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THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

ETV Joint Verification Statement

TECHNOLOGY TYPE:	**COLIFORM DETECTION**
APPLICATION:	**ANALYSIS OF *E. COLI* IN DRINKING WATER**
TECHNOLOGY NAME:	**ENDETEC TECTA™ B-16**
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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 system for coliform detection in drinking water (DW). This verification statement provides a summary of the test results for Pathogen Detection System, Inc.'s ENDETEC TECTA™ B-16.

VERIFICATION TEST DESCRIPTION

In February 2013, EPA revised the 1989 Total Coliform Rule (TCR), a national primary drinking water regulation (NPDWR). The revised rule establishes a maximum contaminant level goal (MCLG) of zero for E. coli (EC), a more specific indicator of fecal contamination and potential harmful pathogens than total coliform. EPA has removed the 1989 MCLG and maximum contaminant level for total coliform. In the revised TCR, total coliforms serve as an indicator of a potential pathway of contamination into the distribution system. A PWS that exceeds a specified frequency of total coliform occurrence must conduct an assessment to determine if any sanitary defects exist and, if found, correct them. In order to comply with the revised TCR (RTCR), water utilities need coliform detection technologies that are able to detect EC at concentrations of one colony forming unit (CFU) per 100 milliliters (mL). While it is difficult to determine if a single target organism is present in 100 mL of water, when approximately half of the analyzed replicates are positive and half are negative, the density of the organism has become adequately low so that a positive result can be considered single organism detection. Therefore, for the purpose of this verification, the objective was to prepare spiked DW dilution sets that provided 50 ±25% positive results for EC with the Colilert-18 reference method and then confirm the results from the TECTA™ B-16 (through analysis of the spent media from the initial samples) with the Colilert-18 reference method.

The verification test of the TECTA™ B-16 was conducted from August 21 through August 23, 2013 at the City of Columbus Division of Water (CDW) laboratory in Columbus, Ohio with the reference method analyses being performed at Superior Laboratories in Galloway, Ohio (which is a 15 minute drive from the CDW laboratory). Technology operation and sample handling and analysis were performed according to the operating documentation and method description provided by the vendor. Both reference method and TECTA B-16 sample analysis results were reported in presence/absence format, consistent with the requirements of the RTCR.

Sample analysis results from the TECTA B-16 were evaluated by calculating the true positive (and true negative) results through confirmation analyses using Colilert-18 as described above. These calculations include the comparison of false positive rate (or specificity) and false negative rate (or sensitivity). In addition, statistical testing was performed on the initial reference method and TECTA B-16 results. Sustainable operational factors such as ease of use, required reagents, analysis time, and laboratory space and utilities required are 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 25% of the test data. This verification statement, the full report on which it is based, and the test/QA plan for this verification test are all available at www.epa.gov/etv/centers/center1.html.

TECHNOLOGY DESCRIPTION

The TECTA B-16 is a bench top detection and data logging system for the analysis of TC and EC in water samples. It utilizes an enzyme substrate test to simultaneously detect the presence of TC (β-galactosidase enzyme) and EC (β-glucuronidase enzyme). The system consists of single-use cartridges that contain pre-measured reagents and an embedded optical sensor. A 100 mL water sample is added to the cartridge and then up to 16 of the cartridges are incubated in and analyzed by the TECTA B-16.

The enzymes produced by TC and EC bacteria cleave the fluorogenic substrates in the growth media, resulting in the release of fluorescent products. The fluorescent product molecules rapidly accumulate in an optical sensor formed from a polymeric material embedded at the center of the test cartridge base, which is continuously illuminated by an ultraviolet light source in the bottom of each TECTA B-16 sample chamber. The light emitted by the polymer when fluorescent indicator products are present is detected at wavelengths specific to each fluorescent product that are in turn specific to detection of TC and EC bacteria. Optical detection is performed automatically by a charge-coupled device. Test management software within the TECTA B-16 interprets these optical signals continuously throughout the test cycle, and provides an alert of a positive sample detection for both EC and TC as soon as a threshold level of fluorescence is detected.

Samples not displaying detectable fluorescence at the wavelengths of interest are determined to be absent of bacteria after 18 hours. Due to its continuous monitoring capability, positive sample results can be detected in less than 18 hours.

In continous mode, the TECTA B-16 can analyze up to 16 samples in 18 hours (h). The vendor provided three units for testing, providing simultaneous sample analysis capacity of 48 samples. The TECTA B-16 can also be operated in read mode where the detector in the sample chamber can be used to make an instantaneous measurement of light emission from the polymer. Read mode can be used to confirm results obtained in continuous mode or if a sample cartridge has been incubated under appropriate conditions outside of the TECTA B-16. In read mode, the results display on the screen in the same way as for the continuous measurements, except that a "time-to-result" indication is not available for samples processed in read mode.

