to detect 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 quality would help to control microbial outbreaks, expedite remediation efforts, and protect public health.

The verification test of the Bactiquant®-test technology was conducted from June 2 through June 27, 2011 at Battelle in Columbus, Ohio. Technology operation, sample handling, and analyses were performed according to the vendor's instructions.

For this verification, the Bactiquant®-test technology was verified for repeatability and inter-assay reproducibility by detecting bacteria in water samples. Linearity was assessed using dilutions of stock cultures in dechlorinated tap water. In addition, sustainable operational factors such as ease of use, required reagents, analysis time, laboratory space, and utilities required were reported.

QA oversight of verification testing was provided by Battelle and EPA. Battelle and EPA QA staff conducted technical systems audits of the testing and Battelle QA staff conducted a data quality audit of at least 10% of the test data. This verification statement, the full report on which it is based, and the quality assurance project plan for this verification test are available at www.epa.gov/etv/centers/center1.html.

TECHNOLOGY DESCRIPTION

According to the vendor, Bactiquant®-test is designed to provide a rapid method to estimate total bacteria in water samples. The Bactiquant®-test rapid bacteria detection technology is based on fluorogenic detection of enzyme activities found predominantly in a taxonomic group of organisms. Water samples (typically 250 mL) are passed through a membrane filter. A synthetic enzyme substrate is added to the filter and left to react over a period of time based on temperature. The enzyme present in the bacterial cells hydrolyzes the synthetic enzyme substrate. When the synthetic substrate molecule is cleaved into two molecules by the enzyme, one of the molecules can be made to fluoresce upon excitation with ultraviolet (UV) light (365 nanometers). The amount of fluorescence is measured using a handheld fluorometer. This fluorescence semi-quantitatively correlates to a measure of the bacterial biomass. Fluorescence measurements can be captured electronically and may be downloaded to a computer or can be transcribed by hand. The sample preparation and analyses can be performed on site in less than one hour.

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 used for surface and air samples. For Bactiquant®-test, the filter material is critical for both sampling and the enzyme reaction that takes place directly on the filter. It is important, therefore, 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.

VERIFICATION RESULTS

Table 1 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 1 linearity is evaluated for Bactiquant BQ values against concentration. The BQ values are fluorescence unit (fu) readings standardized for reaction time, temperature, and sample volume. During the lake water indigenous bacteria test, the vendor provided information that fluorescence readings above 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, the linearity data for indigenous bacteria from lake water were

Table 1. Linearity Results for Bactiquant®-test: BQ Value vs. Concentration

Test Organism	Concentration Range (CFU/mL)	Range of Average Adjusted Fluorescence Units (fu)	Range of Average BQ values	Slope	Y-intercept	Coefficient of Determination (R^2)
Lake Water Indigenous Bacteria- with all test solutions	3.7 x 10^2 to 6.0 x 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 x 10^2 to 3.0 x 10^3	2408 to 17343	1542 to 11106	3.55	739	0.9689
P. aeruginosa ATCC 27853	8.7 x 10^2 to 8.0 x 10^3	1321 to 11645	868 to 7655	0.95	-136	0.9923

examined both with and without the most concentrated test solution that had fluorescence readings consistently greater than 20,000 fu.

Table 2 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 2. Bactiquant®-test Repeatability and Inter-Assay Reproducibility

Test Iteration	Adjusted Fluorescence Units (fu)			
	Indigenous Bacteria from Lake Water (3.7 x 10^2 CFU/mL)		*P. aeruginosa* ATCC 27853 (4.7 x 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	

Operational Factors. The verification staff found that the Bactiquant®-test was easy to use. A Mycometer A/S representative came to Battelle to train the verification staff in the use of the 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, 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. 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.

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. To process 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. Pricing for the fluorometer, reagent kits and filteration apparatus can be obtained from the provided vendor contacts. 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 quantification of 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.

Signed by Tracy Stenner 01/06/2012	Signed by Cynthia Sonich-Mullin 02/01/2012
Tracy Stenner Date	Cynthia Sonich-Mullin Date
Manager	Director
Environmental Solutions Product Line	National Risk Management Research Laboratory
Energy, Environment, and Material Sciences	Office of Research and Development
Battelle	U.S. Environmental Protection Agency