Battelle
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Environmental Technology Verification Program

Advanced Monitoring Systems Center

Test/QA Plan for Verification of Coliform Detection Technologies for Drinking Water

TEST/ QUALITY ASSURANCE PLAN

for

Verification of
Coliform Detection Technologies for Drinking Water

Version 1.0

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SECTION A

PROJECT MANAGEMENT

A1 VENDOR APPROVAL PAGE

ETV Advanced Monitoring Systems Center

Test/QA Plan for Verification of
Coliform Detection Technologies for Drinking Water

APPROVAL:

Name _____

Company _____

Date _____

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A3 LIST OF ACRONYMS

ADQ	audit of data quality
AMS	Advanced Monitoring Systems
ATCC	American Type Culture Collection
BGLB	brilliant green lactose bile
COC	chain of custody
DANETV	Danish ETV
DDW	dechlorinated drinking water
DQA	data quality audit
DW	drinking water
E. coli	*Escherichia coli*
EPA	U.S Environmental Protection Agency
ETV	Environmental Technology Verification
FN	false negative
FP	false positive
h	hours
LRB	laboratory record book
LTB	lauryl tryptose broth
MB	method blank
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
MUG	4-methyllumbelliferyl-β-D-glucorinide
N	number
NA	nutrient agar
PA	presence/absence
PDF	portable document format
PO	project officer
QA	quality assurance
QC	quality control
QMP	Quality Management Plan
RB	reagent blank
RMO	Records Management Office
SM	Standard Methods
SSDW	spiked, stressed drinking water
SWTP	Southerly Wastewater Treatment Plant
TC	total coliform
TCR	Total Coliform Rule
TSA	technical systems audit

A4 DISTRIBUTION LIST

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A5 VERIFICATION TEST ORGANIZATION

The verification test will be conducted under the U.S. Environmental Protection Agency (EPA) Environmental Technology Verification (ETV) Program. It will be performed by Battelle, which is managing the ETV Advanced Monitoring Systems (AMS) Center through a cooperative agreement with EPA. The scope of the AMS Center covers verification of monitoring technologies for contaminants and natural species in air, water, and soil. This verification testing is also being conducted in cooperation with the Canadian and Danish (DANETV) ETV programs as a possible ETV verification in those countries. The criteria for ETV cooperation are outlined in a cooperative verification process document prepared by the respective cooperating ETV programs. At least one of the technology vendors has agreed to participate in the verification with the possibility that their technology be considered for verification. It should be noted however that neither U.S. ETV verification, nor the cooperation with the Canadian or Danish ETV programs, represents an approval of methods for regulatory compliance. This verification test is being conducted to provide a verification of technologies that are not already approved for compliance monitoring in the U.S., or elsewhere, such that the vendors have an opportunity to have their technologies tested under similar set of test conditions that might be required for method approval.

The day to day operations of this verification test will be coordinated and supervised by Battelle, with the participation of the vendors who will have the performance of their technologies verified. Testing of the technologies being verified will be conducted at Battelle laboratories in Columbus, Ohio. Each vendor will provide Battelle with their respective technologies and will train the verification staff. Staff from Battelle will operate the technologies during verification testing.

Quality Assurance (QA) oversight will be provided by the Battelle Quality Manager and also by the EPA AMS Center Quality Manager, at her discretion. The organization chart in Figure 1 identifies the responsibilities of the organizations and individuals associated with the verification test. Roles and responsibilities are defined further below.

This test/QA plan will be subjected to review by the vendors, EPA, and expert peer reviewers referred to as verification test stakeholders in this test/QA plan. The reviews of this test/QA plan will help to improve the design of the verification test and the resulting report(s) such that they better meet the needs of potential users of these technologies. After all peer review comments are received, revisions will be made to the test/QA plan, and it will be submitted to EPA for approval.

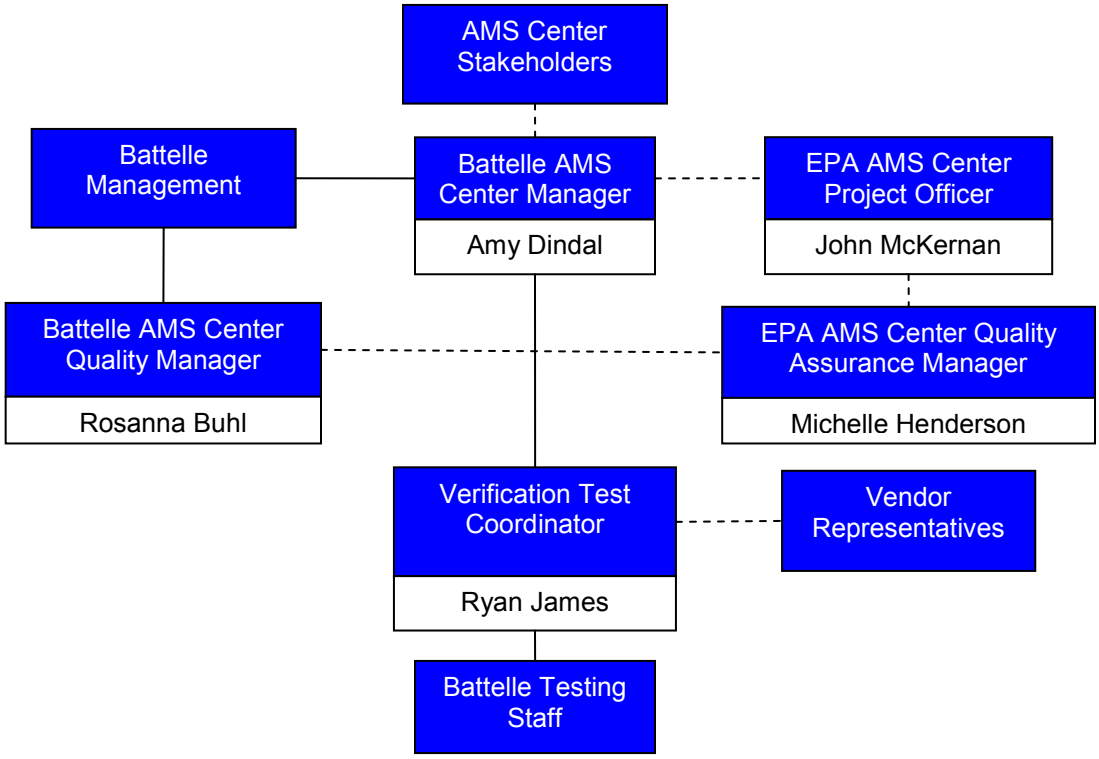

Figure 1. Organization Chart for the Verification Test

A5.1 Battelle

Dr. Ryan James is the AMS Center's Verification Test Coordinator for this test. In this role, Dr. James will have overall responsibility for ensuring that the technical, schedule, and cost goals established for the verification test are met. Specifically, Dr. James will:

- Prepare the draft test/QA plan, verification reports, and verification statements
- Establish a budget for the verification test and manage staff to ensure the budget is not exceeded

- Revise the draft test/QA plan, verification reports, and verification statements in response to reviewer comments
- Assemble a qualified technical staff to conduct the verification test
- Direct the staff in performing the verification test in accordance with this test/QA plan
- Hold a kick-off meeting approximately one week prior to the start of the verification test to review the critical logistical, technical, and administrative aspects of the verification test. Responsibility for each aspect of the verification test will be reviewed to ensure each participant understands his/her role
- Ensure that all quality procedures specified in this test/QA plan and in the AMS Center Quality Management Plan[1] (QMP) are followed
- Maintain real-time communication with the Battelle AMS Center Manager and EPA AMS Center Project Officer and Quality Assurance Manager on any potential or actual deviations from the test/QA plan
- Serve as the primary point of contact for vendor representatives
- Ensure that confidentiality of sensitive vendor information is maintained
- Assist vendors as needed during verification testing
- Become familiar with the operation and maintenance of the technologies through instruction by the vendors
- Direct the 100% validation and verification of data by technical staff prior to submission for data quality audit
- Respond to any issues raised in assessment reports, audits, or from verification staff observations, and institute corrective action as necessary
- Coordinate distribution of the final test/QA plan, verification reports, and verification statements.

Ms. Amy Dindal is Battelle's Manager for the AMS Center. As such, Ms. Dindal will oversee the various stages of verification testing. Ms. Dindal will:

- Review the draft and final test/QA plan
- Review the draft and final verification reports and verification statements

- Ensure that necessary Battelle resources, including staff and facilities, are committed to the verification test
- Ensure that confidentiality of sensitive vendor information is maintained
- Support Dr. James in responding to any issues raised in assessment reports and audits
- Maintain communication with EPA's technical and quality managers
- Issue a stop work order if Battelle or EPA QA staff discovers adverse findings that will compromise test results.

Mr. Daniel Lorch and other technical staff in Battelle's microbiological laboratories will support Dr. James in planning and conducting the verification test. They will:

- Assist in planning for the test, and making arrangements for the receipt of and training on the technologies
- Attend the verification test kick-off meeting
- Assist vendor staff as needed during technology receipt and training
- Conduct verification testing using each participating technology, following all aspects of the ETV AMS Center QMP as well as the test/QA plan for this verification
- Coordinate activities of Superior Laboratories, Inc., the laboratory performing the reference measurements
- Confirm that Superior Laboratories, Inc. is completing all required QC samples and corresponding documentation
- Support Dr. James in the preparation of the test/QA plan and reports, as necessary
- Support Dr. James in responding to any issues raised in assessment reports and audits related to technical performance, statistics, or data reduction as needed.

