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Environmental Technology Verification Program

Advanced Monitoring Systems Center

Test/QA Plan for Verification of Rapid PCR Technologies

TEST/QA PLAN

for

Verification of
Rapid PCR Technologies

May 2004

Prepared by

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Test/QA Plan for Verification of Rapid PCR Technologies

Version 1.0

May 2004

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1.0 INTRODUCTION

1.1 Test Objective

This test/quality assurance (QA) plan provides procedures for a performance verification test of rapid polymerase chain reaction (PCR) technologies for the analysis of biological agents and pathogens in drinking and distilled water, under a specific set of test conditions. The verification test will be conducted under the auspices of the U.S. Environmental Protection Agency (EPA) through the Environmental Technology Verification (ETV) Program. 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. ETV verification does not imply approval, certification, or designation by EPA or Battelle, but rather a quantitative assessment of the performance of a technology under specified test conditions. The objective of this verification test of rapid PCR technologies is to evaluate their ability to rapidly detect specific biological agents and pathogens that are particularly toxic to humans and their susceptibility to interferents in several drinking water matrices.

1.2 Test Description

The verification test 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. In performing the verification test, Battelle will follow the procedures specified in this test/QA plan and will comply with the data quality requirements in the "Quality Management Plan for the ETV Advanced Monitoring Systems Center" (AMS QMP)[1].

Rapid PCR technologies are based on polymerase chain reactions combined with real-time detection capabilities. These technologies can be both lab-based (e.g., weighing over 75 lbs. and measuring 20 in. x 21 in. x 15 in.) and field portable (e.g., weighing 50 lbs. and measuring 19 in. x 14 in. x 10 in.). The PCR process involves enzyme-mediated reactions which allow for target DNA replication and amplification through a series of temperature cycles. Each series of temperature cycles, which can consist of up to 30 or more cycles, constitutes a run on a given rapid PCR technology. Depending on the design of the technology, multiple samples can be measured in one PCR run. For most rapid PCR technologies, reactions are monitored in real-time during the PCR process through the use of hybridization probes and DNA-specific dyes in

combination with fluorescence detection. Other rapid PCR technologies can involve separate detection devices that offer quick turnaround times over conventional techniques. Results from these methods can be qualitative or quantitative. Because rapid PCR technologies are anticipated to serve mostly as screening tools in water monitoring scenarios, providing rapid results as to whether or not a pathogen or biological agent is present in the water, only qualitative results from rapid PCR technologies will be considered for this test. Quantitative PCR testing often involves more extensive user participation and takes longer to conduct.

This test will determine the accuracy, specificity, precision, and false positive and negative rates of rapid PCR technologies in detecting selected biological agents and pathogens in American Society of Testing and Materials (ASTM) Type II deionized (DI) water, in the presence of possible interferents added to ASTM Type II DI water, and in drinking water obtained from a variety of geographically dispersed U.S. water utilities that use various water treatment processes. Qualitative characteristics of each rapid PCR technology, such as ease of use, sample throughput, and cost, will also be assessed and reported. While most of the testing will occur in a laboratory, the rapid PCR technologies that are designed for field use will be tested outside of the laboratory by an experienced operator.

1.3 Organization and Responsibility

The verification test will be performed by Battelle, with the participation of the vendors who will be having the performance of their rapid PCR technologies verified. The testing will occur at Battelle's West Jefferson and Columbus, Ohio laboratories and at a non-laboratory (i.e., field) location in the Columbus, Ohio, area. 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.

1.3.1 Battelle

Dr. Stephanie Buehler is the AMS Center Verification Test Coordinator. In this role, Dr. Buehler will have overall responsibility for ensuring that the technical, schedule, and cost goals established for the verification test are met. Specifically, she will

- Assemble a team of qualified technical staff to conduct the verification test.
- Direct the team performing the verification test in accordance with the test/QA plan.
- Ensure that all quality procedures specified in the test/QA plan and in the AMS QMP are followed.

- Prepare the draft and final test/QA plan, verification reports, and verification statements.
- Revise the draft test/QA plan, verification reports, and verification statements in response to reviewers' comments.
- Respond to any issues raised in assessment reports and audits, including instituting corrective action as necessary.
- Serve as the primary point of contact for vendor representatives.
- Coordinate distribution of the final test/QA plan, verification reports, and statements.
- Establish a budget for the verification test and monitor staff effort to ensure that the budget is not exceeded.
- Ensure that confidentiality of vendor information is maintained.

