Battelle
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Environmental Technology Verification Program
Advanced Monitoring Systems Center

Quality Assurance Project Plan for Verification of Mycometer®-test Rapid Fungi Detection and BactiQuant®-test Rapid Bacteria Detection Technologies

QUALITY ASSURANCE PROJECT PLAN

for

Verification of
Mycometer®-test Rapid Fungi Detection
and
BactiQuant®-test Rapid Bacteria Detection
Technologies

Version 1.0

April 5, 2011

Prepared by

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SECTION A

PROJECT MANAGEMENT

A1 VENDOR APPROVAL PAGE

ETV Advanced Monitoring Systems Center

Quality Assurance Project Plan for Verification of
Mycometer®-test Rapid Fungi Detection
and
BactiQuant®-test Rapid Bacteria Detection Technologies

Version 1.0
April 5, 2011

APPROVAL:

Name _____

Company _____

Date _____

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A3 LIST OF ACRONYMS AND ABBREVIATIONS

%RSD	percent relative standard deviation
ADQ	audit of data quality
A. flavus	*Aspergillus flavus*
AMS	Advanced Monitoring Systems
APS	Aerodynamic Particle Sizer
ARCA	Aerosol Research and Component Assessment
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
C. herbarum	*Cladosporium herbarum*
CDC	Centers for Disease Control and Prevention
CFU	colony forming units
CIP	cleaning in place
COA	certificates of analysis
COC	chain of custody
DQIs	data quality indicators
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
L	liter
LPM	liter per minute
LRB	laboratory record book
µL	microliter
mL	milliliter
MQO	measurement quality objective
N	number
NA	nutrient agar
NIOSH	National Institute for Occupational Safety and Health
P. aeruginosa	*Pseudomonas aeruginosa*
PBS	phosphate buffered saline
PDA	Potato Dextrose Agar
PE	performance evaluation
QA	quality assurance
QAPP	Quality Assurance Project Plan
QM	Quality Manager
QC	quality control
QMP	Quality Management Plan
RMO	Records Management Office
RPD	relative percent difference
SM	Standard Methods
SOP	Standard Operating Procedure
TSA	technical systems audit
VTC	Verification Test Coordinator

A4 DISTRIBUTION LIST

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A5 VERIFICATION TEST ORGANIZATION

The verification tests described in this document will be conducted under the 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 the U.S. Environmental Protection Agency (EPA). The scope of the AMS Center covers verification of monitoring technologies for contaminants and natural species in air, water, and soil.

These verification tests will be conducted by Battelle in cooperation with the EPA. Testing will be conducted between April and June, 2011 in Battelle laboratories in Columbus, Ohio. The testing will involve the evaluation of Mycometer®-test rapid fungi detection and BactiQuant®-test rapid bacteria detection technologies that are commercially available from Mycometer A/S and Mycometer, Inc.

Quality assurance (QA) oversight of these EPA Category III verification tests will be provided by the Battelle AMS Center Quality Manager (QM), and by the EPA AMS Center QM at their discretion. The organization chart in Figure 1 identifies the responsibilities of the organizations and individuals associated with these verification tests. Roles and responsibilities are defined further below.

A5.1 Battelle

Ms. Mary Schrock is the AMS Center Verification Test Coordinator (VTC) for these tests. In this role, Ms. Schrock will have overall responsibility for ensuring that the technical, schedule, and cost goals established for the verification tests are met. Specifically, she will:

- Assemble a team of qualified technical staff to conduct the verification tests,
- Hold a kick-off meeting approximately one week prior to the start of the verification tests to review the critical logistical, technical, and administrative aspects of the verification tests. Responsibility for each aspect of the verification tests will be confirmed,
- Direct the team (Battelle testing staff and vendor) in performing the verification tests in accordance with this Quality Assurance Project Plan (QAPP),
- Ensure that all quality procedures specified in the QAPP and in the AMS Center Quality Management Plan[1] (QMP) are followed,