Ms. Rosanna Buhl is Battelle's Quality Manager for the AMS Center. Ms. Buhl will:

- Review the draft and final test/QA plan
- Attend the verification test kick-off meeting and lead the discussion of the QA elements of the kickoff meeting checklist

- Prior to the start of verification testing, verify the presence of applicable training records for Battelle and Superior Laboratories, Inc. staff, including any vendor training on test equipment
- Contact Superior Laboratories, Inc. before the verification test to confirm adequate quality practices are in place
- Conduct a technical systems audit at least once during the verification test, or designate other QA staff to conduct the audit (Section C1.1)
- Conduct audits of verification data quality or designate other QA staff to conduct the data audit (Section C1.2).
- Prepare and distribute an assessment report for each audit
- Verify that audit responses for each audit finding and observation are appropriate and that corrective action has been implemented effectively
- Communicate to the test coordinator and/or technical staff the need for immediate corrective action if an audit identifies test/QA plan deviations or practices that threaten data quality
- Review and approve test/QA plan amendments, deviations and audit reports
- Verify implementation of any necessary corrective action
- Request that Battelle's AMS Center Manager issue a stop work order if audits indicate that data quality is being compromised
- Provide a summary of the QA/QC activities and results for the verification reports
- Review the draft and final verification reports and verification statements.
- Delegate QA activities to other Battelle quality staff as needed to meet project schedules

A5.2 Technology Vendors

The responsibilities of the technology vendors are as follows:

- Review and provide comments on the draft test/QA plan
- Accept (by signature of a company representative) the final test/QA plan prior to test initiation

- Provide their technology for evaluation during the verification test
- Provide all other equipment/supplies/reagents/consumables needed to operate their technology for the duration of the verification test
- Supply training on the use of the technology, and provide written consent and instructions for verification staff to carry out testing, including written instructions for routine operation of their technology
- Provide maintenance and repair support for their technology, on-site if necessary, throughout the duration of the verification test
- Review and provide comments on the draft verification report and statement for their respective technology
- Recognize that results in the verification report will be reviewed by the cooperating ETV programs in order to determine if verification is applicable in those countries
- Allow for publication of the verification results by the cooperating ETV programs, if vendors are considered for verification in those countries.

A5.3 EPA

EPA's responsibilities in the AMS Center are based on the requirements stated in the "Environmental Technology Verification Program Quality Management Plan" (ETV QMP)[2]. The roles of specific EPA staff are as follows:

Ms. Michelle Henderson is EPA's AMS Center Quality Manager. For the verification test, Ms. Henderson will:

- Review the draft test/QA plan
- Perform at her option an external technical systems audit during the verification test
- Notify the EPA AMS Center Project Officer of the need for a stop work order if the external audit indicates that data quality is being compromised
- Prepare and distribute an assessment report summarizing results of the external audit
- Perform at her option audits of data quality

- Notify the EPA AMS Center Project Officer of the need for a stop or modify work order if the audit of data quality indicates that data quality is being compromised
- Review draft verification reports and verification statements.

Dr. John McKernan is EPA's Project Officer for the AMS Center. Dr. McKernan or designee will:

- Review the draft test/QA plan
- Approve the final test/QA plan
- Be available during the verification test to authorize any test/QA plan deviations by phone and provide the name of a delegate to the Battelle AMS Center Manager should he not be available during the testing period
- Review the draft verification reports and verification statements
- Oversee the EPA review process for the test/QA plan, verification reports, and verification statements
- Coordinate the submission of verification reports and verification statements for final EPA approval
- Post the test/QA plan, verification reports, and verification statements on the ETV web site.

A5.4 Verification Test Stakeholders

This test/QA plan and the verification report(s) and verification statement(s) based on testing described in this document will be reviewed by experts in the fields related to coliform detection. The following experts have been providing input to this test/QA plan and have agreed to provide a peer review:

- Rick Sakaji, East Bay Municipal Water District
- Jim Sinclair, Sandhya Parshionikar, Jennifer Best, and Keya Sen, EPA Office of Water

The responsibilities of verification test stakeholders (or expert peer reviewers) include:

- Participate in technical panel discussions (when available) to provide input to the test design
- Review and provide input to the test/QA plan
- Review and provide input to the verification report(s)/verification statement(s).

In addition, the general approach for verification testing was reviewed with the broader AMS Center Stakeholder Committee as a presentation during regular stakeholder teleconferences, including the November 12, 2009 meeting, and input from the committee was solicited.

A5.5 Cooperating ETV Programs

This test/QA plan will be reviewed by the cooperating ETV programs to confirm that the experimental design meets the requirements for possible verification in those countries. In addition, the verification report(s) based on testing described in this document will be reviewed to determine if verification is applicable. The following people from the cooperating ETV programs will review these documents and provide input

- John Neate and Mona El Hallak, ETV Canada
- Mette Tjener Andersson, Claus Jørgensen, and Christian Grøn, DANETV

Representatives of the cooperating ETV programs will:

- Participate in technical discussions during development of the test/QA plan to provide input to the test design
- Review and provide input to the test/QA plan
- Review and provide input to the verification report(s)
- Draft the verification statements for their respective program if results are determined to be acceptable.

A6 BACKGROUND

A6.1 Technology Need

The ETV Program's AMS Center conducts third-party performance testing of commercially available technologies that detect or monitor natural species or contaminants in air, water, and soil. The purpose of ETV is to provide objective and quality assured performance data on environmental technologies, so that users, developers, regulators, and consultants can make informed decisions about purchasing and applying these technologies. The ETV Water Stakeholder Committee, made up of buyers and users of such technologies recommend technology categories, and technologies within those categories, as priorities for testing. Among the technology categories recommended for testing are coliform detection technologies. In particular, the use of coliform detection technologies for the monitoring of drinking water (DW) was identified as an area of interest for technology verification. It is important to note that this verification test is independent of any activities related to approval of technologies for use in meeting regulatory requirements for the detection of total coliforms (TC) or *Escherichia coli (E. coli)*.

Fecal pollution can introduce disease-causing (pathogenic) bacteria, viruses, and parasites into receiving waters, which may serve as private/public DW supplies. Through the consumption of these waters, enteric and other pathogens can be transmitted to humans, resulting in gastrointestinal illness. 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 serving fecally polluted water to consumers. Based on the 1989 Total Coliform Rule (TCR), assessment of this health risk is based on the detection and enumeration of fecal indicator bacteria, such as TC and *E. coli,* whose presence indicates that the water may be contaminated with sewage or animal wastes. In this study, TC are defined as all facultative anaerobic, gram-negative, non-spore forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 hours (h) at 35^{o}C, and that develop red colonies with a metallic (golden) sheen within 24 h at 35^{o}C on a Endo-type medium containing lactose.[3] *E. coli* is defined as a species of coliform bacteria that possesses the enzyme β-glucuronidase and is capable of cleaving the fluorogenic substrate 4-methyllumbelliferyl-β-D-glucorinide (MUG) with the release of fluorogen when grown in a

MUG-based medium at 44.5°C within 24 ± 2 h or less, producing a blue fluorescence within the media and/or around the periphery of the colony.[3]

The 1989 TCR sets both goals and legal limits for the presence of TC in DW. The rule also details the type and frequency of testing that all public water systems must implement. The Maximum Contaminant Level Goal (MCLG[i]) is no detectable TC in DW. The Maximum Contaminant Level (MCL[ii]) is no more than 5% of samples positive for TC in a month. For water systems that collect fewer than 40 routine samples per month, no more than one sample can be total coliform-positive per month. If a sample is positive for TC, each total coliform-positive routine sample must be tested for the presence of fecal coliforms (fecal coliforms will be removed from the revised TCR) or *E. coli*, and repeat samples must be collected and analyzed for TC. As mentioned above, this verification test is not being conducted to provide data that will be used to approve technologies for use in meeting regulatory requirements for the detection of total coliform or *E. coli* as required by either the 1989 TCR or the upcoming revision to the TCR. It is being conducted, based on feedback from ETV AMS Center stakeholders, to provide a verification test that is similar in requirements to the current TCR approval process, such that technologies that are not already approved have an opportunity to be tested under similar set of test conditions.

Similar to the TCR in Europe, the Official Journal of the European Communities published Council Directive 98/83/EC in 1998 that provided directives on the regulation of public water systems within the European Union. Annex II and III within that document includes the standard of zero *E. coli* per 100 mL and suggests the International Standards Organization Method 9308 as the preferred method for regulatory *E.coli* analysis. More recently, the Colilert-18 method has been shown to be equivalent to the suggested method and therefore will be used to meet the DANETV requirement of European acceptance.

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[i] Maximum Contaminant Level Goal (MCLG) - Level of a contaminant in drinking water below which there is no known or expected risk to health. MCLGs allow for a margin of safety and are non-enforceable public health goals.
[ii] Maximum Contaminant Level (MCL) - The highest level of a contaminant that is allowed in drinking water. MCLs are set as close to MCLGs as feasible using the best available treatment technology and taking cost into consideration. MCLs are enforceable standards.

A6.2 Technology Description

The coliform detection technologies to be tested use chromatogenic and fluorogenic growth media to detect coliforms and *E. coli* based on the enzymatic activity of these organisms. The systems consist of single-use sample containers that contain pre-measured reagents and can be used in conjunction with a bench top incubator/analyzer/data logger system. In short, a 100 mL water sample is added to the containers and then incubated in and analyzed by the bench top instrument. In order to accommodate the large number of samples required for this test, the containers can be incubated in an external incubator, rather than in the bench top instrument, and then returned to the instrument for analysis. The enzymes produced by TC and *E. coli* cleave the chromatogenic or fluorogenic substrates in the growth media, resulting in the release of colored or fluorescent products. The bench top instruments include optical sensors and/or spectrophotometers that take readings of the sample at regular intervals to quantify the bacteria. The sample data and instrument calibration are stored on the instrument's internal computer until it is retrieved with a flash drive or some other portable data transfer device.