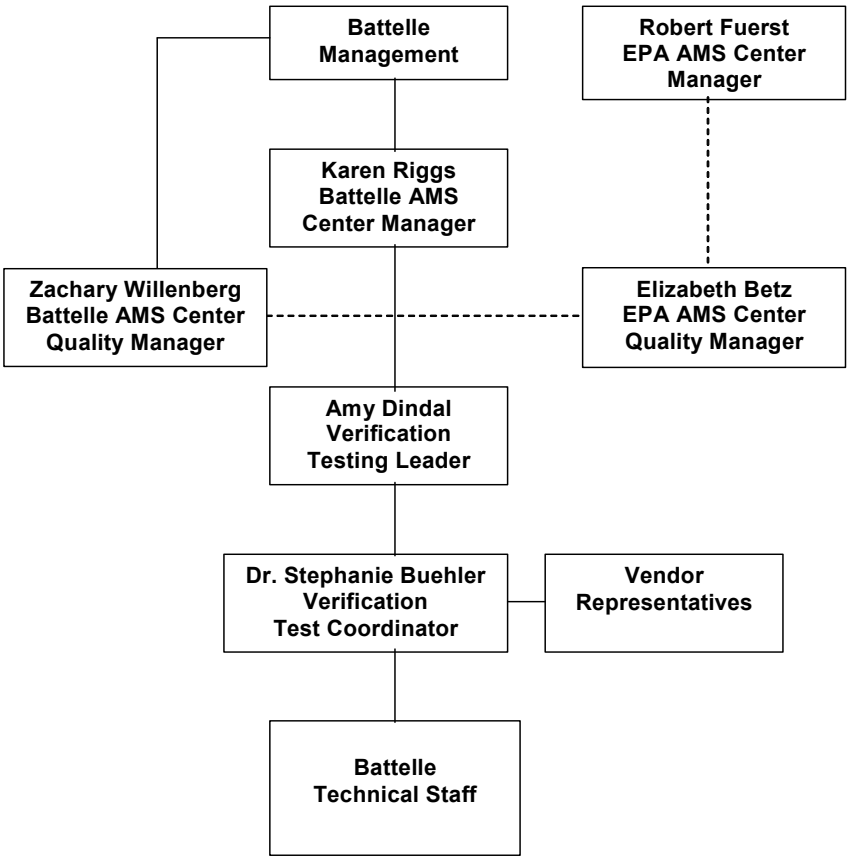

Figure 1. Organization Chart

Ms. Amy Dindal is a Verification Testing Leader for the AMS Center. Ms. Dindal will provide technical guidance and oversee the various stages of verification testing. She will

- Support Dr. Buehler in preparing the test/QA plan and organizing the testing.
- Review the draft and final test/QA plan.
- Review the draft and final verification reports and verification statements.
- Support Dr. Buehler in responding to any issues raised in assessment reports and audits.

Ms. Karen Riggs is Battelle's manager for the AMS Center. Ms. Riggs 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 vendor confidentiality is maintained.
- Support Dr. Buehler in responding to any issues raised in assessment reports and audits.
- Maintain communication with EPA's technical and quality managers.
- Facilitate a stop work order if Battelle or EPA QA staff discovers adverse findings that will compromise test results.

Battelle Technical Staff will conduct the testing of the rapid PCR technologies during the verification test. The responsibilities of the technical staff will be to

- Assist in the collection, receipt, and storage of drinking water samples.
- Prepare drinking water samples, as required for analysis.
- Prepare the stock solutions and the test samples as required.
- Perform plate enumeration to confirm concentrations of stock solutions.
- Perform the rapid PCR technology analyses by following the vendor's protocol.
- Make qualitative observations about the operation of the rapid PCR technology.

Mr. Zachary Willenberg is Battelle's Quality Manager for the AMS Center. Mr.Willenberg will

- Review the draft and final test/QA plan.
- Conduct a technical systems audit once during the verification test, or designate another QA manager to conduct the audit.
- Audit at least 10% of the verification data.

- Prepare and distribute an assessment report for each audit.
- Verify implementation of any necessary corrective actions.
- Issue a stop work order if self audits indicate that data quality is being compromised; notify Battelle's AMS Center Manager if a stop work order is issued.
- Provide a summary of the QA/ quality control (QC) activities and results for the verification reports.
- Review the draft and final verification reports and verification statements.
- Assume overall responsibility for ensuring that the test/QA plan is followed.

1.3.2 Vendors

The responsibilities of the vendor representatives are as follows:

- Review the draft test/QA plan.
- Approve the test/QA plan prior to test initiation.
- Provide off-the-shelf rapid PCR technologies for analysis of all verification test samples.
- Provide all other equipment and consumables needed to complete the PCR analyses on all water samples, including any necessary extraction or purification consumables and/or equipment.
- As desired, provide training to Battelle personnel on operating the rapid PCR technologies and associated equipment prior to testing.
- Provide written instructions for operation of the technology.
- Review the draft verification report and statement.

1.3.3 EPA

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

Ms. Elizabeth Betz is EPA's AMS Center Quality Manager. For the verification test, Ms.Betz will

- Review the draft test/QA plan.
- Perform at her option one external technical system audit during the verification test.

- Notify the EPA AMS Center Manager of the need for a stop work order if external audit indicates that data quality is being compromised.
- Prepare and distribute an assessment report summarizing results of external audit.
- Review draft verification reports and statements.

Mr. Robert Fuerst is EPA's manager for the AMS Center. Mr. Fuerst will

- Review the draft test/QA plan.
- Approve the final test/QA plan.
- Review the draft verification reports and statements.
- Oversee the EPA review process for the verification reports and statements.
- Coordinate the submission of verification reports and statements for final EPA approval.

1.3.4 Supporting Organizations

Physio-chemical characterization including turbidity, organic carbon, specific conductivity, alkalinity, pH, hardness, total organic halides, trihalomethanes and haloacetic acids (Table 3) have been performed for all drinking water samples. Spiked concentrations of humic and fulvic acids in ASTM Type II DI water will also be confirmed. Battelle has established a subcontract with Aqua Tech Environmental Laboratories, Inc. (hereafter called subcontract laboratory) to perform the physio-chemical analyses and humic and fulvic acid concentration confirmations.

The Metropolitan Water District of Southern California has concentrated each drinking water sample by a factor of 400 using ultrafiltration concentration techniques.

2.0 VERIFICATION APPROACH

2.1 Scope of Testing

This test/QA plan specifically addresses verification of rapid PCR technologies that provide measurements of *Bacillus anthracis* (anthrax), *Escherichia coli* O157:H7 (*E. coli*), *Francisella tularensis* (*F. tularensis*) LVS (ATCC# 29684), *Brucella suis* (ATCC#23444) and/or *Yersinia pestis* CO92 (plague) in drinking water. The contaminants were selected based on the capabilities of the technologies being tested and the availability of the pathogens and biothreat agents. The rapid PCR technologies participating in this test will be evaluated on qualitative results, indicating only the presence or absence of the contaminants within a specified concentration interval. Each rapid PCR technology will be tested only for contaminants for which they are designed to detect, as specified by the vendor. The performance of the rapid PCR technologies will be verified by subjecting each to various concentration levels of individual contaminants in ASTM Type II DI water, a single concentration of contaminant in the presence of possible interferents (i.e., fulvic and humic acids) spiked into ASTM Type II DI water, and a single concentration of each contaminant spiked into drinking water samples obtained from four water utilities from different geographical locations in the United States. Each source of drinking water will represent a unique water treatment process. Also, both the possible interferent samples and the drinking water matrices will be analyzed without the addition of any contaminant to evaluate the potential for false positive results. The performance of each rapid PCR technology will be evaluated based on the parameters outlined below, with confirmation of solutions spiked with known concentrations of contaminants by available reference methods (i.e., plate enumeration).