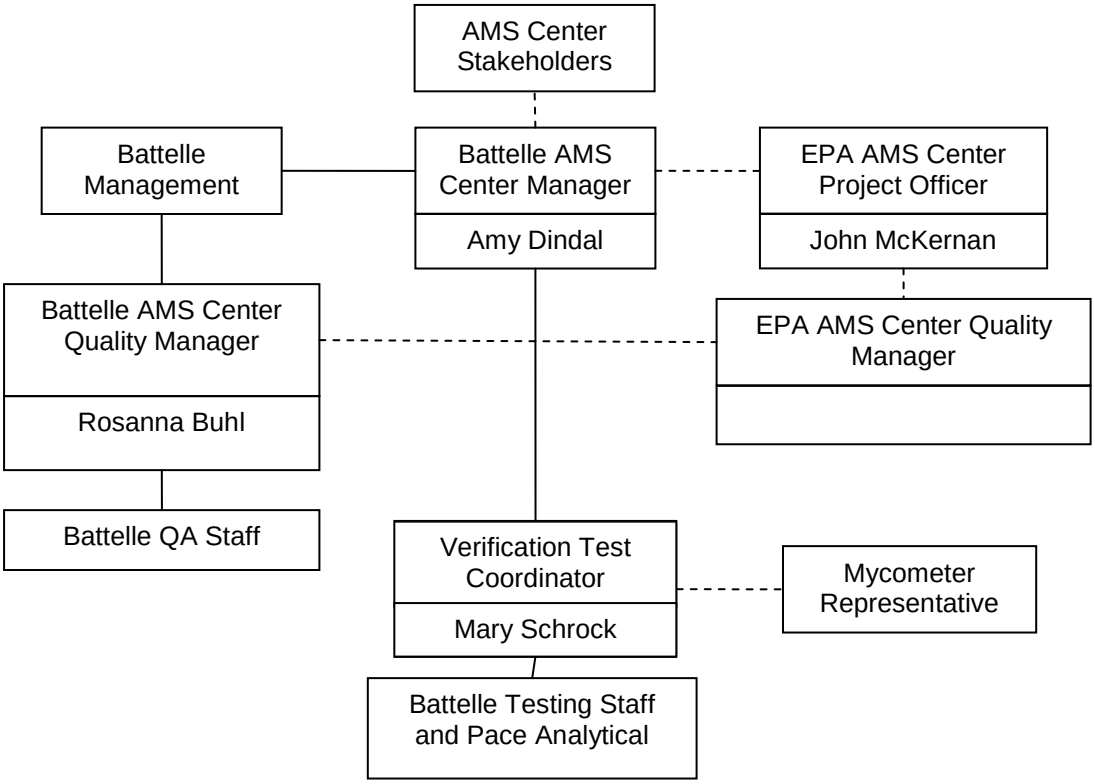

Figure 1. Organizational Chart

- Maintain real-time communication with the Battelle AMS Center Manager, Battelle AMS Center QM, EPA AMS Center Project Officer, and EPA AMS Center QM on any potential or actual deviations from the QAPP,
- Provide test data, including data from the first day of testing, to the Battelle AMS Center Manager, Battelle AMS Center QM, EPA AMS Center Project Officer, and EPA AMS Center QM,
- Prepare the draft and final QAPP, verification report, and verification statements.
- Conduct a technical review of all test data. Designate an appropriate Battelle technical staff member to review data generated by the VTC,
- Revise the draft QAPP, verification report, 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 representative,
- Coordinate distribution of the final QAPP, verification report, and statements, and
- Establish a budget for the verification tests and manage staff to ensure the budget is not exceeded.

Ms. Amy Dindal is Battelle's manager for the AMS Center. Ms. Dindal will:

- Review the draft and final QAPP,
- Review the draft and final verification report and verification statements,
- Ensure that necessary Battelle resources, including staff and facilities, are committed to the verification tests,
- Ensure that confidentiality of sensitive vendor information is maintained,
- Ensure that testing staff respond to QAPP deviations and any issues raised in assessment reports, audits, or from test staff observations, and that any necessary corrective actions have been implemented,
- Maintain communication with EPA's AMS Center Project Officer and QM, and
- Facilitate a stop work order if Battelle or EPA QA staff discover adverse findings that will compromise data quality or test results.

Ms. Carol Riffle will lead Battelle Testing Staff in Battelle's microbiological laboratories to support Ms. Schrock in planning and conducting the verification tests. Battelle Testing Staff will:

- Assist in planning for the tests, and making arrangements for the receipt of and training on the technologies,
- Attend the verification test kick-off meeting, as requested,
- Assist vendor staff as needed during technology receipt and training,
- Participate in training provided by the vendor, as requested,
- Conduct verification testing following all aspects of the ETV AMS Center QMP as well as this QAPP,

- Record qualitative observations about the maintenance and operation of the technologies during testing,
- Ensure that the data from the technologies are immediately reviewed for quality, and after the first day of testing and thereafter on at least a weekly basis, compiled, recorded, and transmitted to the VTC,
- Notify the VTC of any QAPP deviations and institute corrective action as necessary,
- Document and prepare any deviations to the QAPP that may occur during testing,
- Address any comments from reviewers regarding testing or the deviations,
- Support Ms. Schrock in the preparation of the QAPP, report, and verification statements, as necessary, and
- Support Ms. Schrock 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 and will serve as the QA Officer for these verification tests. Ms. Buhl will:

- Review the draft and final QAPP,
- Attend the verification test kick-off meeting and lead the discussion of the QA elements of the kick-off meeting checklist,
- Prior to the start of verification testing, verify the presence of applicable training records, including any vendor training on test equipment,
- Prepare audit checklists,
- Conduct a technical systems audit at least once near the beginning of each verification test,
- Conduct audits to verify data quality,
- Prepare and distribute an audit 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 VTC and/or technical staff the need for immediate corrective action if an audit identifies QAPP deviations or practices that threaten data quality,

- Provide a summary of the QA/quality control (QC) activities and results for the verification reports,
- Review the draft and final verification report and verification statements,
- Maintain real-time communication with the VTC on QA activities, audit results, and concerns,
- Recommend a stop work order if audits indicate that data quality or safety is being compromised,
- Work with the VTC and Battelle's AMS Center Manager to resolve data quality concerns and disputes,
- Delegate QA activities to other Battelle quality staff as needed to meet project schedules, and
- Review and approve QAPP amendments, deviations and audit reports.

A5.2 Mycometer A/S

The vendor's responsibilities are as follows:

- Review and provide comments on the draft QAPP,
- Approve the final QAPP prior to test initiation,
- Provide Mycometer®-test and BactiQuant®-test technologies for evaluation during the verification tests,
- Provide all equipment/supplies/reagents/consumables needed to operate their technologies for the duration of the verification tests,
- Supply a representative to train Battelle staff in operation of their technologies and provide written consent for Battelle staff to operate their technologies during verification testing,
- Provide written instructions for routine operation of their technologies, and
- Review and provide comments on the draft verification report and statements for their technologies.

A5.3 EPA

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

EPA AMS Center Quality Manager. The EPA AMS Center Quality Manager will:

- Review the draft QAPP,
- Review deviations to the approved final QAPP,
- Review the first day of data from the verification tests and provide immediate comments if concerns are identified,
- Perform at her option one external technical systems audit and/or audit of data quality during the verification tests,
- Notify the EPA AMS Center Manager of the need for a stop work order if the external audit indicates that data quality or safety is being compromised,
- Prepare and distribute an assessment report summarizing results of any external audits, and
- Review the draft verification report and statements.

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

- Review the draft QAPP,
- Approve the final QAPP,
- Review and approve deviations to the approved final QAPP,
- Appoint a delegate to review and approve deviations to the approved final QAPP in his absence, so that testing progress will not be delayed,
- Review the first day of data from the verification tests and provide immediate comments if concerns are identified,
- Review the draft verification report and statements,
- Oversee the EPA review process for the verification report and statements, and
- Coordinate the submission of verification report and statements for final EPA approval.

A5.4. Verification Test Stakeholders

This QAPP and the verification report and verification statement based on testing described in this document will be reviewed by experts in the fields related to fungi and bacteria detection and/or analytical instrumentation. The following experts have been providing input to this QAPP and have agreed to provide a peer review.

- Nancy Clark Burton, Centers for Disease Control and Prevention/National Institute of Occupational Safety and Health,
- Connie Schreppel, Mohawk Valley Water Authority, and
- Timothy Dean, EPA, Air Pollution Prevention and Control Division.

The responsibilities of verification test stakeholders include:

- Participate in technical panel discussions (when available) and/or review an outline of the verification tests to provide input to the test design,
- Review and provide input to the QAPP, and
- Review and provide input to the verification report and verification statements.

In addition, the QAPP in general was reviewed with the broader AMS Center Stakeholder Committee as a presentation during regular stakeholder teleconferences, including the September 11, 2008 and January 22, 2009 meetings. Input from the water and air committees was also solicited during email updates of AMS Center activities in June 2010.

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. Stakeholder committees 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 rapid fungal and bacterial detection technologies.