If no target bacteria are present in the sample, the sample color remains unchanged or fluorescence is not produced. If the target organisms are present, there is a progressive change in the color or fluorescence produced in the sample. For some technologies, results may be available within 60 minutes after sample collection/test initiation, depending on the number of bacteria present in the sample. However, the typical analyses are not completed until after 14 to 24 hours of incubation.

A7 VERIFICATION TEST DESCRIPTION AND SCHEDULE

This verification test will assess the performance of the coliform detection technologies relative to key verification parameters, including specificity and sensitivity. In performing the verification test, Battelle will follow the technical and QA procedures specified in this test/QA plan and will comply with the data quality requirements in the AMS Center QMP (refer to Table 11 in Section B).

A7.1 Verification Test Description

The objective of this verification test is to evaluate the technologies' performance detecting the presence or absence of TC and *E. coli* against spiked concentrations of target organism in DW. This verification test will take place at Battelle in Columbus, Ohio. Technologies undergoing verification will be used to analyze spiked DW samples for TC and *E. coli*. The presence/absence results from the technologies will be compared to the results from the reference methods, Standard Methods (SM) 9221B and F^3. These reference methods are recognized in the U.S. and Canada as the standard for regulatory purposes. Each technology in the test will be considered for possible verification in Canada. For those vendors that select the optional verification in Denmark, their results will also be compared with Colilert®-18 as this product has recently been shown to be equivalent to the European Union approved method and therefore will be used to meet the DANETV requirement of European acceptance.

Subsequent to the verification test, verification reports describing the test will be drafted. These reports will be reviewed by the vendor and by peer reviewers, revised, and submitted to EPA for final approval. In performing the verification test, Battelle will follow the technical and QA procedures specified in this test/QA plan and will comply with the data quality requirements in the AMS Center QMP. As a point of clarification, an ETV verification does not imply EPA approval for use to meet regulatory requirements by current or future regulations (such as the TCR).

A7.2 Proposed Verification Test Schedule

Table 1 shows the proposed schedule of testing, auditing, and data analysis/reporting activities to be conducted during this verification. The verification of coliform detection technologies is planned to be completed over the course of approximately three weeks after the start of laboratory testing. The verification test is expected to be performed in July-August 2010. The technical systems audit (TSA) will take place during testing; the data quality audit (DQAs) will take place after the data are reviewed by the Test Coordinator, or designee. A daily overview of the technical activities to be performed during the verification test is presented in Section B1.2.

Table 1. Proposed Verification Test Schedule

Approximate Date(s)	Testing Activities	Data Analysis and Reporting
January – June 2010	Test/QA plan design and approval	Not applicable
July and August 2010	Verification testing Perform Technical Systems Audit Audit of data (1st batch) Reference analysis	Compile data from technologies Review and summarize testing staff observations Compile reference method results Perform data analysis Begin draft reports
August 2010	Audit of data (remaining data) Prepare draft verification reports and statements	Complete draft verification reports and statements
August 2010	Coordinate reviews of draft verification reports and statements	Complete peer review and vendor review of draft reports
September 2010	Prepare final verification reports and statements	Revise draft verification reports and statements Submit final reports for EPA approval[1]

[1] Indicates EPA approval of ETV reports for release and does not imply approval for regulatory use

A8 QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA

In performing the verification test, Battelle will follow the technical and QA procedures specified in this test/QA plan and will comply with the data quality requirements in the AMS Center QMP. QA Category II, Highly Visible Project, has been specified for this test by the EPA Project Officer.

To ensure that this verification test provides suitable data for a robust evaluation of performance, a variety of quality control (QC) samples will be incorporated for this test. Their acceptance criteria are defined in Section B5. The acceptance criteria for the QC samples indicate the minimum required performance to meet the objectives of the verification test and are used as the QC criteria for laboratory measurements. The QC samples and acceptance criteria for this verification test were established to assess the performance of the coliform detection technologies relative to the reference method. In order to provide a suitable benchmark for comparison, the reference measurements must meet the minimum requirements of the QC sample acceptance criteria.

Method and reagent blank samples will be required to demonstrate freedom from contamination, and positive and negative control cultures (American Type Culture Collection

[ATCC]) will be processed to demonstrate the laboratories can accurately identify the target organism and that the media and confirmation testing are providing appropriate results. ATCC strains shown in Table 2, which are commonly used for water quality testing, will be acquired from MicroBioLogics®. A Certificate of Analysis is provided with each strain that demonstrates culture purity based on macroscopic, microscopic, and biochemical (phenotypic) testing.

Table 2. Quality Control Strains

Targeted Coliform	Positive Control	Negative Control
Total Coliform	*Enterobacter aerogenes* ATCC 13048	*Pseudomonas aeruginosa* ATCC 10145
Fecal Coliform	*Escherichia coli* ATCC 8739	*Enterobacter aerogenes* ATCC 13048

The Battelle Quality Manager or designee will perform a technical systems audit (TSA) at least once during this verification test to augment these QC criteria. The EPA Quality Manager also may conduct an independent TSA, at her discretion.

A9 SPECIAL TRAINING/CERTIFICATION

Documentation of training related to technology testing, data analysis, and reporting is maintained for all Battelle technical staff in training files at their respective Battelle location. Prior to the start of verification testing, the Battelle Quality Manager will verify the presence of applicable training records, such as documentation, chain-of-custody, reference and technology test methods, and equipment training. The vendors will be required to train the Battelle technical staff prior to the start of testing. Battelle will document this training with a consent form, signed and dated by the vendor, which states which Battelle technical staff have been trained to use their technology and can train other staff. In the event that other staff members are required to use the technologies, they will be trained by the operators that were trained by the vendors. All technical staff will have a minimum of a bachelor's degree in science or equivalent work experience (e.g., experience performing bacterial analysis).

Battelle will conduct all verification testing following the biosafety guidelines[4] established at the Battelle laboratories (Battelle Building 20 Biosafety Manual, Version 4.0). Exposure to biological organisms will be minimized, personal protective equipment will be worn, and safe laboratory practices will be followed.

A10 DOCUMENTATION AND RECORDS

The documents for this verification test will include the test/QA plan, vendor instructions, reference methods, verification reports, verification statements, and audit reports. The project records will include laboratory record books (LRB) and data collection forms, supporting laboratory records, training records, electronic files (both raw data and spreadsheets), and QA audit files. Table 3 summarizes the types of data to be recorded. Documentation of Battelle staff training by vendors and copies of other project specific training will also be included in the project files. All of these records will be maintained in the Verification Test Coordinator's office during the test and will be transferred to permanent storage at Battelle's Records Management Office (RMO) at the conclusion of the verification test. All Battelle LRBs are stored indefinitely with the project files by Battelle's RMO. Section B10 further details the data management practices and responsibilities.

All data generated during the conduct of this project will be recorded directly, promptly, and legibly in ink. All data entries will be dated on the date of entry and signed or initialed by the person entering the data. Any changes in entries will be made so as not to obscure the original entry, will be dated and signed or initialed at the time of the change and shall indicate the reason for the change. Project-specific data forms will be developed prior to testing to ensure that all critical information is documented in real time. The draft forms will be provided to the Battelle QA Manager for review.

Table 3. Summary of Data Recording Process

Data to Be Recorded	Responsible Party	Where Recorded	How often recorded	Disposition of Data
Dates, times of test events	Battelle	Laboratory record books or data collection sheets	Start/end of test, and at each change of a test parameter	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Test parameters	Battelle	Laboratory record books or data collection sheets	When set or changed, or as needed to document test notable details during testing	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Sample collection data	Battelle	Laboratory record books or data collection sheets	During each sampling event	Used to document collection, handling, and storage of sewage and drinking water samples
Reference sample data	Battelle	Laboratory record books or data collection sheets	When test samples are aliquotted for the reference analysis	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Reference method sample analysis, chain of custody, and results	Superior Laboratories, Inc.	Laboratory record books, chain of custody forms, data collection sheets, or data acquisition system, as appropriate	Throughout sample handling and analysis process	Transferred to spreadsheets/agreed upon report; project files. Retained for documentation of reference method performance

SECTION B
MEASUREMENT AND DATA ACQUISITION

B1 EXPERIMENTAL DESIGN

Technologies undergoing verification will be used to analyze spiked DW samples for TC and *E. coli*. Technology operation and sample handling and analysis will be performed according to the vendor's instructions. The results from the coliform detection technologies will be evaluated by comparing the proportion of positive results to the proportion of positive results produced by SM9221B and F which includes the comparison of false positive rate (or specificity) and false negative rate (or sensitivity. In addition, operational factors such as ease of use, required reagents, analysis time, and laboratory space and utilities required will be documented and reported.

B1.1 Verification Test Sample Preparation

The preparation of verification test samples includes the collection of raw sewage as the source of the target organisms, collection of the DW sample, the fortification of the DW sample with target organisms, and the chlorine stressing and dilution of samples for analysis. The sample preparation steps and timeline are illustrated in Table 4 and described in detail in the sections B1.1.1 through B1.1.3.