The rapid PCR technologies are designed to be operated by users with technical training. Therefore, for this verification test, experienced operators, i.e., operators that have previous experience using PCR technologies, that have been trained by the vendor in the operation of the PCR technologies, will analyze the test samples on the technology. For those technologies which are designed to be field portable, an experienced operator trained by the vendor will also perform tests outside of the laboratory environment.

A variety of natural and contaminant-fortified water samples (i.e., unspiked and spiked) will be analyzed using the rapid PCR technologies. These matrices are examples of drinking water types that could be monitored using rapid PCR technologies; however, this is not intended to be an exhaustive study or to represent all possible water types that could be tested.

The rapid PCR technologies will be evaluated for the following parameters:

- Accuracy

- Specificity
- Precision
- Matrix effects
- Occurrence of false positive and false negative results
- Field portability by experienced operator
- Ease of use by experienced operator
- Sample throughput.

The PCR technologies will not be evaluated for method detection limits (MDL) because only the qualitative aspects of each technology will be evaluated in this verification test. However, the lowest concentration performance test sample to produce consistently positive results will be reported to help end users better understand the sensitivity of the technologies.

2.2 Experimental Design

This verification test will determine the performance capabilities of rapid PCR technologies to detect individual contaminants in three types of samples—performance test (PT), drinking water (DW), and quality control (QC). PT samples will include samples prepared in ASTM Type II DI water, including contaminant PT samples and interferent PT samples. The contaminant PT samples will be fortified with each individual contaminants at five concentrations. Concentrations will include the infective/lethal dose concentration given in Table 1 for each contaminant and approximately 2, 5, 10, and 50 times the vendor reported limit of detection (LOD) for each technology. The infective/lethal dose of each contaminant was determined by calculating the concentration at which 250 mL of water is likely to cause the death of a 70-kg person based on human LD50 or ID50 data [3]. The results from quadruplicate analysis of the contaminant PT samples and comparison with the known concentrations will provide information on the accuracy and precision of the rapid PCR technologies. The interferent PT samples will consist of humic and fulvic acid at two concentrations, both spiked and unspiked with each contaminant, each analyzed in quadruplicate.

Table 1. Infective/Lethal Dose of Target Contaminants

Contaminant (common name)	Infective/Lethal Dose Concentration
Bacillus anthracis (anthrax)	200,000 spores/L
Yersinia pestis (Plague)	280 organisms/L
Francisella tularensis (*F. tularensis*)	4 x 10^8 organisms/L
Brucella suis	40,000 organisms/L
Escherichia coli O157:H7 (*E. coli*)	200 bacteria/L

DW samples have been collected from four water systems that get water from various sources and employ different treatment processes. The DW samples disinfected by chlorination were obtained from surface (filtered and unfiltered) and groundwater sources. A DW sample from a surface water source disinfected by chloramination was also collected. These same water samples were concentrated by a factor of 400 at the Metropolitan Water District of Southern California using their ultrafiltration concentration system. Each of these water samples will be analyzed in quadruplicate, both unspiked and spiked with each contaminant at a single concentration level approximately 10 times greater than the LOD of each rapid PCR technology.

QC samples will include method blanks consisting of unspiked ASTM Type II DI water, as well as vendor-provided positive and negative controls or internal controls. Positive, negative, and/or internal control samples will be run with each PCR run, per the vendor instructions, and will be used as a quality check to ensure the rapid PCR technology's performance. If controls are not provided by the vendor, positive control samples will be prepared in ASTM Type II DI water with a known concentration of a toxin specified by the vendor and will be used as a quality check to ensure the PCR technology performance. The number and frequency of QC samples will vary with each rapid PCR technology, but 10% of all samples will be method blanks and at least 1 positive and 1 negative control will be part of every batch of samples run on each PCR technology.

Performance parameters, such as ease of use and reliability, will be based on documented observations of the operators and by the Verification Test Coordinator. Sample throughput will

be estimated based on the time required to analyze a sample set. All rapid PCR technologies will be tested in the laboratory; while applicable field-portable rapid PCR technologies will be analyzed for their performance and ease of use outside of the laboratory. Because many of the contaminants of interest cannot be safely handled outside of special facilities, the samples analyzed in the field will include method blank samples and non-toxic positive and negative control samples as provided by the vendor. An experienced operator will perform analyses in the field, which will also include evaluations of ease of use while dressed in personal protective equipment (PPE) (i.e., Level C PPE consisting of a splash suit and a filter-type respirator).

Given the agent facility restrictions, vendors will not be able to operate their rapid PCR technologies during this verification test. Each rapid PCR technology will be operated by a Battelle staff member and tested independently. All operators will have experience with and knowledge of PCR technologies in general. The vendor will train the operators by means of a visit to Battelle or a conference call prior to starting the verification test and then will be asked to sign a consent form stating the names of the Battelle staff they have trained. Each operator will manipulate the water samples and reagents to generate solutions that can be analyzed by the rapid PCR technologies. More than one operator may be used by Battelle, but operators will be restricted to only operating rapid PCR technologies on which they have been trained. Because of the potential time requirements for this test, more than one operator may be assigned to each participating technology for the duration of the test.

