EPA/600/R-14/307
February 2007

Environmental Technology Verification Report

ENDETEC TECTA™ B-16 BY PATHOGEN DETECTION SYSTEMS, INC.

Prepared by

Battelle
The Business of Innovation

Under a cooperative agreement with

EPA

ETV ETV ETV

Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

ENDETEC TECTA™ B-16 BY PATHOGEN DETECTION SYSTEMS, INC.

by

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Notice

The U.S. Environmental Protection Agency, through its Office of Research and Development, funded and managed, or partially funded and collaborated in, the research described herein. It has been subjected to the Agency's peer and administrative review and has been approved for publication. Any opinions expressed in this report are those of the author (s) and do not necessarily reflect the views of the Agency, therefore, no official endorsement should be inferred. Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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.

Acknowledgments

The authors wish to thank Jim Sinclair, Sandhya Parshionikar, Jennifer Best, Keya Sen, and Mark Rodgers of the U.S. EPA, and Rick Sakaji of the East Bay Municipal Utility District for their review of the test/quality assurance (QA) plan and Mark Rodgers and Stanley States of the Texas A&M Engineering Extension Service for their review of this verification report. QA oversight was provided by Holly Ferguson, U.S. EPA, and Betsy Cutié, Zachary Willenberg, and Rosanna Buhl, Battelle.

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List of Abbreviations

ADQ	Audit of Data Quality
AMS	Advanced Monitoring Systems
ATCC	American Type Culture Collection
CDW	Columbus Division of Water
CFU	colony forming unit
cm	centimeters
COC	chain of custody
DDW	dechlorinated drinking water
DQA	data quality audit
DW	drinking water
EA	*Enterobacter aerogenes*
EC	*Escherichia coli*
EPA	U.S Environmental Protection Agency
ETV	Environmental Technology Verification
FN	false negative
FP	false positive
h	hour(s)
L	liter
MB	method blank
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
min	minute(s)
mL	milliliter
MUG	4-methyllumbelliferyl-β-D-glucorinide
N	number
NA	not applicable
NPDWR	National Primary Drinking Water Regulation
NRMRL	National Risk Management Research Laboratory
PA	*Pseudomonas aeruginosa*
PDS	Pathogen Detection Systems
QA	quality assurance
QC	quality control
QMP	Quality Management Plan
RTCR	revised Total Coliform Rule
TC	total coliforms
TCR	Total Coliform Rule
TN	true negative
TP	true positive
TQAP	Test Quality Assurance Plan
TSA	technical systems audit
USB	Universal Serial Bus

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 permitting agencies; 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 the ENDETEC™ TECTA B-16, a bench top incubator/analyzer/data logger system for the analysis of total coliforms (TC) and *Escherichia coli* (EC) manufactured by Pathogen Detection Systems, Inc. (PDS), a subsidiary of Veolia Water Solutions & Technologies.

Chapter 2
Technology Description

This report provides results for the verification testing of the ENDETEC™ TECTA B-16 by PDS (hereafter referred to as the TECTA™ B-16). The following is a description of the TECTA B-16, based on information provided by the vendor.

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 which is shown in Figure 2-1.

Figure 2-1. TECTA B-16 during analysis (left); Operator holding test cartridge next to TECTA B-16 ready for insertion of sample cartridges (right).

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 (see Figure 2-2), 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

Figure 2-2. Sample cartridge exhibiting positive (top) and negative (bottom) test results.

to be absent of bacteria after 18 hours. As shown in Figure 2-3, positive results are indicated by a red light surrounding the symbol for each sample chamber on the touch screen (green indicates negative) when the presence of TC or EC is detected. The results are stored on the TECTA B-16 and can be downloaded with a universal serial bus (USB) drive for viewing with the TECTA B-16 software or any internet browser. Due to its continuous monitoring capability, positive sample results can be detected in less than 18 hours.

Figure 2-3. Red and green indicate results around sample chambers and on touch screen.

In continuous mode, the TECTA B-16 can analyze up to 16 samples in 18 hours (h). PDS 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.

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.