Table 4. Sample Preparation Timeline

Day	Process	Battelle Key Activities
1	Collect Sewage	• Collect sewage in early AM from wastewater treatment plant • Remove excess solids by filtration • Characterize sewage - Enumerate total coliform (TC) and *E. coli* via Standard Method (SM) 9222B/G - Enumerate total heterotrophic bacteria via standard spread plating (R2A agar) • Measure pH (SOP GEN.V-003-10), free and total chlorine (HACH Method 8021 & 8167)
	Collect Drinking Water	• Collect approximately 50 L drinking water from tap in Battelle laboratory • Measure pH (SOP GEN.V-003-10), free and total chlorine (HACH Method 8021 & 8167) • Adjust 30 L DW to 2.0-2.5 mg/L free chlorine for chlorine stressing
	Chlorine Stressing (stress bacteria; reduce TC/*E. coli* by 2-4 orders of magnitude) (prepare spiked, stressed TC or *E. coli* DW)	• Spike three 10 L DW samples with 200 mL filtered sewage (targeting 10^{4-5} org./100 mL for TC or *E. coli*) • Dechlorinate after 2.5, 5, and 10 minutes • Enumerate each spiked, stressed, DW sample (SSDW) • Refrigerate SSDW
2	Prepare dilutions for sample analysis	• Based on Day 1 enumeration results, prepare three 10 L dilutions of SSDW targeting 0.1, 1, and 10 TC or *E. coli*/100 mL from one of the three SSDW 10 L aliquots
	Complete Sample Preparation; Initiate Verification Testing	• Dispense 20 replicate 100 mL aliquots of each dilution into sterile reservoirs (total of 60 samples plus 1 negative matrix control, 1 negative TC control, , 1 positive TC/negative *E. coli*, and 1 positive *E. coli* for a total of 64 samples per method) • Promptly transfer required samples to the reference laboratory[a] on wet ice to begin Reference testing (SM 9221B/F) and Colilert®-18 testing • Initiate vendor analysis at Battelle

[a] Superior Laboratories, Inc., 1075 Amity Road, Galloway, OH 43119-9304, (614- 793-2172). Attn: James Gossard

B1.1.1 Sewage and Drinking Water Sample Collection

A single raw sewage sample (approximately 1 L), will be collected in the morning hours from the influent sewage line entering the Southerly Wastewater Treatment Plant (SWTP) in Columbus, OH by SWTP staff. The sewage will be immediately stored on wet ice, and transported by Battelle staff to Battelle laboratories. Upon receipt, the sewage will be filtered through a Whatman No. 2 pleated filter under vacuum using a Buchner funnel to remove excess solids, shaken vigorously for 1 minute to insure homogeneity, and then immediately characterized as follows:

- Measure pH using calibrated pH meter (SOP GEN.V-003-10)[5]

- Measure free chlorine and total chlorine using a HACH Chlorine test kit [(HACH Method 8021 (free) and Method 8167 (total); procedures are equivalent to U.S. EPA Standard Method 4500-CL G for drinking water]
- Quantify the total, culturable heterotrophic bacteria by enumerating onto R2A agar using a standard spread plating method as described in the AOAC's Bacteriological Analytical Manual[6].
- Quantify TC and *E. coli* (organisms per 100 mL) using quantitative methods for TC (SM 9222B – m-Endo) and *E. coli* (SM 9222G – NA-MUG)[3].
  - Based on recent analysis of sewage samples collected from SWTF, the concentration of total coliform and *E. coli* is expected to be in the range of 10^{6-7} organisms/100 mL.
  - To obtain readable filters, serial dilutions of the sewage will be prepared
  - Total coliform concentrations will be based on the number of typical coliform colonies (pink to dark-red with metallic surface sheen) observed on m-Endo.
  - To determine *E. coli* concentrations, the filter membranes will be transferred from m-Endo onto NA-MUG, incubated for 4 hours at 35±0.5°C, and then held under a long-wave length ultraviolet light. Colonies that fluoresce blue are counted as *E. coli*.

A single DW sample (approximately 62 L) will be collected into multiple, sterile carboys the same day the sewage sample is collected. This volume will accommodate the preparation of a complete set of 100 mL aliquots for one round of verification testing for TC and *E. coli*. Table 5 shows that approximately 62 L of DW will be required to prepare three 10.5 L volumes for chlorine stressing (32 L) and to prepare the three dilutions for sample analysis from one of the chlorine stressed samples (30 L). These diluted samples will be the source of the 100 mL samples that will be analyzed by the technologies being tested as well as the reference methods.

The DW sample will be collected as follows:

(1) Remove faucet screen if present, and surface decontaminate with 70% isopropanol.

(2) Purge line for 2 to 3 minutes with cold water.

(3) Collect approximately 62 L DW from the tap into multiple sterile (autoclaved) carboys equipped with a spigot and containing large stir bars.

Once collected, a 100 mL aliquot will be collected from the carboy's spigot and used to characterize the DW by measuring the pH (SOP GEN.V-003-10) and the concentration (mg/L) of free and total chlorine using a HACH Chlorine kit (HACH Methods 8021 and 8167). Since recent chlorine tests of the tap water indicate the water generally contains 0.2 to 1.5 mg/L free chlorine, sodium thiosulfate will not be added prior to the chlorination step. The DW sample will be used the same day as collected for the chlorine stressing and stored at 2°C to 8°C and used the following day to prepare the diluted samples for analysis. DW used to dilute the stress samples will be dechlorinated prior to use.

Table 5. Volume of Drinking Water Required for Verification Testing

Step	Description	DW Volume Needed
Chlorine Stressing	Prepare three 10.5 L spiked aliquots for three timed exposures	32L
Dilution and Sample Preparation	Prepare three 10.5 L dilutions from one of the 10.5 L Chlorinated aliquots above ; 2 L required for each method (60 x 100 mL); -control preparation -replicate 100 ml samples will be generated from these three dilutions	Approx. 30 L (Volume required for prep. of three dilutions depends on concentration of chlorinated sample selected for sample prep.)
	Total=	62L

B1.1.2 Chlorine Stressing and Preparation of Samples for Verification Testing

The goal of the chlorine stressing step is to reduce the number organisms in the spiked DW samples by two to four orders of magnitude, starting at approximately 10^5 target organisms/100 mL. In addition, this step also stresses the organisms with chlorine. Three spiked DW samples will be chlorinated for 2.5, 5, or 10 minutes, after which time they will be dechlorinated with sodium thiosulfate and subsequently enumerated to determine the reduction of target organisms in each spiked, stressed drinking water (SSDW) sample. Chlorine stressing will be initiated the day the sewage and DW samples are collected in order to minimize the storage time between sewage and DW collection and the start of sample analysis. Following

chlorine stressing, one of the three 10 L dilutions of SSDW will be selected to prepare a set of three 10 L dilutions prepared into dechlorinated drinking water targeting a range of 0.1 to 10 target organisms/100 mL. The chlorine stressing and sample preparation steps are detailed below:

Chlorine Stressing Procedure:

(1) Dispense 10.5 L of the original DW sample into each of three, sterile 10 L carboys containing stir bars. Place carboys on stir plates and mix at medium speed.

(2) Measure the total residual and free residual chlorine concentration using a HACH Chlorine test kit (approved for SM 4500-Cl- G[3]) in each carboy. If necessary, adjust the free residual chlorine level to 2.0-2.5 mg/L using a 4% solution (40,000 mg/L) of sodium hypochlorite. Since the spiked DW sample will generally have a low chlorine demand, overstressing or killing the organisms by prolonged exposure to free chlorine must be avoided. The total residual chlorine level should not exceed 2.5 mg/L.

(3) Measure pH by collecting a 10 mL aliquot from each carboy and measure using a calibrated pH meter, following SOP GEN.V-003-10[5].

(4) Measure temperature of water in each carboy using a calibrated thermometer. Thermometers are calibrated according to SOP GEN.V-013-04[7]. [Surface decontaminate thermometer with 70% isopropanol before each use].

(5) Start chlorine stressing by spiking each 10.5 L DW sample with 200 mL filtered sewage to achieve a concentration of approximately 10^5 target organisms/100 mL. The concentration of TC or *E. coli* will be assumed to be 10^4-10^5 org./mL (as indicated by analysis of sewage collected over the past several weeks leading up to the verification study).

(6) At each of three time points (2.5, 5, and 10 minutes after spiking with sewage):

   a. Add 8.6 mL 10% (w/v) sodium thiosulfate solution (i.e., 0.8 mL per L) to stop chlorine oxidation (dechlorinate). Allow to mix for at least 1 minute.

   b. Measure pH and temperature of sample.

   c. Measure free residual chlorine and total chlorine.

(7) Enumerate the target organism densities of each SSDW via SM 9222B for TC and SM 9222G for *E. coli*. .

a. Enumerate triplicate 100 mL aliquots of undiluted sample, and triplicate 100 mL aliquots of 10^{-1} and 10^{-2} dilutions of the sample; using phosphate buffered water as the diluent.

b. Calculate TC and *E. coli*/100 mL in each 10 L sample.

(8) Calculate the reduction of TC and *E. coli* based on the estimated spiking (starting) concentration and the concentrations after each exposure time.

If none of the exposure times used was adequate to achieve the desired reduction of two to four orders of magnitude, the entire process will be repeated using a freshly collected sewage sample and DW. The measured density of chlorine-stressed target organisms confirms that a two to four order of magnitude reduction has been attained, and can then be used to prepare three dilutions that target a density of 0.1 to 10 target organisms/100 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, the ultimate objective is to have one of the prepared dilution sets provide 50 ±25% positive results for TC or *E. coli* (depending on which is being targeted) with the reference method(s).

Based on the results of the chlorine stressing step, three 10.5 L volumes of DW with TC or *E.coli* concentrations of 0.1 org./100 mL, 1 org./100 mL, and 10 org./100 mL, respectively, will be prepared, and replicate 100 mL aliquots of each dilution will be subsampled for verification testing. Preparation of the dilution samples will be performed as follows:

(1) Calculate the volume of the original DW needed to prepare 10.5 L of each of three dilutions, and dispense this volume into one or more carboys. Measure the free chlorine concentration, and dechlorinate if the free chlorine concentration is in the range of 0.2 – 0.5 mg/L by adding 0.8 mL 10% sodium thiosulfate per 1 L. Use this dechlorinated DW (DDW) to prepare the dilutions.

(2) Accurately dispense the appropriate volume of DDW to each of three sterile carboys having spigots and containing large stir bars.

(3) Spike the DDW with the appropriate volume of SSDW sample to each of the three carboys to generate suspensions of 0.1 org./100 mL, 1 org./100 mL, and 10 org./100 mL.