2.3 Test Samples

Test samples to be used in this verification test will include PT samples, DW samples, and QC samples. Table 2 lists the number and type of each sample to be analyzed for each contaminant in the verification test. Each type of test sample is described further below.

2.3.1 PT Samples

PT sample types (listed in Table 2) will be prepared in ASTM Type II DI water. The first type of PT sample will consist of ASTM Type II DI water spiked at five concentration levels of each individual contaminant. The contaminant PT sample concentrations will range from the infective/lethal dose concentration to 50 times the vendor-stated LOD. The infective/lethal dose concentration will be analyzed to document the response of each technology at that important concentration level. Four concentration levels in addition to the infective/lethal dose concentration will be analyzed by each technology. Those concentration levels will be

approximately 2, 5, 10, and 50 times the LOD of each individual technology. The maximum and minimum concentrations may be limited by the available standards. The PT samples will be prepared for each rapid PCR technology based on the vendor-defined LOD such that samples used for a particular technology contain 2, 5, 10, or 50 times the LOD of that technology. Each concentration level for the PT samples will be analyzed in quadruplicate using the appropriate rapid PCR technology for that sample.

Table 2. Summary of Test Samples for Rapid PCR Technology Verification

Performance Test (PT)	**Performance Factor**	**Sample Description**	**Reps**
ASTM Type II DI Water	Accuracy, Specificity and Precision	Contaminant PT sample @ infective/lethal dose	4
		Contaminant PT sample @ 2 times the LOD[(a)]	4
		Contaminant PT sample @ 5 times the LOD	4
		Contaminant PT sample @ 10 times the LOD	4
		Contaminant PT sample @ 50 times the LOD	4
	Interferent	Fulvic and humic acids @ a total concentration of 1 mg/L	4
		Fulvic and humic acids @ a total concentration of 1 mg/L + contaminant @ 10 times the LOD	4
		Fulvic and humic acids @ 5 mg/L	4
		Fulvic and humic acids @ 5 mg/L + contaminant @ 10 times the LOD	4
	Ease of Use, Field Portability	Analysis of method blank in level C suit	3
		Analysis of unspiked, unconcentrated DW in level C suit	3
		Analysis of method blank not in suit	3
Drinking Water (DW)	**Performance Factor**	**Sample Description**	**Reps**
Filtered chlorinated surface water	Matrix Effect	Concentrated unspiked	4
		Concentrated and spiked with contaminant @ 10 times the LOD	4
Unfiltered chlorinated surface water		Concentrated unspiked	4
		Concentrated and spiked with contaminant @ 10 times the LOD	4

Filtered chlorinated groundwater		Concentrated unspiked	4
		Concentrated and spiked with contaminant @ 10 times the LOD	4
Filtered chloraminated surface water		Concentrated unspiked	4
		Concentrated and spiked with contaminant @ 10 times the LOD	4
Quality Control (QC)	**Performance Factor**	**Sample description**	**Reps**
Method Blank	Quality Check	DI water - 10% of all samples	8
Positive Control	Quality Check	Provided by vendor - at least 1 on every PCR run	Var[(b)]
Negative Control	Quality Check	Provided by vendor - at least 1 on every PCR run	Var[(b)]
Approximate total number of samples per contaminant			**85**

(a) LOD in all cases is limit of detection provided by the vendor for their rapid PCR technology.
(b) Number of positive and negative controls will vary based on the number of samples that can be analyzed by the PCR technology in each run.

The second type of PT sample will be potential interferent samples. Because it is anticipated that humic and fulvic acids will be major interferents in real-world uses of rapid PCR technologies for water monitoring, four replicates of each interferent PT sample will be analyzed to determine each rapid PCR technology's susceptibility to these commonly found interferents in DW. The interferent PT samples will contain humic and fulvic acids obtained from the International Humic Substances Society spiked into ASTM Type II DI water. Each of these interferent mixtures will be prepared at two different concentration levels. One concentration will be near the upper limit of what would be expected in drinking water (5 mg/L) and one concentration at a mid-low range of what would be expected (1 mg/L). These spiked interferent levels will be confirmed through analysis of aliquots by the subcontract laboratory. Also, each contaminant will be added to these samples along with the potential interferent, at a concentration of 10 times the LOD, and analyzed in quadruplicate.

2.3.2 Drinking Water Samples

Drinking water samples were collected from four geographically distributed municipal sources (Ohio, New York, California, and Florida) to evaluate the performance of the rapid PCR

technologies with various sample matrices. These samples vary in their source and treatment and disinfection process. All samples have undergone either chlorination or chloramination disinfection prior to receipt. Samples were collected from water utility systems with the following treatment and source characteristics:

- Chlorinated filtered surface water source
- Chlorinated unfiltered surface water source
- Chlorinated filtered groundwater source
- Chloraminated filtered surface water

All samples were collected in pre-cleaned high density polyethylyne (HDPE) containers. After sample collection, to characterize the DW matrix, an aliquot of each DW sample was sent to a subcontract laboratory to determine the following water quality parameters: concentration of trihalomethanes, haloacetic acids, total organic halides, calcium, magnesium, pH, conductivity, alkalinity, turbidity, organic carbon, and hardness. The DW samples were dechlorinated upon arrival to the Metropolitan Water District of Southern California with sodium thiosulfate pentahydrate to prevent the degradation of some of the contaminants by chlorine. Because real-world applications of PCR technologies to screen water samples rely on pre-concentration of the water sample to be analyzed, approximately 100 L of each of the above sources of DW were dechlorinated and then concentrated through ultrafiltration techniques to a final volume of 250 mL. As shown in Table 2, each DW sample will be analyzed without adding any contaminant, as well as after fortification with each individual contaminant at a single concentration level (10 times the vendor-stated LOD).