Chapter 3
Test Design and Procedures

3.1 Introduction

The ETV AMS Center Water Stakeholder Committee identified the use of coliform detection technologies for the monitoring of drinking water (DW) as an area of interest for technology verification. Fecal pollution can introduce disease-causing (pathogenic) bacteria, viruses, and parasites into receiving waters, which may serve as private/public DW supplies. Utilities fully recognize the possibility of this waterborne pollution and take every precaution (filtering, treatment with disinfectants such as chlorine and chloramines, and regulatory compliance sampling and analysis) to avoid fecal contamination in DW. Assessment of this health risk is based on the detection and enumeration of fecal indicator bacteria, such as TC and EC, where its presence indicates a potential pathway for contamination (e.g., sewage or animal waste) of the distribution system which is designed to provide a physical barrier to contamination of DW. To evaluate the ease of use of the TECTA B-16, as well as the applicability of the instrument to non-microbiologist users under field / in-plant conditions, the testing was conducted by a Columbus, OH water utility staff member who did not have any prior training or certification in microbiological analyses.

In February 2013, EPA revised the 1989 Total Coliform Rule (TCR)[1], a national primary drinking water regulation (NPDWR). The revised rule establishes a maximum contaminant level goal (MCLG) of zero for *E. coli*, 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.

3.2 Test Overview

This verification test was conducted according to procedures specified in the Test/QA Plan for Verification of Coliform Detection Technologies for Drinking Water[2] (TQAP) and adhered to the quality system defined in the ETV AMS Center Quality Management Plan (QMP)[3].

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 a single organism detection. Therefore, for the purpose of this verification test, the objective was to prepare spiked DW dilution sets that provided 50 ± 25% positive results for EC using the Colilert-18 reference method. However, because of the very small number of organisms in the suspension, the mixture is not homogeneous, and therefore, representative sampling cannot be done reliably from a bulk solution. The implication is that replicate 100 mL samples cannot be compared. That is, the ratio of positive and negative results in 20 samples analyzed by the TECTA B-16 and 20 samples analyzed by Colilert-18 cannot be compared directly to confirm the performance against an accepted reference method. In order to confirm presence or absence in each sample, the positive and negative spent media from each replicate analyzed by the TECTA B-16 was inoculated into sterilized water for analysis by Colilert-18. The Colilert-18 results confirmed the presence or absence of TC and EC for the samples that had been analyzed by the TECTA B-16.

The overall ETV test of the TECTA B-16 was conducted from August 21-23, 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 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.

3.3 Experimental Design

3.3.1 Verification Test Sample Preparation

The preparation of verification test samples included the collection of the DW sample, the inoculation of the DW sample with target organisms, and the dilution of samples for analysis. A detailed description of the sample preparation steps is provided in the TQAP[1].

3.3.1.1 Drinking Water Sample Collection

A single DW sample was collected from the tap at a Battelle laboratory as directed in the TQAP. The DW sample was collected by first removing the faucet screen and decontaminating the surface with 70% isopropanol. Next the line was purged for 5 minutes with cold water and 18 liters (L) of DW were collected from the tap into a sterile (autoclaved) carboy equipped with a spigot. Once collected, an aliquot (several hundred milliliters) was collected from the carboy

and used to measure the pH, free chlorine, and total chlorine. The analysis methods and subsequent results are provided in Table 3-1.

Table 3-1. Methods, Equipment, and Results for the Characterization of the Drinking Water (DW) Sample

Parameter	Equipment/Media	SOP/Method	DW Results
pH	calibrated pH meter	SOP GEN.V-003-10[4]	7.24
free chlorine	HACH Chlorine test kit	HACH Method 8021	1.51 mg/L
total chlorine	HACH Chlorine test kit	HACH Method 8167	1.54 mg/L

3.3.1.2 Preparation of Samples for Verification Testing

To test the coliform technologies, separate DW samples of EC containing concentrations of approximately 1 organism per 100 mL were prepared. To ensure that these concentrations would be attained, a range of concentrations were prepared. Two separate aliquots, approximately 5 L each, of dechlorinated DW (DDW) were added to carboys (sterilized by autoclaving) containing stir bars and spiked with a suspension of EC (American Type Culture Collection [ATCC] 25922) to generate target suspensions of 0.5 CFU/100 mL and 5 CFU/100 mL. Each dilution was mixed on a stir plate for 5 to 10 minutes, and then 100 mL aliquots were dispensed into sterile 100 mL bottles using 50 mL and/or 100 mL graduated pipettes (sterile, individually wrapped, and disposable). Twenty replicate samples were prepared at each concentration level. Each bottle was labeled with a unique sample identification number. Once all forty 100 mL aliquots were dispensed for technology verification, they were immediately transported on ice to CDW where verification testing was conducted upon receipt. All laboratory work was performed within certified Class II biological safety cabinets by analysts wearing disposable laboratory coats and non-latex gloves to minimize the potential for inadvertent contamination. Polypropylene sample bottles used were either sterilized via autoclaving or purchased sterile.