VERIFICATION RESULTS

Positive Results. The positive TC and EC test results for the TECTA B-16 and reference method (Colilert-18) are presented in Table 1. One of the two dilutions (0.5 CFU/100 mL) yielded the target 50 ± 25% split in responses from the reference method. The other dilution generated results that were 100% positive.

Table 1. Results Summary for Positive TECTA B-16 Results for TC and EC

Dilution (target concentration)	TECTA B-16 TC and EC		Colilert-18	
	N	% of total samples	N	% of total samples
A (5 CFU/100 mL)	20	100%	20	100%
B (0.5 CFU/100 mL)	6	30%	11	55%

Because the reference method results were between 25% and 75% positive, the reference method results suggested that the 0.5 CFU/100 mL solutions prepared for the evaluation were at the single organism per 100 mL concentration level. Therefore the TECTA B-16 results were able to be used along with the reference method confirmation data to determine the effectiveness of the TECTA B-16 in detecting such low concentrations. Specifically, following analysis using the TECTA B-16, 1 mL of the resulting suspension was inoculated into 99 mL of sterilized water and analyzed by the reference method. The result of these analyses provided confirmation of the presence/absence results for each replicate sample. Table 2 summarizes the confirmed true positive and true negative TC and EC results for the TECTA B-16.

Table 2. TECTA B-16 True Positive and Negative Results Summary

Dilution (target concentration)	N	Confirmed (True Positive)	Confirmed (True Negative)	False Results
A (5 CFU/100 mL)	20	20	0	0
B (0.5 CFU/100 mL)	20	6	14	0

N= Number of replicates

Sensitivity, Specificity, False Positive (FP) Rate, False Negative (FN) Rate. Table 3 summarizes the specificity, sensitivity, FP rate, and FN rate for TC and EC for 18 and 24 h incubations. Sensitivity is defined as the percent of positive samples correctly identified as positive and specificity is defined as the percent of negative samples correctly identified as negative. For the 0.5 CFU/100 mL sample, the sensitivity and specificity of the TECTA B-16 was 100% with no false positives or false negatives. For the 5 CFU/100 mL samples, the sensitivity was also 100% (with no false negatives. For both concentrations, the results were all confirmed by the reference method.

Table 3. Results Summary of TECTA B-16

Incubation Time (h)	0.5 CFU/100 mL	5 CFU/100 mL
Sensitivity	100%	100%
Specificity	100%	NA
False Positive	0%	NA
False Negative	0%	0%

NA – not applicable because zero in denominator of calculation

Comparability. In another approach of comparison, a chi-square test for independence was performed to compare the TECTA B-16 against the reference method (Colilert-18). Because only the 0.5 CFU/100 mL dilution had both positive and negative results, the chi-squared analysis was only performed for that solution. This analysis generated a p-value that was greater than 0.05 indicating that the TECTA B-16 results were not significantly different from the initial Colilert-18 results (at the 95% confidence level), a result that is consistent with the confirmatory analyses described above (which indicated identical results between the TECTA B-16 and Colilert-18 confirmatory analysis of each TECTA B-16 replicate).

Additional Concentrations in Continuous Operation. The objective of this component of the testing was to verify the TECTA B-16 capability of reporting analysis results as soon as determined by the TECTA B-16 rather than waiting for the end of an incubation time period such as 18 or 24 h. Four concentrations of EC ATCC 25922 (8, 100, 1,000, and 8,600 CFU/100 mL) were analyzed four times each. The TECTA B-16 generated positive TC and EC responses for all of the samples. The required analysis time for TC ranged from 10 to 14 h and for EC ranged from 9 to 13 h. The amount of time until detection for the TC and EC samples decreased with each increasing concentration level and generally the EC took about 30 to 50 minutes less time for detection.

Operational Factors. The TECTA B-16 was operated in continuous measurement mode for the simultaneous measurement of TC and EC in up to 16 different samples. To initiate analysis, 100 mL of each individual water sample were dispensed into each cartridge and the cartridge was snapped firmly shut, then swirled to dissolve the contents. The cartridges were loaded into the TECTA B-16 in the same manner and the 18 h incubation/analysis was started by closing the lid. The samples (16 at a time) were incubated within the TECTA B-16 at 35 °C and results were reported on the screen (and available to the operator as electronic alerts) as soon as the TECTA B-16 was able to make a conclusive positive determination of TC and/or EC based on the fluorescence measurement. The result of each measurement was displayed on the screen and the operator recorded the result on a sample data sheet. Each result could also be downloaded for review and viewed on a computer containing the TECTA B-16 software or a standard web browser. The CDW operator noted that the technology was very user friendly and eliminated the need for a technician to be present outside of working hours to read the results.

The TECTA B-16 has dimensions of 48 cm wide × 62 cm deep × 34 cm high (18.8 inches wide × 24.5 inches deep × 13.5 inches high) and weighs approximately 28 kilograms (61.7 pounds). The TECTA B-16 is completely self-contained and does not require any additional equipment or materials to perform analyses.

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