2.3.3 QC Samples

QC samples will include method blank (MB) samples consisting of ASTM Type II DI water, and positive and negative controls, as provided by the vendor. All of the MB QC samples will be exposed to identical sample preparation and analysis procedures as the test samples. Positive and negative controls will be prepared and used according to protocol provided by the vendor. The MB samples will be used to ensure that no sources of contamination are introduced in the sample handling and analysis procedures. At least 10% of the test samples (8 samples) will be MB samples. The vendor provided control samples will indicate to the operator whether the rapid PCR technology is functioning properly and will be added to each run of each technology. To the extent practicable, the test samples will be analyzed blindly by the operator such that at a minimum samples used by the technician for the analysis are marked with a non-identifying number.

2.3.4 Field Portability Samples

Those PCR technologies that are designed to be field portable will be tested outside of the laboratory by an experienced operator. Because many of the contaminants being tested are highly toxic and unsafe to be handled outside of a special facility, method blank samples and non-toxic positive and negative control samples as provided by the vendor will be analyzed in the field portability test. Unconcentrated drinking water samples will also be tested to simulate real-world applications. Because these technologies are meant to be used by first-responders, their performance in the field will also be evaluated under simulated first response conditions by having the operator dressed in Level C PPE. One set of MB samples will be tested without the use of a protective suit.

2.4 Reference Method

For all contaminants, a plate enumeration technique of quantifying bacteria will be followed to confirm the concentration of the stock solution of these contaminants. If necessary, aliquots of the organism to be used for testing may be frozen during the plate enumeration process to ensure that the proper solution concentration is being tested and prevent the organism from multiplying during storage while awaiting the results of the confirmation analysis. For anthrax and plague plate enumeration, the Battelle standard operating procedure (SOP) to be followed is SOP No: MREF X-054[4] "Standard Operating Procedure (SOP) for the Enumeration of BL-2 and BL-3 Bacteria Samples Via the Spread Plate Technique." For *E. coli*, *F. tularensis*, and *Brucella suis*, general laboratory practices for bacteria plate enumeration will be followed.

3.0 MATERIALS AND EQUIPMENT

In general, this verification test will rely on rapid PCR technology materials and equipment provided by the vendors. Battelle will provide the following equipment and materials for the collection, preparation, storage, and shipment of test samples.

3.1 Testing Supplies

The following supplies will be needed throughout testing for sample collection and preparation of the DW and QC samples:

- ASTM Type II DI water
- Various laboratory supplies necessary for accurate preparation of the test samples (i.e., volumetric pipettes, pipette bulbs, Eppendorf micro pipettes/pipette tips, volumetric flasks, disposable pipettes, etc.)
- Various smaller sizes of pre-cleaned HDPE and glass containers for sample aliquot storage
- Standards of contaminant and interferents with a known level of purity (NIST traceable or equivalent)
- Sodium thiosulfate pentahydrate
- n,n-diethyl-p-phenylenediamine (DPD) tablet
- Personal protective equipment.

3.2 Field Analysis Supplies

For the analysis of the method blank samples and unconcentrated, unspiked drinking water samples in the field, Battelle will provide the water used for analysis and the Level C personal protective equipment (PPE). The operators will depend on only supplies provided by the vendor to analyze the samples.

3.3 Special Facilities

The contaminants to be evaluated in this verification test require special handling and/or special facilities. Plague, anthrax, and *Brucella suis* can only be handled in laboratories that are specially designed and certified for the use of chemical and biological agents and by operators

who are trained in their use. Battelle's Medical Research and Evaluation Facility (MREF), which is a Department of Defense laboratory-scale facility conducting research with chemical and biological agents, will provide the facilities and staff for verification testing of plague, anthrax, and *Brucella suis*. The MREF is licensed to ship, receive, and handle select agents, as defined by the Centers for Disease Control and Prevention (CDC)[5]. The facility maintains state-of-the-art equipment and professional and technical staffing trained to safely conduct testing and evaluation of hazardous chemical and biological materials.

The MREF and its personnel have the demonstrated capability for storing and safely handling plague, anthrax, and *Brucella suis*. Biological agent use will be according to the CDC Select Agents Program (32 CFR 626 and 627)[5] administered through the Biological Defense Safety Program and the Battelle MREF Facility Safety Plan. All PCR technologies used in this building will have to go through a decontamination process involving hydrogen peroxide vapor spray to eliminate the possibility of any anthrax or plague remaining on or in the instrument. The decontamination step has previously been used on computers and other sensitive electronic equipment without harm to the technology.

E. coli and *F. tularensis* are not required to be used in a specially designed facility as are anthrax, plague, and *Brucella suis,* but they do require special handling, thus testing of these bacteria will take place in qualified laboratories in either the Battelle Columbus Operations or MREF facilities.

4.0 PROCEDURES

4.1 Test Sample Collection, Preparation, and Storage

Stock solutions of each contaminant and interferent will be prepared in ASTM Type II DI water or appropriate reagent from certified standards. The concentration of all stock solutions will be confirmed using a plate enumeration method. If possible, solutions will be prepared on a daily basis for all bacteria. However, because the concentration of some organisms can change over the course of a day, aliquots of the solution to be used for spiking the samples may be prepared and frozen while the confirmation analyses are performed. Samples confirmed to contain adequate levels of the organism would then be thawed and used as needed.

PT samples will be prepared in ASTM Type II DI water using the aforementioned stock solutions. Aliquots of each stock solution will be diluted to the appropriate concentration using volumetric pipettes and glassware. The DW samples were collected as described in Section 2.3.2. Because free chlorine will degrade many of the contaminants and interferences during storage, the samples were immediately dechlorinated with sodium thiosulfate pentahydrate (or other dechlorination reagents as per vendor protocol). The dechlorination of the DW was qualitatively confirmed by adding a diethyl-p-phenylene diamine (DPD) tablet to an aliquot of DW. If the water did not turn pink, the dechlorination process was determined to be successful. If the water did turn pink, additional dechlorinating reagent was added and the dechlorination confirmation procedure was repeated. Once dechlorination was confirmed, 100 L of each DW sample was concentrated as described previously, and approximately 25 L remained unconcentrated. The dechlorinated concentrated DW samples will be analyzed unspiked and spiked. Aliquots of each bacteria stock solution will be diluted with DW samples to the appropriate concentration. All spiked DW samples will go through the appropriate vendor-specified DNA preparation/isolation procedure on the same day as they are spiked.