In addition to the samples to be used for verification, a second set of 100 mL aliquots were prepared in the same manner as described above (forty samples in total; twenty from each carboy) for the reference method analysis. Immediately after being dispensed and labeled with unique sample identification numbers, all reference samples were transported by car in coolers packed with ice packs to Superior Laboratories, Inc.

Quality control samples (listed in Table 3-2) were also prepared. Positive and negative ATCC control cultures were purchased from MicroBioLogics. Control organisms included the EC negative control *Enterobacter aerogenes* (EA) (ATCC 13048), EC (ATCC 25922), and the non-coliform *Pseudomonas aeruginosa* (PA) (ATCC 10145). All control cultures were prepared on tryptic soy agar and incubated overnight. The QC samples were then prepared by inoculating triplicate 990 mL filter sterilized DDW aliquots each with 10 mL of a 100 colony forming unit (CFU)/mL suspension prepared from the agar cultures in DDW. Control samples were used to confirm the accurate response (positive response for positive control and negative response for the negative controls) of the TECTA B-16 and reference methods at relatively high concentrations. The QC samples were shipped with the test samples that went to both laboratories. This resulted in 48 samples (Table 3-3) prepared and shipped to each laboratory.

Sample custody for all samples transferred to CDW and Superior Laboratories were documented using a chain-of-custody (COC) form following Battelle SOP ENVS-6-055 for Chain of Custody[5]. The COC form was signed once receipt of all samples was confirmed. Reference method analysis was initiated using Colilert-18 on the same day as arrival at the laboratory, within 2 h of initiation of the TECTA B-16 sample analysis.

3.3.2 Sample Analysis

The ability of the TECTA B-16 to determine the presence of EC was challenged using 20 replicates of the two concentrations of EC in DW samples. Positive/negative control samples spiked with quality control (QC) cultures listed in Table 3-2 as well as method blank samples were included during testing. PDS provided three TECTA B-16s to perform testing of the replicate samples shown in Table 3-3. Each of the TECTA B-16s contained 16 sample chambers for incubating and measuring the fluorescence from the sample cartridges. Therefore, all 48 samples in the primary technology evaluation were analyzed simultaneously in continuous detection mode. In continuous mode, the sample cartridges were inserted into the TECTA B-16s at the start of the incubation and remained in the unit for the full 18 h analysis period. All of the samples were assayed by the Colilert-18 reference method and the TECTA B-16 concurrently.

Table 3-2. Quality Control Strains and Expected Results

Targeted Coliform	Description	Expected Result
Sterilized Water	Method Blank	TC- and EC-
Pseudomonas aeruginosa (PA)ATCC 10145	TC and EC negative control	TC- and EC-
Enterobacter aerogenes (EA) ATCC 13048	TC positive control EC negative control	TC+ and EC-
Escherichia coli ATCC 25922	TC positive control EC positive control	TC+ and EC+

Table 3-3. Replicate Samples by each Analysis Method

Sample Description	Replicate Analyses by TECTA B-16	Replicate Analyses by Colilert-18
Dilution A Target conc.= 5 CFU/100 mL	20	20
Dilution B Target conc.= 0.5 CFU/100 mL	20	20
Method Blank (sterilized water)	3	3
EC Positive control Target conc.=100 CFU/100 mL	3	3
TC Positive control Target conc.= 100 CFU/100 mL	1	1
Negative control Target conc.= 100 CFU/100 mL	1	1
Total Replicate Analyses	48	48

3.3.2.1 Confirmation of Results

As described in Section 3.2, each TECTA B-16 result was confirmed as definitively positive or negative with the Colilert-18 reference method to verify the result obtained TECTA B-16. In summary, 1 mL of each 100 mL sample resulting from the 18 h incubation during TECTA B-16 analysis was inoculated into 99 mL of filter sterilized water, dechlorinated tap water and analyzed using Colilert-18. The Colilert-18 result provided definitive confirmation of presence or absence of EC in the initial samples.