(4) Each dilution will be treated as follows:

a. Mix on stir plate for 5-10 minutes.

b. During continuous mixing on the stir plate, dispense 100 mL aliquots into sterile 100 mL bottles using 50 mL and/or 100 mL graduated pipets. All bottles will be pre-labeled with a unique sample ID.

c. Store bottles at 2 °C to 8 °C as they are generated.

d. Immediately after all samples have been dispensed for the reference methods, transport them in coolers packed with ice packs to Superior Laboratories, Inc., which is approximately a 15 to 20 minute drive from the sample preparation laboratory. Reference method analysis following SM9221B/F and Colilert®-18 will commence the same day as arrival at the laboratory.

Once all 100 mL aliquots are dispensed for technology verification (20 at each dilution level for a total of 60 replicates per technology), verification testing will begin. Figure 2 illustrates the experimental design for the sample dilution. Not shown in Figure 2 are the QC samples which will be prepared and analyzed at the same time.

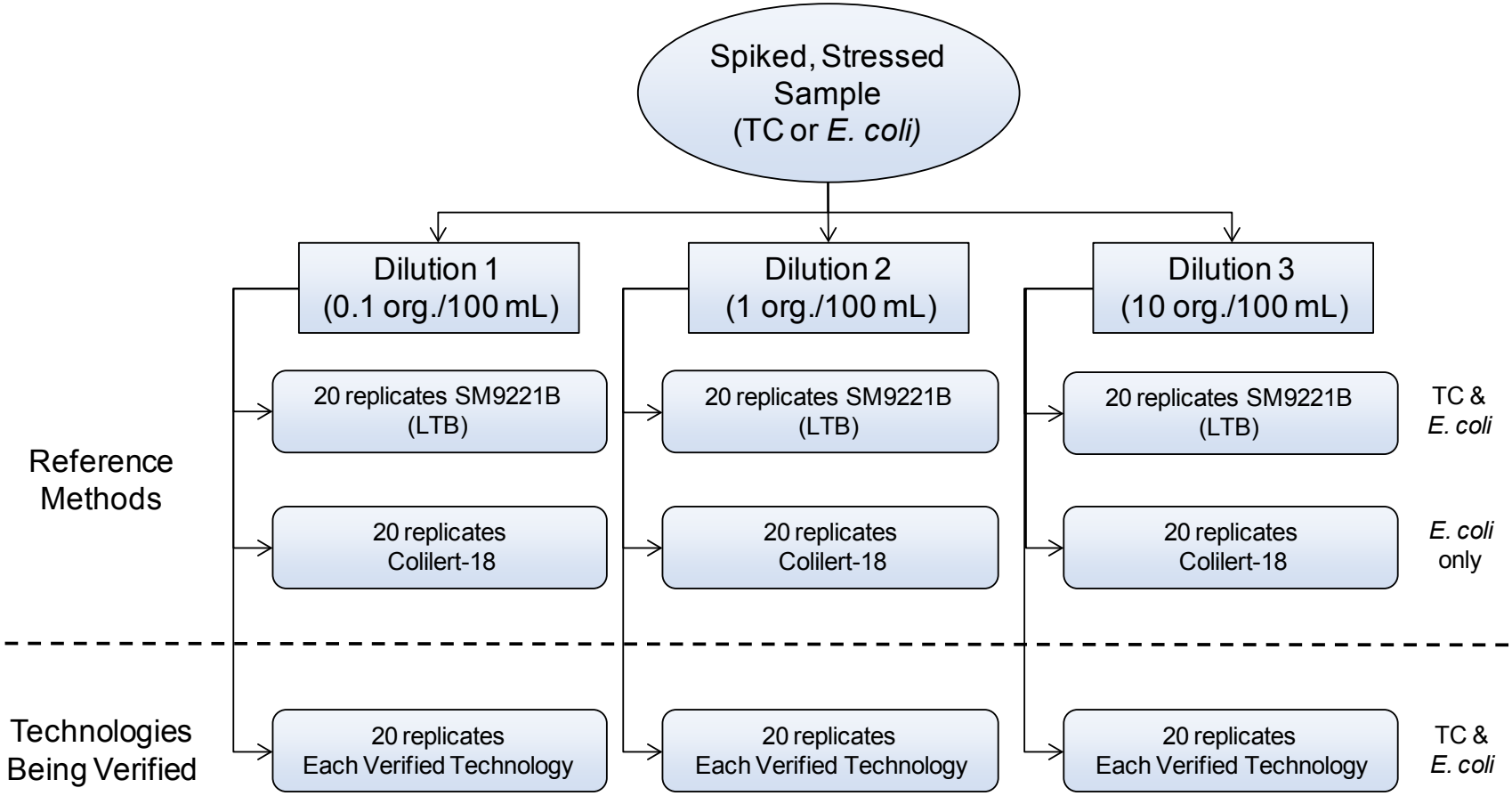

Figure 2. Experimental Design for Sample Dilution and Analysis

B1.2 Sample Analysis

The number of replicates to be analyzed by each technology and reference method was selected following a power analysis based on a Pearson chi-square test for equality of proportions associated with two independent populations, where the test is two-sided (i.e., test for equality versus any type of non-equality). The populations being compared are the two test types (reference and technologies being verified), and the proportion corresponds to the proportion of positive results out of the total tests performed. Conducted using the POWER procedure in the SAS System, the power analysis determined the number of replicate tests (across both test types) that is 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 will be 0.25 to 0.75 for this test), and a significance level of 0.05 for the test. Table 6 gives the power analysis results for reference method positive proportions of 0.25, 0.5, and 0.65. The results indicate that approximately 20 replicate samples of the technologies being tested will be adequate to determine significant differences in the proportion of positive results generated by the technologies being tested and the reference method. The absolute differences in proportion relative to the reference test that is shown through this power analysis to be detectable as significantly difference were 0.45, 0.4, and 0.325 (shaded results) for the three reference method positive proportions of 0.25, 0.50, and 0.65, respectively. Differences in positive proportion less than those may be detectable, but not with 80 percent power under the assumptions of the test.

As shown in Table 6, the use of approximately 20 replicates provides a likelihood that a significant difference can be determined between the reference method and the technologies being tested. If a significant difference is determined based on the results of this ETV test, the result means that the reference method and technology being tested are at least different by the shaded ratios shown in the table. Upon discussion with the peer reviewers for this verification test, it was determined that use of 20 replicates would provide data that was useful to the end user of these technologies. The reviewers determined that, because this test does not have regulatory implications, the result will be valuable as a screening tool to help with purchase decisions.

Table 6. Power Analysis Results for Determination of Replicates

Reference Method Ratio of Positive Results	Reference Method N	Tested Technology Ratio of Positive Results	Difference in Positive Proportion Between Reference and Technology	Required Tested Technology N for Significant Difference	Test Method Positives	Test Method Negatives
0.25	71	0.025	0.225	71	2	69
	152	0.10	0.15	152	15	137
	1,251	0.20	0.05	1,251	251	1,001
	1,251	0.30	0.05	1,251	375	876
	152	0.40	0.15	152	61	91
	58	0.50	0.25	58	29	29
	31	0.60	0.35	31	19	12
	19	0.70	0.45	19	13	6
0.50	15	0.05	0.45	15	1	15
	20	0.10	0.4	20	2	18
	39	0.20	0.3	39	8	31
	93	0.30	0.2	93	28	65
	388	0.40	0.1	388	233	155
	388	0.60	0.1	388	155	233
	93	0.70	0.2	93	28	65
	39	0.80	0.3	39	31	8
	20	0.90	0.4	20	18	2
0.65	22	0.325	0.325	22	7	15
	39	0.390	0.26	39	15	24
	189	0.520	0.13	189	98	91
	804	0.715	0.065	804	575	229
	189	0.780	0.13	189	147	42
	39	0.910	0.26	39	36	3
	22	0.975	0.325	22	21	1

The ability of each coliform detection technology to determine the presence of TC and *E. coli* will be challenged using spiked stressed DW samples. Positive/negative control samples spiked with quality control cultures listed in Table 2 will be included during testing. The technologies will be operated according to the manufacturer's instructions and vendor will provide the necessary equipment and supplies to analyze up to sixty 100 mL samples at a given time. The dilution samples generated in Section B1.1 will be assayed by the reference methods and the technology methods concurrently. The reference analyses will be conducted as described

in Section B4. Table 7, which is continued from Table 4, presents an overview of the daily verification test activities during days 3 through 7. The details of the activities are described in sections B1.2.1 and B1.2.2.

Table 7. Sample Analysis Timeline

Day	Battelle and Superior Key Activities				
	9221B (Superior)	**9221F (Superior)**	**Colilert®-18 (Superior)**	**Vendor Technologies (Superior)**	**Vendor Technologies (Battelle)**
1	Sewage and DW collection, and chlorination (refer to Table 4)				
2 (Dilutions already prepared)	100 mL SSDW into LTB		Start analysis on replicate 100 mL samples		Replicate 100 mL samples into vendor supplied containers; start analysis
3	24h LTB into BGLB /EC-MUG	24 h LTB into EC-MUG	Complete tests	Receive Vendor analyzed samples; start Confirmed phase by inoculating LTB	Vendor Results; transport all samples to Superior Labs
4	48h LTB into BGLB /EC-MUG	48 h LTB into EC-MUG		24h LTB into BGLB /EC-MUG	
5	Complete tests from Day 3	Complete with UV test		48h LTB into BGLB /EC-MUG	
6	Complete tests from Day 4			Complete tests from Day 4	
7	(Optional) Additional completed tests if deemed appropriate			Complete tests from Day 5	
8				(Optional) Additional completed tests if deemed appropriate	
9					

The data used for verification will be from the dilution(s) which produces results closest to an equal number of positive (50%) and negative (50%) results for the reference method. A 50 ± 25% split in responses (in either direction; i.e., 25:75 or 75:25% split) will be sought. For comparability, the evaluated results for the coliform detection technologies will be from the same dilution level as the 50 ± 25% positive reference method results. If none of the dilutions

produces an acceptable split in positive and negative results for the reference method, the process may be repeated starting with the collection of fresh sewage and DW. The results from the dilution sets not used for the data evaluation will be documented, but the samples will be discarded. Confirmation tests will be conducted on the dilutions to be used for the data evaluation.