4.2 Sample Identification

Aliquots to be analyzed by each rapid PCR technology will be drawn from the PT, QC or DW samples and placed in sample containers with unique identification (ID) numbers. A master log of the samples and sample ID numbers for each rapid PCR technology will be maintained by Battelle. The ID number, date, person collecting, sample location, and time of collection was recorded on a chain-of-custody form for all field samples.

4.3 Sample Analysis

4.3.1 Drinking Water Characterization

Table 3 lists the methods used to characterize the DW samples collected from the various water sources by the subcontract laboratory. An aliquot of each DW sample was sent to Aqua Tech Environmental Laboratory prior to concentration to determine the water quality parameters listed in Table 3. Because the water samples were collected and then transported and tested at later dates, some of the water quality parameters may have changed slightly prior to testing with the rapid PCR technologies. Information produced on physio-chemical parameters of drinking water by the subcontract laboratory will be utilized for verifying performance of the rapid PCR technologies.

Table 3. Physio-Chemical Characterization of Drinking Water[a]

Parameter	Method	Method Detection Limits[(b)]
Turbidity	EPA 180.1[6]	0.067 ntu
Organic carbon	SM 5310[7]	0.7 mg/L
Specific conductivity	SM 2510[7]	2 µmho
Alkalinity	SM 2320[7]	2 mg/L
pH	EPA 150.1[8]	NA
Hardness	EPA 130.2[8]	5 mg/L
Total organic halides	SM 5320[7]	5 µg/L
Trihalomethanes	EPA 524.2[9]	0.5 µg/L/analyte
Haloacetic acids	EPA 552.2[10]	1.0 µg/L/analyte

(a) Physio-chemical DW characterization to be performed by the subcontract laboratory
(b) Method detection limits based on standard methods.

4.3.2 Stock Solution Confirmatory Methodologies

The concentration of all contaminants will be confirmed by a plate enumeration method following Battelle SOP No.: MERF X-054 and Battelle general laboratory practices for plate enumeration. For the interferent samples, the concentration of humic and fulvic acid will be confirmed by Standard Method 5310[7] for total and dissolved organic carbon by the subcontract laboratory.

4.4 Rapid PCR Technologies

Each vendor will provide rapid PCR technologies, consumables (e.g., reagents and controls), and other necessary equipment (e.g. DNA extraction and purification reagents and/or equipment) for the analysis of all samples. The full set of samples listed in Table 2, unless otherwise noted, will be analyzed by each rapid PCR technology for each applicable contaminant. The analyses will be performed according to the vendor's recommended procedures as described in the standard written instructions or manual provided with the rapid PCR technologies. Calibration and maintenance of the rapid PCR technologies will be performed as specified in the written instructions or manual.

Rapid PCR technology results will be recorded manually by the operators on data sheets designed specifically for this verification test. Where applicable, electronic results from the supplied technology software will also be coded and stored. In addition to the rapid PCR technology results, the data sheets will include records of the time required for sample analysis and the operator observations concerning the use of the rapid PCR technology (e.g., ease of use, maintenance, etc.).

4.5 Schedule

The verification test described here will take place during approximately four to seven weeks in late May - July 2004 at Battelle's laboratories in West Jefferson and Columbus, Ohio, and at a nearby non-laboratory location. It will be necessary for participating vendors to provide their rapid PCR technologies to Battelle prior to testing so staff may become familiar with operating the rapid PCR technologies before testing begins. Vendor staff are requested to provide training in operating the rapid PCR technologies either in person or by teleconference. Unused rapid PCR technologies and associated equipment will be returned to the vendors at the completion of report writing. As appropriate, rapid PCR technologies will be decontaminated by being exposed to a vapor of hydrogen peroxide before being returned to the vendor.

5.0 DATA HANDLING AND REPORTING

5.1 Data Acquisition and Review

Various types of data will be acquired and recorded electronically or manually by Battelle during this verification test. Table 4 summarizes the type of data to be recorded. All data and observations will be documented by Battelle staff on data sheets or in laboratory record books. Results from the reference methods will be compiled in written or electronic format.

Records received by or generated by Battelle staff in the verification test will be reviewed by a another Battelle staff member within two weeks of receipt or generation, respectively, before the records are used to calculate, evaluate, or report verification results. 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 a hard copy of the record being reviewed. This hard copy will then be returned to the Battelle staff member who will be storing the record. In addition, data calculations performed by Battelle 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 independently for each rapid PCR technology. The Verification Report prepared and provided to the vendor will address only the vendor's technology submitted for performance verification (not other technologies submitted by other vendors that might also have been verified under the ETV program).

Table 4. Summary of Data Recording Process

Data to Be Recorded	Where Recorded	How Often Recorded	Disposition of Data[(a)]
Dates and times of test events	ETV data 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
Calibration information and results for physico-chemical parameters (temperature, salinity, pH, conductivity, etc.)	ETV data sheets	Prior to sample preparation	Manually incorporated in data spreadsheets as necessary
Sample collection and preparation information, including chain-of-custody	ETV data sheets and chain-of-custody forms	At time of sample collection and preparation	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Rapid PCR technology procedures and sample results	ETV data sheets	Throughout test duration	Manually incorporated in data spreadsheets
Reference method procedures and sample results	ETV data sheets, or data acquisition system, as appropriate	Throughout sample analysis process	Transferred to spreadsheets

(a) All activities subsequent to data recording are carried out by Battelle.