3.3.3 Detection of Additional Concentration Levels in Continuous Operating Mode

Another component of the ETV test was performed to verify the capability of the TECTA B-16 to detect EC ATCC 25922 at various concentration levels in continuous operating mode which provides positive results as soon as determined by the TECTA B-16. A target inoculation was prepared in DDW that contained approximately 10^4 EC per 100 mL, and then a serial dilution (1:10, 1:100, and 1:1,000) of the stock was prepared to obtain four separate samples for testing (10, 100, 1,000 and 10,000 EC per 100 mL). The data from these tests were intended to identify: (1) whether or not the TECTA B-16 detected the presence of EC at higher concentrations and (2) to evaluate the time required for detection of positive results. Triplicate aliquots at each concentration level were analyzed using a quantitative spread plate enumeration method for EC to confirm the concentration of the samples.

Chapter 4
Quality Assurance/Quality Control

QA/QC procedures were performed in accordance with the TQAP for this verification test[1] and the QMP for the AMS Center[2]. QA/QC procedures and results are described in the following sections.

During testing, there were five minor deviations from the TQAP. These included:

- Adjusting the number of QA samples due to the limited number of sample chambers (48) within the TECTA B-16 instrument package provided for the trial on-site. The number of QA samples was adjusted so the total number of test and QA samples combined did not exceed 48. The original plan called for two of each QA sample. The number was adjusted to three method blanks and three EC positive controls (therefore there was one sample per TECTA B-16 unit) and one EC negative control and one EC positive control overall. Analysis of the planned number of QA samples would have been preferable. However, inclusion of triplicate samples of the EC positive control provided positive control results for both TC and EC. Also, the EC negative control and the negative control had densities of greater than 100 CFU/100mL, a rather high concentration of possible interfering organisms that would be more likely to cause a false positive than a lower density. Lastly, confirmation analysis also adds certainty to the correct result of the control samples.
- Changing the SM 9226 (nutrient Agar-4-methyllumbelliferyl- β-D-glucorinide [MUG]) method to the standard spread plate enumeration method because EC source was a pure culture and therefore the differential enumeration technique was not required.
- Changing target suspensions of EC to 0.5 CFU/100 mL and 5 CFU/100 mL from 1 CFU/100 mL and 10 CFU/mL based on preliminary results from use of the methods planned to attain the target presence/absence ratios.
- Using *Pseudomonas aeroginosa* ATCC 27853 as the negative control rather than ATCC 10145 because of supplier availability.
- Correcting the phone number of Superior Laboratories.

Each of these deviations was judged by the Battelle Verification Test Coordinator to not result in any adverse impacts on the quality of the data generated. The deviation was reviewed and approved by the EPA ETV AMS Center Project Officer and EPA ETV AMS Center Quality Manager.

4.1 Quality Control Samples

The Colilert-18 reference method required the inclusion of method blanks (MBs) and positive and negative control organisms. Three MB samples were taken across the 48 total test samples. The MB samples consisted of 100-mL dechlorinated, sterilized tap water processed as a sample. MB samples were exposed to identical handling and analysis procedures as the rest of the test samples. These samples were used to ensure that no sources of contamination were introduced in the sample handling and analysis procedures. All three MB samples analyzed by the TECTA B-16 as well as the reference method were negative, indicating the absence of TC and EC.

Positive and negative control samples were also analyzed using each method. The control cultures were enumerated by membrane filtration. The EC positive control was 116 CFU EC/100 mL (three replicates), the EC negative control was 146 CFU EA/100 mL (one replicate), and the negative control was 276 CFU PA/100 mL (one replicate).

The EC negative control was determined to be negative (TC+EC-) using the reference method and the TECTA B-16 (and confirmed with the Colilert-18 confirmation analysis). In addition, all three EC positive controls were determined to be positive (TC+EC+) using the reference method and the TECTA B-16 (and confirmed with the Colilert-18 confirmation analysis). The non-coliform negative control resulted in only negative results which were also confirmed.

4.2 Audits

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

4.2.1 Technical Systems Audit

The Battelle AMS Center Quality Auditor performed a TSA on August 21, 2013 at CDW's water quality laboratory in Columbus, OH and at the reference laboratory, Superior Laboratories located in Galloway, OH. The TSA consisted of interviews with Battelle and Superior Laboratories personnel, observations of test sample preparation and testing at Battelle and Superior Laboratories, and observation of sample analysis. The purpose of the audit was to verify that:

- Sample preparation procedures were performed by Battelle according to the TQAP requirements
- Reference laboratory methods for analyzing test samples conformed to the TQAP and reference method requirements
- Technology testing was performed according to the TQAP and vendor instructions
- Test documentation provided a complete and traceable record of sample preparation and analysis

- Equipment used in the test was calibrated and monitored according to TQAP requirements and standard laboratory procedures.