B1.2.1 Confirmation of Results

All sample results must be confirmed with more definitive tests in order to adequately compare results between the reference method and the technologies being tested. Confirmation for both the SM 9221B and F reference methods, as well as the technology methods, is described below. Figure 3 illustrates the process by which all positive and negative samples from the verification technologies and SM 9221B and F are confirmed. Although all samples will be processed through the confirmation phase, only the results from the dilution of reference samples that gives the 50 ± 25% positive result will be used to compare the technologies to the reference methods.

Confirmation of reference method (SM 9221B and F) results

1) All lauryl tryptose broth (LTB) bottles showing acidic reaction (distinct yellow color) within 24 ± 2 hours of incubation will be submitted for confirmatory testing according to SM 9221B using inoculation into brilliant green lactose bile (BGLB) broth. Negative LTB samples will continue to incubate.
    - If acidic reaction in LTB is displayed at the end of a 48 ± 3 hour incubation period, these samples will also be submitted to the confirmed phase by simultaneous inoculation into BGLB and EC-MUG broth
    - The absence of acidic reaction in LTB at the end of 48 ± 3 hours of incubation constitutes a negative test for TC and *E. coli*.
2) Formation of gas in the BGLB broth fermentation tube at anytime within a 48 ± 3 hour incubation period constitutes a positive confirmed phase for TC.
3) After incubation in EC-MUG, the appearance of a blue fluorescence under long-wave (approximately 366 nm) UV light after 24 h incubation at 44.5^{o}C constitutes a positive confirmed phase for *E. coli*.

4) As additional, optional confirmation, a complete test for TC may be performed by inoculating MacConkey media and then selecting suspected TC colonies and inoculating into LTB, as described by SM 9221B.

No confirmation testing is required for Colilert®-18. Positive Colilert®-18results for TC are indicated by presence of yellow color equal to or greater than the Colilert®-18comparator control after 18 h at 35°C. The appearance of blue fluorescence when exposed to long-wave UV light represents a positive reaction for *E. coli*.

Confirmation of the samples analyzed by the vendor technologies will be performed as follows:

(1) Transport all vendor samples to Superior Labs after all vendor analyses have been performed.

(2) .For each vendor samples following proper incubation time and result determination (regardless if positive or negative): inoculate 10 mL LTB into test tubes containing Durham tubes with 1 mL of vendor sample to initiate confirmation tests.

(3) Confirm results as illustrated in Figure 3 and following SM 9221B and SM9221F.

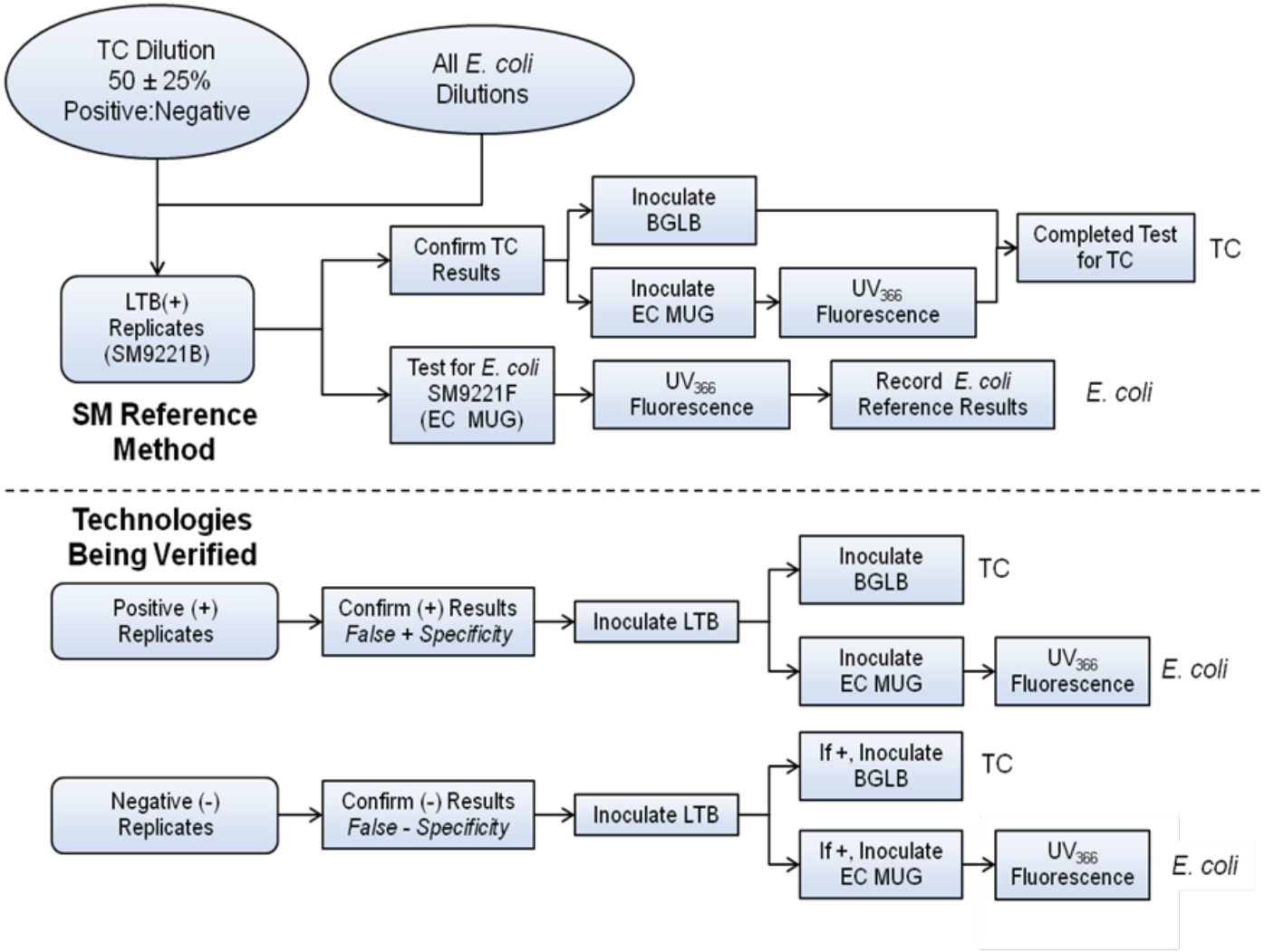

Figure 3. Flowchart describing confirmation analyses for both the technologies being verified and SM 9221B

B1.3 Statistical Analysis

B1.3.1 False Positive Rates, False Negative Rates, Sensitivity, and Specificity

False positive (FP) and false negative (FN) rates of reference methods will be evaluated when assessing comparability. During this test, true positives are those positive results from the technologies being tested that are confirmed, and false positives are those positive results from the technologies being tested that are not confirmed as directed by the reference method. Conversely, true negative results are those negative results that are confirmed as negative, and false negative results are those negative results that were shown to be positive by the confirmatory method. Performance of the coliform detection technologies will be tested by comparing the proportion of true positive results from those technologies to the proportion of positive results from the SM 9221B and F and Colilert®-18.

Specificity is defined as the percent of negative samples correctly identified as negative, and sensitivity is defined as the percent of positive samples correctly identified as positive. Estimates of sensitivity, specificity, false positive rates, and false negative rates as percentages for the two methods will be calculated as follows:

$$\text{Sensitivity}_i = \frac{TP_i}{TP_i + FN_i} \times 100\%$$

$$Specificity_i = \frac{TN_i}{TN_i + FP_i} \times 100\%$$

$$False\ positive\ rate_i = \frac{FP_i}{TN_i + FP_i} \times 100\% = \left(1 - \frac{TN_i}{TN_i + FP_i}\right) \times 100\%\ 1 - Specificity_i$$

$$False\ negative\ rate_i = \frac{FN_i}{TP_i + FN_i} \times 100\% = \left(1 - \frac{TP_i}{TP_i + FN_i}\right) \times 100\% = 1 - Sensitivity_i$$

Where,

F = False
N = Negative
P = Positive
T = True
i = specified method (i = 1 for technology being tested, i = 2 for reference method)

B1.3.2 Method Comparability

The chi-square test will be used to compare the percent of false positives and false negatives in the coliform detection technologies with the percent of false positives and false

negatives produced by the reference method. To perform the chi-square test, the data will first be arranged in the format shown in Tables 8 and 9:

Table 8. False Negative Rate Comparison

		Method		
		Tested Technology	**Reference**	**Total**
Result	**True +**	TP_1	TP_2	$TP_1 + TP_2$
	False -	FN_1	FN_2	$FN_1 + FN_2$
	Total	$TP_1 + FN_1$	$TP_2 + FN_2$	$TP_1 + TP_2 + FN_1 + FN_2$

Table 9. False Positive Rate Comparison

		Method		
		Tested Technology	**Reference**	**Total**
Result	**True +**	FP_1	FP_2	$FP_1 + FP_2$
	False -	TN_1	TN_2	$TN_1 + TN_2$
	Total	$FP_1 + TN_1$	$FP_2 + TN_2$	$FP_1 + FP_2 + TN_1 + TN_2$

In order to assess whether the false positive and false negative rates differ between methods, chi-square tests will be run over all samples. For false negative rates, the chi-square test(s) indicate whether the proportions of negative samples correctly identified as negative by the two methods are significantly different, and for false positive rates, the chi-square test(s) indicate whether the proportions of true positive results correctly identified as positive for the two methods are significantly different.