5.2 Data Analysis

The technologies participating in this verification test will only be evaluated for qualitative results (i.e., yes/no responses to samples) based on the expected application of these technologies as rapid screening tools. All data analyses will be based on these qualitative results.

5.2.1 Accuracy

The accuracy will be assessed by evaluating how often the rapid PCR technology result is positive in the presence of a concentration above the LOD. An overall percent agreement will be

determined by dividing the number of positive responses to the overall number of analyses of spiked samples.

5.2.2 Precision

Precision measures the repeatability and reproducibility of PCR technology responses. The precision of the four replicates of each sample set will be assessed. Responses will be considered inconsistent if one or more of the four replicates differs from the response of the other samples in the replicate set. The overall precision for each rapid PCR technology will be assessed by calculating the overall number of consistent responses of all the sample sets.

5.2.3 Specificity

The specificity will assess the rapid PCR technology's ability to detect the absence of the contaminant when it is truly absent. An overall specificity rate will be determined by dividing the number of negative responses by the total number of unspiked samples or method blanks.

5.2.4 False Positive/False Negative Responses

A false positive response will be defined as a detectable or positive rapid PCR technology response when the ASTM DI water, including interferent samples, or drinking water sample is not spiked at all. Because most of the contaminants being tested can occur naturally in water, and because rapid PCR technologies cannot distinguish between live and dead organisms, each DW sample will be plate enumerated to verify the presence or absence of the contaminant of interest. A false positive rate will be reported as the frequency of false positive results out of the total number of unspiked samples or method blanks, as in the specificity calculation.

A false negative response is defined as a non-detectable response or negative response when the sample was spiked with a contaminant at a concentration greater than the LOD. Reagent blanks, PT (contaminant and interferent) samples, and DW samples will be included in the analysis. A false negative rate will be evaluated as the frequency of false negative results out of the total number of spiked samples for a particular contaminant.

5.2.5 Matrix Interferences

The potential effect of the DW matrix on the rapid PCR technology performance will be evaluated qualitatively by comparing the results for the spiked and unspiked DW samples to those for the PT samples. The results indicating the correct or incorrect reporting of the presence of a contaminant will be evaluated.

5.2.6 Field Portability

The results obtained from the measurements made on samples in the laboratory and field setting will be compiled independently for each rapid PCR technology that is designated as being field portable and assessed for comparability of the measurements under the different environmental conditions. The results obtained from unspiked, unconcentrated drinking water samples in the field will also be evaluated. Also, qualitative observations of each technology's performance in a non-laboratory setting will be made by the Verification Test Coordinator and operators. Factors such as the ease of transport and set-up, demand for electrical power, and space requirement will be documented and discussed in the report. Dexterity issues and ease of use will also be evaluated under a first-responder scenario where the operator will test samples in a Level C PPE. These results will be compared to field portability tests done without the use of a protective suit.

5.3 Reporting

The data obtained in the verification test will be compiled separately for each rapid PCR technology, and the data evaluation methods described in Section 5.2 will be applied to each data set without comparison to any other rapid PCR technology. Following completion of the data evaluation, a draft verification report will be prepared for each rapid PCR technology. The verification report will individually address each rapid PCR technology submitted for performance verification, not other technologies submitted for verification under the same test. The verification report will describe the verification test procedures and document the results. Each draft verification report will be submitted to the corresponding vendor for review and comment. Each draft report will be revised in response to the comments provided by the vendor. The revised reports will be submitted for external peer review, revised again to address the peer review comments, and submitted to EPA for final approval.

A verification statement will also be prepared for each rapid PCR technology. The verification statement is a two to four page summary of the rapid PCR technology, test procedures, and results. The verification statement will follow the same review and revision process as the verification reports. Upon final approval by EPA, each verification statement will be signed by a senior Battelle manager and an EPA laboratory director. Final verification reports and statements are expected to be posted on the ETV website (http://www.epa.gov/etv), and original signed verification statements will be provided to the vendor.

6.0 QUALITY ASSURANCE /QUALITY CONTROL

The QA/QC activities associated with this verification test will focus primarily on sample preparation and handling, data recording and analysis, and reference laboratory analysis. An independent audit covering each of these areas will be performed by the Battelle Quality Manager to ensure the quality of the verification test.

6.1 Sample Chain-of-Custody Procedures

Sample custody was documented throughout collection, shipping, and analysis of the samples from the water utility to Battelle laboratories. Similar documentation will be recorded for shipping and analysis of samples to the subcontract laboratories. Sample chain-of-custody procedures will be in accordance with the Battelle SOP ASAT II-007[11], *Sample Chain-of-Custody for Dioxin/Furan Analysis.* The chain-of-custody form summarizes the samples collected and analyses requested. The chain-of-custody form will track sample release from the field to the Battelle laboratory, and from the Battelle laboratory to the subcontract laboratory. Each chain-of-custody form will be signed by the person relinquishing samples once that person has verified that the chain-of-custody form is accurate. The original sample chain-of-custody forms accompany the samples; the shipper will keep a copy. Upon receipt at the sample destination, chain-of-custody forms will be signed by the person receiving the samples once that person has verified that all samples identified on the chain-of-custody forms are present in the shipping container. Any discrepancies will be noted on the form and the sample receiver will immediately contact the Verification Test Coordinator to report missing, broken, or compromised samples. The recipient will retain a copy of the form while the original chain-of-custody form will be sent back to the Verification Test Coordinator for storage in the study file.

6.2 Rapid PCR Technology Calibration

Prior to analysis, the Verification Test Coordinator will identify which rapid PCR technologies require calibration. All such rapid PCR technologies will be calibrated according to vendor directed procedures. Other equipment provided by the vendor will be calibrated based on vendor requirements.