The TSA revealed no findings and just one observation. The observation involved an inconsistency with the reference method procedure being followed by the reference laboratory and a description of the reference method included in the reference method given in the appendix of the TQAP. The method summary provided in the appendix gave an incubation time range of 24 to 28 h rather than the correct range of 18 to 22 h. As the reference lab followed the proper procedure, there was no negative impact to the test results. No action was required.

A TSA report was prepared and distributed to the Verification Test Coordinator, the Battelle AMS Center Manager, the EPA AMS Center Project Officer, and the EPA AMS Center Quality Manager.

4.2.2 Data Quality Audit

Records generated in the verification test were reviewed by a second verification staff member before these records were used to calculate, evaluate, or report verification results. The person performing the review added his/her initials and the date to a hard copy of the record being reviewed. In addition, an audit of data quality (ADQ) was conducted on October 17-18, 2013. During the audit, laboratory data generated at the reference laboratory, Superior Laboratories, Inc., and data generated by the TECTA B-16 were reviewed and verified for completeness, accuracy and traceability. The verification of coliform detection technologies was determined by the EPA AMS Center Project Officer to be Category III test. Accordingly, at least 25% 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. All calculations performed on the data undergoing the audit were checked.

The ADQ revealed no findings and two observations. The first observation was that the TQAP specified grouping results to determine proportions of results that are positive, negative, false positive, and false negative, then running chi-square tests for determination of significance. Instead, the chi-squared test was modeled in SAS using the FREQ procedure for determining significance (without presenting the specified groupings). While a completely appropriate approach to completing a chi-squared test, a deviation will be completed to document this slight change in data treatment. The second observation is that all project entries could be linked to personnel, but there was no signature page to link initials and signature to a specific individual. A signature page will be added to the data binder to link the initials to individual performing the work. None of these had an adverse impact on the test results.

A data audit report was prepared, and a copy was distributed to the EPA AMS Center Quality Manager.

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 False Positive Rates, False Negative Rates, Sensitivity, and Specificity

False positive (FP) and false negative (FN) rates of the TECTA B-16 were evaluated when assessing comparability. During this test, true positives (TPs) were those positive results from the TECTA B-16 that were confirmed as positive by the reference method, and false positives were those positive results from the TECTA B-16 that were not confirmed by the reference method. Conversely, true negative (TN) results were those negative results that were confirmed as negative, and false negative results were those negative results that were shown to be positive by the confirmatory method.

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. Estimates of sensitivity, specificity, false positive rates, and false negative rates as percentages for the two methods were calculated as follows:

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100\%$$

$$\text{Specificity} = \frac{TN}{TN+FP} \times 100\%$$

$$\text{False positive rate} = \frac{FP}{TN+FP} \times 100\% = \left(1 - \frac{TN}{TN+FP}\right) \times 100\% = 1 - \text{Specificity}$$

$$\text{False negative rate} = \frac{FN}{TP+FN} \times 100\% = \left(1 - \frac{TP}{TP+FN}\right) \times 100\% = 1 - \text{Sensitivity}$$

5.2 Method Comparability

In order to assess whether the proportion of positive and negative samples was significantly different between the TECTA B-16 and the reference method, chi-square tests for independence were conducted. The chi-squared test was modeled in SAS® (ver. 9.1.3), using the FREQ

procedure. If the calculated chi-square value is less than the critical value, the sample results between the two methods are not significantly different (95% confidence, alpha = 0.05, p-value > 0.05). If the Chi-square value is greater than the critical value (based on a significance of 0.05), the results between the two methods are significantly different, and it is concluded that there is a difference between the two methods.

Prior to testing, a power analysis was conducted to determine the number of replicates required to determine possible significant differences between the technologies being tested and the reference method. Conducted using the POWER procedure in SAS, the power analysis determined the number of replicate tests (across both test types) that would be necessary to detect a specified difference in proportions of a specified size with 80% power, given a specified value of the proportion for the reference test (the acceptable range of reference test positive proportions was 25% to 75% for this test), and a significance level of 0.05 for the test. To summarize, the power analysis shows that for approximately 20 replicates, if the reference method was approximately 50% positive (10 positive results and 10 negative results), then the technology being tested would be required to be 90% positive (18 positives and two negative results) or 90% negative (two positives and 18 negatives) to have a significant difference. The TECTA B-16 results are discussed in the context of this power analysis.