B1.3.3 Operational Factors

Operational factors such as maintenance needs, calibration frequency, data output, consumables used, ease of use, repair requirements, waste production, and sample throughput will be documented based on operator and Verification Test Coordinator observations.

B1.4 Additional Concentration Levels

An optional component of the ETV test will be performed to verify the capability of each technology to detect *E. coli* ATCC 8739 at various concentration levels. Four target inoculations will be prepared in dechlorinated DW. A stock solution will be prepared that contains

approximately 10^4 *E. coli* per 100 mL, and then a serial dilution (1:10, 1:100, and 1:1,000) of the stock will be prepared to obtain four separate samples for testing (10, 100, and 1,000 *E. coli* per 100 mL). Four replicates of each sample will be analyzed to establish kinetic data and "early warning" capabilities of these technologies. The data from these tests will identify: 1) whether or not each technology detects the presence of *E.coli* and 2) the time required for detection. Triplicate aliquots at each will also be analyzed using a quantitative method for *E. coli* (SM 9222G – NA-MUG).

B1.5 Reporting

Separate verification reports and verification statements are planned for each vendor that is participating in the verification testing. The statistical comparisons described above will be conducted separately for each of the technologies being tested, and information on the operational factors will also be compiled and reported separately for each technology. The verification reports will present the test procedures, test data as statistical evaluation of those data, and discuss any deviations from the approved test/QA plan.

Operational aspects of the technologies will be recorded by the testing staff at the time of observation during the verification test, and summarized in the verification reports. The verification report and verification statements will briefly describe the ETV program, the AMS Center, and the procedures used in verification testing. The results of the verification test regarding coliform detection technology performance will be stated quantitatively. Each draft verification report and verification statement will be subjected to review by the vendor, EPA, and other peer reviewers. The resulting review comments will be addressed in a subsequent revision of the report or statement, and the peer review comments and responses will be tabulated to document the peer review process and submitted to EPA. The reporting and review process will be conducted according to the requirements of the AMS Center QMP. The cooperating ETV programs will deem whether the results are acceptable. If acceptable, a separate verification statement will be prepared by the cooperating ETV program.

B2 SAMPLING METHOD REQUIREMENTS

Sampling method requirements are described above in Section B1.1.1.

B3 SAMPLE HANDLING AND CUSTODY REQUIREMENTS

Sample custody will be documented for the DW, SWTP water, and reference test samples following Battelle SOP ENV-ADM-009 for Chain of Custody[8]. A chain-of-custody (COC) form will include details about the sample such as the time, date, location, and person collecting the sample. The COC form will track sample release from the sampling location to the testing laboratory. The COC form will be signed by the person relinquishing samples once that person has verified that the COC form is accurate. Upon arrival at the testing laboratory, COC forms will be signed by the person receiving the sample once that person has verified that all samples identified on the COC forms are present. Copies of all COC forms will be delivered to the Verification Test Coordinator and maintained with the test records.

B4 LABORATORY REFERENCE METHODS

Technology verification will involve comparison of the results from each coliform detection technology being verified to the results obtained from appropriate reference methods which are described further in Table 10. Superior Laboratories, Inc. will perform the reference analysis, including all QA/QC procedures, and will comply with this test/QA plan and all requirements of the ETV QMP. Prior to testing, the Battelle QA Manager will contact them and confirm that adequate quality systems are in place and then a TSA will include that laboratory. Any options described in the method will be considered and determined prior to testing and documented in the laboratory records. All laboratory records required by the method will be maintained. The reference methods chosen for the U.S. verification test are presence/absence methods for TC and *E. coli* per SM 9221B (TC) and 9221F (*E. coli*). These methods utilize selective and/or chromatogenic liquid growth media to detect TC and *E. coli*. In addition, an optional verification of these technologies for the detection of *E. coli* will be performed using Colilert®-18. The two reference methods (SM and Colilert®-18) will be run in parallel during the verification test, and the results for both will be reported as presence/absence. Reference data from the Colilert®-18 method will be compared to only those technologies for which the vendor has agreed to verification of *E. coli* results.

Table 10. Reference Methods

Analyte	Method Format	Approving Agency	Reference Method	Citation
Total coliforms	P/A	U.S. EPA	SM9221B	40 CFR 141.21[iii]
E. coli	P/A	U.S. EPA	SM9221F	40 CFR 141.21[iv]
E. coli	P/A	European Union	Colilert-18	Drinking Water Directive 98/83/EC (3 November 1998)

P/A = presence/absence
Colilert®-18method is an optional reference method and will only be included in the verification test for each of the technologies upon the vendor's agreement.

B5 QUALITY CONTROL REQUIREMENTS

Quality control procedures will follow the requirements described in this test/QA plan, ETV QMP, and any vendor specified requirements for the coliform detection technologies. Some of the technologies may require calibration before testing begins. If so, the calibration method and results will be documented.

The reference method requires the use of method blanks (MB), positive and negative control organisms, and result confirmation (Table 11). One MB will be performed during the analysis for every 20 samples analyzed. The MB will consist of 100-mL dechlorinated tap water processed as a sample. MB samples will be exposed to identical handling and analysis procedures as the DW samples, including the addition of all reagents. These samples will be used to help ensure that no sources of contamination are introduced in the sample handling and analysis procedures. The MB should be rejected if the target organisms are detected.

Three positive and negative control samples will also be analyzed using each method. Positive and negative control samples will be obtained from ATCC. Control organisms include the total coliform *Enterobacter aerogenes* (ATCC 13048), *E. coli* (ATCC 8739), and the non-coliform *Pseudomonas aeruginosa* (ATCC 10145). All control cultures will be prepared at Battelle following the manufacturer's instructions. In short, freeze dried cultures will be rehydrated using the medium and incubation conditions specified in the manufacture's catalog. The QC control samples will be prepared by diluting the cultures in sterile deionized water for

[iii] 40 CFR 141.21 - Coliform sampling. Code of Federal Regulations - Title 40: Protection of Environment (December 2005)

analysis. Control samples will be used to determine the specificity and sensitivity of the coliform detection technologies and reference methods.

In addition, reference results for one of the three dilutions sets for each target organism must result in a ratio of 50 ± 25% positive results. If these requirements are not met, a new set of samples may be analyzed by the reference method. If the results are still outside the required limits, the repeat of the appropriate parts of the verification test may be considered.

B6 EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

The equipment used for the reference analyses will be tested and inspected as per the equipment manuals, the standard operating procedures of the analysis laboratory, or the methods being used to make each measurement; results will be documented. Operation of the technologies during the verification test will be performed by Battelle as directed by the vendor.

Temperatures for the refrigerators and freezers will be logged on a daily basis while test samples are in storage. If found to be outside of the specified range above, the samples will be

Table 11. Quality Control Samples for Reference and Technology Methods

Performance Test	Method of Assessment	Dilution	Applicable Method	Frequency	Total Number per Analysis and Target Organism	Acceptance Criteria	Corrective Action
Method Blank	Analysis of filter-sterilize, dechlorinated tap water	N/A	Reference and Technology	Once every 6-0 samples	3	No growth	Review data and analysis for possible sources of contamination. Re-analyze QC sample and document corrective action.
TC Positive control	Analysis of ATCC *E. aerogenes*	N/A	Reference and Technology	Once every 60 samples	3	Target organisms detected	Recheck controls for evidence of cross contamination or deterioration.
E. coli Positive control	Analysis of ATCC *E. coli*	N/A	Reference and Technology	Once every 60 samples	3	Target organisms detected	Recheck controls for evidence of cross contamination or deterioration.
Negative Control	Analysis of ATCC *P. aerugenes*	N/A	Reference and Technology	Once every 60 samples	3	Target organisms not detected	Recheck controls for evidence of cross contamination or deterioration.

Note: Sterility control testing on every lot of reagents (buffers and media) used will be performed during each batch (iteration) of testing.

transferred to an acceptable refrigerator or freezer and the deviation will be noted in the LRB, in a deviation report, and in the final verification reports.

B7 CALIBRATION VERIFICATION

Balances, thermometers, pH meters, autoclaves, incubators, pipettes, etc. used during sample preparation and analysis will be maintained and calibrated as required by the Battelle or reference laboratory SOPs. A list of relevant SOPs is attached to this test/QA plan. Equipment maintenance logs and calibration records will be reviewed by the Battelle Quality Manager prior to the verification test.

One laboratory clock will be used as the standard for all time recording. Any other time pieces must be calibrated to it. In particular, the sewage and DW sample collection time must be determined based on a calibrated time piece.

If necessary, the technologies undergoing testing will be calibrated initially by the respective vendors at the time of set-up, at the vendor's discretion.

B8 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

All materials, supplies, and consumables will be ordered by the Verification Test Coordinator or designee. Where possible, Battelle will rely on sources of materials and consumables that have been used previously as part of ETV verification testing without problems. Battelle will also rely on previous experience or recommendations from its technical staff to guide selection of manufacturers and materials. The manufacturer's criteria for acceptance/purity/sterility will be required to be met. Supplies must meet the following criteria:

- Solvent and reagent grades are based on the intended use. All materials must meet the purity requirements of the method.
- Equipment used to generate data must provide appropriate sensitivity.
- A certificate of analysis must be retained for reagents and standards.
- A certificate of sterility must be retained for sample containers and growth media.
- The quality and purity of expendable materials must be adequate to meet the data quality objectives of the client.

These same requirements for quality must be implemented by the reference laboratory. The laboratory manager is responsible for verifying the quality of supplies used for this test.

B9 NON-DIRECT MEASUREMENTS

Data published previously in the scientific literature will not be used during this verification test.

B10 DATA MANAGEMENT

Various types of data will be acquired and recorded electronically or manually by verification staff during this verification test. Table 3 summarizes the type of data to be recorded. All data and observations for the operation of the coliform detection technologies will be documented by the verification staff on data sheets or in LRBs. Results from the laboratory analyses will be compiled by laboratory staff in electronic format and submitted to the Verification Test Coordinator or other verification staff upon obtaining results within 7 days of test completion. A dedicated shared folder within the ETV AMS Center SharePoint site will be established for all project records.