6.3 Equipment Calibration

For physio-chemical characterization, analytical equipment was calibrated by the subcontract laboratory according to the procedure specified in the standard method. Similar calibration will be performed for interferent conformation. Pipettes will be calibrated according to the procedure outlined in Battelle SOP No: VI-025[12]. Pipettes will be calibrated semiannually and the calibration service will provide a calibration certificate.

6.4 QC of Stock Solutions Preparation and DW Characterization

Battelle QA staff will provide oversight of the stock solution preparation for all contaminants. The concentration of the stock solutions of all contaminants will be confirmed by the plate enumeration method. Additionally, each unspiked DW sample will be analyzed by this method to confirm the absence of these bacteria. Method blanks and control spikes will be analyzed by the subcontract laboratory performing confirmation analysis on the humic and fulvic acid samples and water quality parameters. Method blanks and control spikes will be analyzed with every batch of samples processed. Method blank and control spikes of interferences will be analyzed in accordance with standard methods and QC limits specified therein.

6.5 Audits

6.5.1 Technical Systems Audit

The Battelle Quality Manager will conduct a technical systems audit at least once during the course of the verification test. The purpose of this audit is to ensure that the verification test is being performed in accordance with this test/QA plan and the AMS QMP[1], and that all procedures described in this test/QA plan are being followed. This audit will review the standards and methods used, compare actual test procedures to those specified in this test/QA plan, and review data acquisition and handling procedures. An independent technical systems audit may also be performed by EPA Quality Management staff during the verification test at EPA's discretion.

Before using an outside laboratory to perform stock solution confirmation analyses, the Battelle Quality Manager conducts an audit of the laboratory's quality documents. The subcontract laboratory for this test has already been audited and has been deemed qualified to conduct confirmatory analyses as specified previously.

6.5.2 Data Quality Audit

At least 10% percent of the data acquired during the verification test will be audited during the verification test. Battelle's Quality Manager will trace the data from its 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 will be checked.

6.6 QA/QC Reporting

Each assessment and audit will be documented in accordance with Section 3.3.4 of the AMS QMP[1]. The results of the technical systems audit will be submitted to EPA. Assessment reports will include the following:

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

6.7 Corrective Action

During the course of any assessment or audit, the Battelle Quality Manager will inform the technical staff of any immediate corrective action that should be taken. If serious quality problems exist, the Battelle Quality Manager is authorized to stop work. Once the assessment report has been prepared, the Verification Test Coordinator will ensure that a response is provided for each adverse finding or potential problem, and will implement any necessary follow-up corrective action. The Battelle Quality Manager will ensure that follow-up corrective action has been taken.

7.0 HEALTH AND SAFETY

7.1 Standard/Test Sample Preparation

All handling of solid and highly concentrated aqueous solutions of contaminants and possible interferences will be done inside of a laboratory hood with hood sash set to the lowest height that still allows for safe manipulation of materials. The following guidelines should be adhered to:

- Personal protective equipment shall include safety glasses with side shields, a laboratory coat, and nitrile lab gloves. Gloves shall be immediately changed if they become contaminated.
- All contaminated waste shall be handled as hazardous waste and disposed of according to facility regulations.

7.2 Handling During Verification Testing

Laboratory and field handling of any solutions used during the verification test will be accomplished by taking the following precautions:

- All containers shall be stored and transported in double containment.
- Safety goggles, nitrile gloves with long cuffs, and a chemical resistant disposable lab coat shall be worn when handling all chemicals. Gloves shall be immediately changed if they become contaminated.

7.3 Testing of Anthrax, *E. coli*, *F. tularensis*, *Brucella suis*, and Plague

Use of these contaminants in the verification of rapid PCR technologies will be done following the safety procedures required at the MREF facility as noted in Section 3.3 and Battelle Columbus Operation facilities.

8.0 REFERENCES

1. Quality Management Plan (QMP) for the ETV Advanced Monitoring Systems Center, Version 5.0. EPA Environmental Technology Verification Program, prepared by Battelle, Columbus, Ohio, March, 2004.

2. Environmental Technology Verification Program Quality Management Plan, December 2002 (EPA/600/R-03/021).

3. Burrows, W. D.; Renner, S. E. Biological Warfare Agents as Threats to Potable Water, *Environmental Health Perspectives*, 107, 975-984, 1999.

4. Battelle MREF X-054. Enumeration of BL-2 and BL-3 Bacterial Samples via the Spread Plate Technique. July 2003.

5. Code of Federal Regulations, Title 32, Chapter 5, Part 626, Biological Defense Safety Program and Part 627, Biological Defense Safety Program, Technical Safety Requirements.

6. EPA/600/R-93-100. EPA Method 180.1. Turbidity (Nephelometric), Methods for the Determination of Inorganic Substances in Environmental Samples. 1993.

7. American Public Health Association, et al. Standard Methods for Examination of Water and Wastewater. 19th Edition. 1997. Washington D.C.

8. EPA/600/4-79-020. Methods for Chemical Analysis of Water and Wastes. March 1983.

9. EPA/600/R-95-131. EPA Method 524.2. Purgeable Organic Compounds by Capillary Column GC/Mass Spectrometry. Methods for the Determination of Organic Compounds in Drinking Water, Supplement III. August 1995.

10. EPA/600/R-95-131. EPA Method 552.2. Haloacetic Acids and Dalapon by Liquid-Liquid Extraction, Derivatization and GC with Electron Capture Detector. Methods for the Determination of Organic Compounds in Drinking Water, Supplement III. August 1995.

11. Battelle SOP ASAT II-007. Sample Chain-of-Custody for Dioxin/Furan Analysis. October 2000.

12. Battelle SOP VI-025. Operation, Calibration, and Maintenance of Fixed and Adjustable Volume Pipettes. January, 2003.