In summary, the smallest difference that is able to be determined with 20 replicates is approximately a 30% to 40% change in positive results. The power analysis revealed that differences of 5% or 10% of positive results could be determined, but between 150 and 1,250 replicates may be required.

Chapter 6
Test Results

This verification test included both quantitative and qualitative evaluations. The quantitative evaluation was conducted to confirm the presence/absence TECTA B-16 results with those generated by the presence/absence result from the reference method in the same replicate sample as well as in parallel sample sets. The qualitative evaluation was performed to document the operational aspects of the TECTA B-16 when it was used during verification testing. The following sections provide the results of the quantitative and qualitative evaluations. The TC and EC results are presented together in this section because (1) a pure EC culture was used as the source of EC for this test (rather than sewage), (2) positive TC results under the RTCR no longer trigger an acute MCL violation (but can trigger the requirement for sanitary surveys), and (3) the results for TC and EC were the same in each sample. Tables presenting the raw data presence/absence results for the TECTA B-16, the Colilert-18 initial reference method result, and the Colilert-18 confirmation analysis results are provided in Appendix A.

6.1 TECTA B-16 Confirmed Results

The positive TC and EC test results for the TECTA B-16 and reference method (Colilert-18) are presented in Table 6-1. 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 6-1. TC and EC Positive Results

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%

N – Number of replicates

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. Tables 6-2 and 6-3 summarize the confirmed true positive and true negative TC and EC results for the TECTA B-16.

Table 6-2. TECTA B-16 TC and EC Data Summary - Positives

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

Table 6-3. TECTA B-16 TC and EC Data Summary - Negatives

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

The sensitivity, specificity, false-positive, and false-negative rates for the TECTA B-16 results for both the 0.5 CFU/100 mL and 5 CFU/100 mL dilutions were determined as described in Section 5.1 and are presented in Table 6-4.

Table 6-4. TC and EC Data Summary - Confirmations

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

6.2 Method Comparability

Table 6-5 shows the results from the chi-square test for independence that was performed to compare the TC results from the TECTA B-16 for each incubation time period against the initial results (not the confirmation results) of the reference method (Colilert-18). Because only the

Table 6-5. TC and EC Results

Dilution (target concentration)	TECTA B-16		Colilert-18		Chi-Square	Degrees of freedom	p-Value	Critical Limits (p=0.05)
	+	-	+	-				
A (5 CFU/100 mL)	20	0	20	0	NA			
B (0.5 CFU/100 mL)	6	14	11	9	2.558	1	0.110	3.841

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 interval), 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).

These results are consistent with the power analysis performed before testing and described in Section 5.2. For TC and EC, the reference method generated 55% positive results for Dilution B. When referencing the power analysis when the reference method was 50% positive, significant differences could only occur with TECTA B-16 results of one or two positive results and the rest negative results or one or two negative results with the rest positive results. The TECTA B-16 result of six positive and 14 negative samples did not meet that requirement. Based on the power analysis, a significant difference perhaps could have been determined with an additional 70 or 80 replicates. However, because of the small concentrations involved, confirmation analysis on each replicate will always be the best route of determining the technology performance.

6.3 Detection of Additional Concentration Levels in Continuous Operating Mode

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 18 h incubation time period. Table 6-6 gives the results for the analysis of various concentrations of EC ATCC 25922, including the result provided and the time of result. Without exception, the TECTA B-16 generated positive TC and EC responses at all four concentration levels. Four replicate samples of each concentration were analyzed and the TC and EC positive results were reported between 12-14 h for 8 EC CFU/100 mL, 11-13 h for 100 EC CFU/100 mL, 10-12 h for 1,000 EC CFU/100 mL, and 9-11 h for 8,600 EC CFU/100 mL. As there is no requirement for the TECTA B-16 to run a complete 18-hour cycle, once a positive result has been detected, the detection time represents the test endpoint and operator alerts are generated.

6.4 Operational Factors

Following testing, the CDW operator (a CDW staff member who was a part of the field water sampling team) commented that the TECTA B-16 was a very user friendly technology. They noted that the software interface was extremely straight forward and that the 2 hour training session with PDS staff was more than adequate to train them on the operation. The TECTA

Table 6-6. Results of Analysis of Additional Concentrations in Continuous Operation Mode of the TECTA B-16.