Records received by or generated by any of the verification staff during the verification test will be reviewed by a Battelle staff member within two weeks of receipt or generation, respectively, before the records are used to calculate, evaluate, or report verification results. The review will be documented as the dated initials of the reviewer. Some of the checks that will be performed include:

- QC samples and calibration standards were analyzed according to the test/QA plan and the acceptance criteria were met. Corrective action for exceedances was taken
- 100% hand-entered and/or manually calculated data were checked for accuracy
- Calculations performed by software are verified at a frequency sufficient to ensure that the formulas are correct, appropriate, and consistent
- For each cut and paste function, the first and last data value was verified vs. the source data

- Data are reported in the units specified in the test/QA plan.
- Results of QC samples are reported.

If a Battelle staff member generated the record, this review will be performed by a Battelle technical staff member involved in the verification test, but not the staff member that originally received or generated the record. The review will be documented by the person performing the review by adding his/her initials and date to the hard copy of the record being reviewed. In addition, data calculations performed by verification staff will be spot-checked by Battelle technical staff to ensure that calculations are performed correctly. Calculations to be checked include any statistical calculations described in this test/QA plan. The data obtained from this verification test will be compiled and reported for each technology. Results for technologies from different vendors will not be compared with each other.

Battelle will provide technology test data and associated reference data (including records, data sheets, notebook records) from the first day of testing within one day of receipt to EPA for simultaneous review. The goal of this data delivery schedule is prompt identification and resolution of any data collection or recording issues. These data will labeled as preliminary and will not have had a QA review before their release.

SECTION C
ASSESSMENT AND OVERSIGHT

C1 ASSESSMENTS AND RESPONSE ACTIONS

Every effort will be made in this verification test to anticipate and resolve potential problems before the quality of performance is compromised. One of the major objectives of the test/QA plan is to establish mechanisms necessary to ensure this. Internal quality control measures described in this test/QA plan, which is peer reviewed by a panel of outside experts, implemented by the technical staff and monitored by the Verification Test Coordinator, will give information on data quality on a day-to-day basis. The responsibility for interpreting the results of these checks and resolving any potential problems resides with the Verification Test Coordinator, who will contact the Battelle AMS Center Manager, Battelle AMS Center Quality Manager, EPA AMS Center Project Officer, and EPA AMS Center QA Manager if any deviations from the test/QA plan are observed. The Verification Test Coordinator will describe the deviation in a teleconference or by email, and once a path forward is determined and agreed upon with EPA, the deviation form will be completed. Technical staff have the responsibility to identify problems that could affect data quality or the ability to use the data. Any problems that are identified will be reported to the Verification Test Coordinator, who will work with the Battelle Quality Manager to resolve any issues. Action will be taken by the Verification Test Coordinator and Battelle testing staff to identify and appropriately address the issue, and minimize losses and correct data, where possible. Independent of any EPA QA activities, Battelle will be responsible for ensuring that the following audits are conducted as part of this verification test.

C1.1 Technical Systems Audits

Battelle Quality Manager will perform a technical systems audit (TSA) at least once during this verification test. The purpose of this audit is to ensure that the verification test is being performed in accordance with the AMS Center QMP and this test/QA plan. The primary focus of the audit will be the reference test method and specifically the determination of positive and negative results. During this audit, the Battelle Quality Manager, or designee, will compare

actual test procedures to those specified or referenced in this plan, and review data acquisition and handling procedures. The audit will include a review of the testing facility and equipment (calibration, maintenance, operation), and observation of testing and records (including custody forms). She will also check data acquisition procedures and may confer with the vendor and Battelle technical staff. The TSA will be guided by a project-specific checklist based on the test/QA plan and reference methods.

The Battelle Quality Manager will prepare an initial TSA report and will submit the report to the Verification Test Coordinator within 10 business days after completion of the audit. A copy of this initial TSA report (with no corrective actions documented) will be provided to the EPA AMS Center Project Officer. A copy of the final TSA report (with corrective actions documented) will be provided to the EPA AMS Center Project Officer within 20 business days after completion of the audit. At EPA's discretion, EPA QA staff may also conduct an independent on-site TSA during the verification test. The TSA findings will be communicated to technical staff at the time of the audit and documented in a TSA report.

C1.2 Data Quality Audits

The Battelle Quality Manager, or designee, will audit at least 25% of the sample results acquired in the verification test and 100% of the calibration and QC data vs. the test/QA plan requirements. A checklist based on the test/QA plan will guide the audit. An initial data quality audit will be conducted on the first batch of test data within 10 business days of when data were collected to identify errors early in the data reduction process. Given the short duration of this test, the first batch is defined as the first set of reference test data released by the Verification Test Coordinator. The remaining data will be audited once all data for a technology or method has been posted on the project SharePoint site and once all statistical analyses are complete. The primary focus of the audit will be the reference test method. The Battelle Quality Manager, or designee, will trace the data from initial acquisition, through reduction and statistical comparisons, to final reporting. All formulae applied to the data will be verified. Data for the technologies will be reviewed for calculation and transcription errors and data traceability. Results of each audit of data quality (ADQ) will be documented using the checklist and reported to the Verification Test Coordinator within 10 business days after completion of the audit. The EPA AMS Center Project Officer will be provided a copy of the initial ADQ report (with no

corrective actions documented). A final ADQ that assesses overall data quality, including accuracy and completeness of the verification report, will be prepared as a narrative and distributed to the Verification Test Coordinator within 10 business days of completion of the audit. The EPA AMS Center Project Officer will be provided a copy of the final ADQ report (with corrective actions documented).

C1.3 QA/QC Reporting

Each assessment and audit will be documented in accordance with Section 3.3.4 of the AMS Center QMP. The results of the TSA and DQA will be submitted to EPA. Assessment reports will include the following:

- Identification of Findings and Observations
- Recommendations for resolving problems
- Response to adverse findings or potential problems
- Confirmation that solutions have been implemented and are effective
- Citation of any noteworthy practices that may be of use to others.

C2 REPORTS TO MANAGEMENT

The Battelle Quality Manager, during the course of any assessment or audit, will identify to the technical staff performing experimental activities any immediate corrective action that should be taken. If serious quality problems exist, the Battelle Quality Manager is authorized to notify the Battelle AMS Center Manager who will issue the stop work. Once the TSA or data quality audit report has been prepared, the Verification Test Coordinator will respond to each Finding and Observation following the timeline defined in Section C1 and will implement any necessary corrective action. The Battelle Quality Manager will verify that corrective action has been implemented effectively.

In addition to this test/QA plan, a final report and a verification statement for each technology verified will be prepared and reviewed. The final report is a comprehensive document describing the verification test. The verification statement is a two-to-three page summary of the technology, the test procedures, and the test results. Each draft report and verification statement will be submitted to the respective vendor for review. They are then

reviewed by EPA AMS Center Quality Manager and the EPA AMS Center program Project Officer. Upon approval by EPA, each verification statement will be signed by a senior manager of Battelle and by an EPA laboratory director. Original signed verification statements will be provided to the respective vendors for use in marketing their technology. Upon final review and approval, the final verification report(s) will be submitted to EPA in MS Word and Adobe portable document format (PDF) and subsequently posted on the ETV website (www.epa.gov/etv).

SECTION D
DATA VALIDATION AND USABILITY

D1 DATA REVIEW, VALIDATION, AND VERIFICATION REQUIREMENTS

The key data review requirements for the verification test are the analysis of QC samples as outlined in the test/QA plan, a comparison of laboratory data sheet comments against final data to flag any suspect data, and a review of final data to resolve any questions about apparent outliers. The QA audits, as described within this document, are designed to assure the quality of this data.

D2 VALIDATION AND VERIFICATION METHODS

Section C of this test/QA plan provides a description of the validation safeguards employed for this verification test. Data validation and verification efforts include the analysis of QC samples as required in this document, and the performance of a TSA and DQAs as described in Section C.

D3 RECONCILIATION WITH USER REQUIREMENTS

The purpose of this verification test is to evaluate the performance of coliform detection technology for use in DW monitoring. In part, this evaluation will include demonstrations of the monitoring ability of coliform detection technologies to detect the presence of TC and *E. coli*. To meet the requirements of the user community, input on the tests described in this test/QA plan has been provided by external experts. Additional performance data regarding operational characteristics of the coliform detection technologies will be collected by verification test personnel. To meet the requirements of the user community, these data will include thorough documentation of the performance of the technologies during the verification test. The data review, verification, and validation procedures described above will assure that data meeting these requirements are accurately presented in the verification reports generated from this test, and will assure that data not meeting these requirements will be appropriately flagged and discussed in the verification reports.

This test/QA plan and the resulting ETV verification report(s) will be subjected to review by the vendor(s), EPA, and expert peer reviewers. The reviews of this test/QA plan will help to improve the design of the verification test and the resulting report(s) such that they better meet the needs of potential users of these technologies.

SECTION E

REFERENCES

E1 REFERENCES

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

2. *Environmental Technology Verification Program Quality Management Plan*. January, 2008 (EPA/600/R-08/009).

3. American Public Health Association, American Water Works Association, and Water Environment Federation. 2005. *Standard Methods for the Examination of Water and Wastewater*. 21st Edition.

4. Battelle Building 20 Biosafety Manual, Version 4.0.

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

6. AOAC International. 1995. *Bacteriological Analytical Manual, 8th Edition*.

7. SOP GEN.V-013-04. *SOP for the Calibration and Maintenance of Thermometers*. Battelle.

8. *SOP ENV-ADM-009, Standard Operating Procedure for Sample Chain-of-Custody* Battelle, September 2007.