EC Conc. (CFU/100mL)[a]	TC	Incubation Time TC Detected (h:min)	EC	Incubation Time EC Detected (h:min)
8	X	13:29	X	12:56
	X	13:38	X	12:56
	X	13:09	X	12:39
	X	13:35	X	13:04
100	X	12:18	X	12:00
	X	12:12	X	11:42
	X	12:29	X	11:52
	X	11:53	X	11:25
1,000	X	10:59	X	10:29
	X	11:12	X	10:41
	X	11:05	X	10:37
	X	10:59	X	10:22
8,600	X	10:12	X	9:38
	X	10:18	X	9:44
	X	10:10	X	9:38
	X	10:18	X	9:38

[a]Calculated concentrations
X=Presence; O= Absence
min = minute

B-16 was set up by plugging into standard 110 volt power and powering up. For training purposes, several samples were prepared at a target concentration of 100-200 CFU/100 mL (EA, PA, and EC) and analyzed in triplicate along with an MB (all control samples were prepared and handled by a trained microbiologist). The results were all as expected (EC samples were TC+EC+, PA samples were TC-EC-, method blank samples were TC-EC-) except for two of the EA samples (182 CFU/100 mL) which produced TC+EC+ results and not the expected TC+EC- result. When the sample was immediately repeated in read mode, the EC result became negative. This behavior was also observed in a repeated sample set of EA only (171 CFU/100 mL). PDS assisted in the troubleshooting of the TECTA B-16 and after review of raw data (not available to a standard user) PDS determined that the algorithm that monitors the absorbance of the wavelength produced by the EC was incorrectly producing a EC+ result due to an electronic signal artifact in the presence of high concentrations of EA. The testing was continued and the result was not observed again in the EC negative control samples (146 CFU/100 mL EA). PDS noted that a revision to the algorithm will be made to account for this observation.

Upon first use of a TECTA B-16, analysis of a validation cartridge by each sample chamber was required (following first use, validation is recommended weekly). The validation cartridge does

not contain any liquid, but when inserted into a sample chamber, fluoresces at the proper wavelengths to check for the proper functioning of the TECTA B-16. Once each sample chamber had been validated (each chamber took approximately 30 seconds) with the result reported as "passed", the TECTA B-16 units were ready for the analysis of samples. To further verify the function of the TECTA B-16, the same validation procedure was also successfully completed after the completion of ETV testing.

As previously described, the TECTA B-16 was operated in continuous measurement mode for the simultaneous measurement of TC and EC using PDS 100 mL sample cartridges containing the required reagents. The samples were loaded into the sample chambers and the 18 h incubation/analysis was started by closing the lid of the TECTA B-16. Once a sample was added, the operator swirled the contents (being careful not create bubbles in the bottom of the cartridge) and set the sample down while preparing the rest of the samples. Full dissolution required approximately 5 minutes for each sample. The samples (16 at a time) were incubated within the TECTA B-16 at 35°C and results were reported on the screen 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. A positive result could have been reported at any point during the 18 h analysis, while a negative result would not occur until the end of the incubation time. During the continuous measurement, a countdown timer appeared on the touch screen nearest the sample chambers that were used for the analyses.

Following testing, the CDW operator also noted that the TECTA B-16 does not require staff available after hours and on weekends to read the results of samples after an analysis set of up to 16 had been started during working hours (the incubation function is automatically shut down after 18 hours and results are stored for later evaluation). In addition, according to PDS, the TECTA B-16 can be connected to a network for e-mail or text message alerts upon positive samples.

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 individually on a computer containing the TECTA B-16 software or any standard web browser, but the results from a group of samples could not be exported as a spreadsheet.

Chapter 7
Performance Summary

In order to comply with the RTCR, water utilities need coliform detection technologies that are able to detect EC at concentrations of one organism per 100 mL samples. This ETV test verified the performance of the TECTA B-16 at that level of detection. 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 test, DW dilution sets (inoculated with a pure culture of EC) were prepared to provide 50 ±25% positive results for TC and EC with the reference method. These reference method results confirmed single organism detection. The results from each replicate sample analyzed on the TECTA B-16 were then confirmed with the reference method for definitive presence/absence determination. In addition, the initial results from the reference method were compared through statistical testing with the TECTA B-16. The results of the verification of the TECTA B-16 are summarized in Table 7-1.

Table 7-1. Results Summary for Positive TECTA B-16 Results for TC and EC

Dilution (target concentration)	TECTA 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%

It should be noted that for Dilution B that the observed differences in positive detection rate (30% for the TECTA B-16 and 55% for Colilert-18) are due to statistical differences in organism content in the original samples (i.e., the non-uniform distribution of positive and negative samples from taking 100 ml aliquots from the original sample source) rather than a difference in sensitivity between the methods. Method sensitivity was determined by confirmation - see *Sensitivity*, below.

Specificity, Sensitivity, FP rate, and FN rate. Table 7-2 summarizes the specificity, sensitivity, FP rate, and FN rate for TC and EC for the TECTA B-16 results. 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.

Table 7-2. Confirmed Result Summary of TECTA B-16

EC target concentration:	0.5 CFU/100 mL	5 CFU/100 mL
Sensitivity	100%	100%
Specificity	100%	NA
False Positive	0%	NA
False Negative	0%	0%

NA = undefined results because of zero in denominator

Comparability. In another approach of comparison between the TECTA B-16 and the reference method, a chi-square test for independence was performed. Results from each dilution of EC were tested separately. The chi-square value for the EC solution was less than the critical limit in each case; therefore, for EC and TC, the chi-square test did not detect any differences between the results of the TECTA B-16 and the reference method. In addition, the corresponding p-value was greater than 0.05, indicating that the data did not show a statistically significant difference between the two methods for the detection of EC or TC at the 95% confidence interval. These results were consistent with the power analysis performed before testing and described in Section 5.2.

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.

Chapter 8
References

1. Total Coliform Rule, United States Federal Register, 54 FR 27544-27568, June 29, 1989, Vol. 54, No. 124

2. Test/QA Plan for Verification of Coliform Detection Technologies for Drinking Water, Battelle, Version 2.0, July 16, 2013.

3. Quality Management Plan for the ETV Advanced Monitoring Systems Center, Version 7. U.S. Environmental Technology Verification Program, Battelle, November 2008.

4. SOP GEN.V-003-10. Standard Operating Procedure for the Use of pH meters to Measure pH. Battelle.

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Appendix A

Raw Data from Reference Methods, TECTA B-16, and Confirmation Analyses

Dilution (calculated concentration)	Sample No.	Endetec		Colilert-18		Confirmation of Endetec via Colilert-18	
		TC	EC	TC	EC	TC	EC
A (5 CFU/100ml)	XX01	X	X	X	X	X	X
	XX04	X	X	X	X	X	X
	XX06	X	X	X	X	X	X
	XX07	X	X	X	X	X	X
	XX08	X	X	X	X	X	X
	XX09	X	X	X	X	X	X
	XX13	X	X	X	X	X	X
	XX20	X	X	X	X	X	X
	XX22	X	X	X	X	X	X
	XX25	X	X	X	X	X	X
	XX27	X	X	X	X	X	X
	XX29	X	X	X	X	X	X
	XX30	X	X	X	X	X	X
	XX31	X	X	X	X	X	X
	XX32	X	X	X	X	X	X
	XX33	X	X	X	X	X	X
	XX34	X	X	X	X	X	X
	XX36	X	X	X	X	X	X
	XX38	X	X	X	X	X	X
	XX40	X	X	X	X	X	X
Ratio of Positive		20 of 20	20 of 20	20 of 20	20 of 20	20 of 20	20 of 20
Percent Positive		100%	100%	100%	100%	100%	100%

Dilution (calculated concentration)	Sample No.	Endetec		Colilert-18		Confirmation of Endetec via Colilert-18	
		TC	EC	TC	EC	TC	EC
B (0.5 CFU/100ml)	XX02	O	O	O	O	O	O
	XX03	O	O	O	O	O	O
	XX11	O	O	O	O	O	O
	XX12	O	O	X	X	O	O
	XX14	O	O	X	X	O	O
	XX16	O	O	X	X	O	O
	XX17	O	O	O	O	O	O
	XX18	X	X	X	X	X	X
	XX19	O	O	X	X	O	O
	XX21	O	O	O	O	O	O
	XX24	X	X	X	X	X	X
	XX26	O	O	X	X	O	O
	XX37	O	O	X	X	O	O
	XX39	X	X	O	O	X	X
	XX41	X	X	O	O	X	X
	XX42	X	X	X	X	X	X
	XX43	O	O	X	X	O	O
	XX44	O	O	O	O	O	O
	XX45	O	O	X	X	O	O
	XX47	X	X	O	O	X	X
Ratio of Positive		6 of 20	6 of 20	11 of 20	11 of 20	6 of 20	6 of 20
Percent Positive		30%	30%	55%	55%	30%	30%

X= Presence

O= Absence