Rapid technologies (results available same day of testing) to detect fungi and bacteria from matrices such as surfaces, bulk material and in air or water are of interest to improve the efficiency of delineating and documenting microbial contamination in buildings and water systems and for monitoring progress during cleanup and remediation processes. Microbial contamination has the potential to cause health problems. Fungi are known to produce allergens, irritants, and potentially toxic substances[3] resulting in asthma, respiratory infections and a variety of allergic reactions[4], and bacteria ingested through the water supply can cause gastrointestinal illnesses.[5]

Traditional methods of analysis for bacteria in drinking water include methods for total counts (heterotrophic plate count or direct total microbial counts) and specialized analysis for indicator organisms such as *Escherichia coli*. Plating methods are often time consuming and can take up to seven days for results using traditional methods such as the heterotrophic plate count. Techniques such as direct microbial counts using epifluorescence are much faster, but do not estimate microbial biomass or viability and require an experienced analyst for differentiation of cells from other water constituents. Detection of fungi in air is far more tenuous using methods such as spore trap air sampling and analysis to provide identification of fungi present, quantify the number of spores, and assess background debris, hyphal fragments, and pollen. This technique is subject to variation due to concentrations of airborne particles (spores, hyphae, pollen, debris) and analyst-to-analyst variability associated with microscopic techniques. Technologies providing same day or near "real-time" results indicating changes in water or air quality would help to control microbial outbreaks, expedite remediation efforts, and protect public health.

A.6.2 Technology Description

Mycometer®-test rapid fungi detection and BactiQuant®-test rapid bacteria detection technologies developed and patented by Mycometer A/S are based on fluorogenic detection of enzyme activities found predominantly in a taxonomic group of organisms. In both the Mycometer®-test (measuring fungal biomass) and the Bactiquant®-test (measuring bacterial biomass), a sample (filter, swab or other) is contacted with a test solution containing a synthetic enzyme substrate. The enzyme present in the fungal cells or bacterial cells hydrolyzes the

synthetic enzyme substrate as shown in Figure 2. When the synthetic substrate molecule is cleaved into two molecules by the enzymatic activity, one of the molecules (as indicated by the yellow asterisk in Figure 2) can be made to fluoresce upon excitation with UV light (365 nm). The amount of fluorescence is measured based on time and temperature (e.g., a reaction time of 30 minutes at a temperature of 23 ^{o}C) using a handheld fluorometer. This fluorescence is a measure of the fungal or bacterial biomass. The same fluorometer may be used to measure fluorescence for both the Mycometer®-test and Bactiquant®-test. Fluorescence measurements can be captured electronically and may be downloaded to a computer or can be transcribed by hand. The analyses can be performed on site in less than one hour.

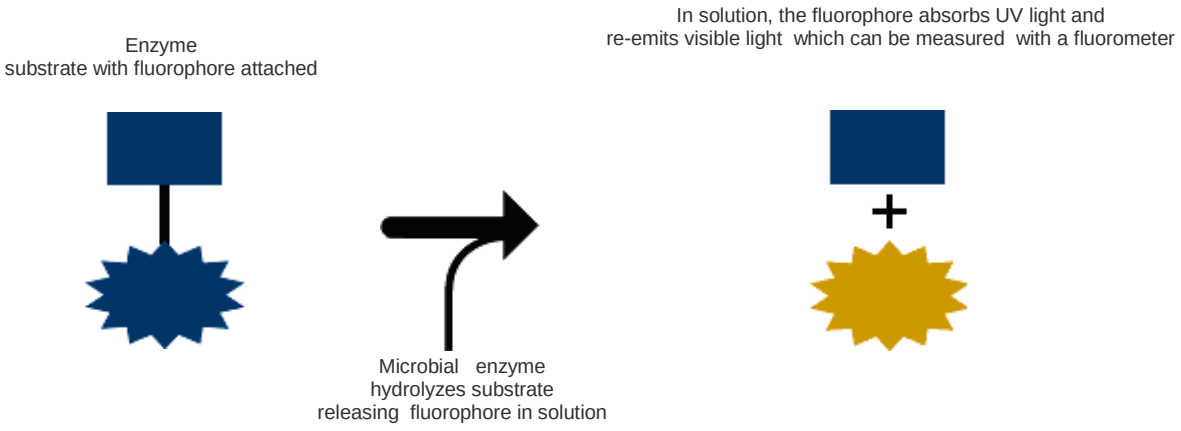

Figure 2. Principle of Fluorogenic Detection of Fungal or Bacterial Related Enzymatic Activity in an Environmental Sample

According to the vendor, the Mycometer®-test for fungi is designed to measure both viable and non-viable spores, hyphae and fungal particles such as hyphal fragments in air, on surfaces, in bulk materials and in solutions to give a comprehensive representation of the contamination in the environment. Although Mycometer®-test cannot distinguish between fungal genera or viable/non-viable fungi, it provides a semi-quantitative measure of the total fungal biomass present. Air samples can be collected with traditional air sampling pumps onto filter media. Enzyme substrate is added to the filter and fungal enzyme reacts with the substrate to release a fluorescent product. The amount of fungi in the sample is estimated by measuring the fluorescence produced.

According to the vendor the BactiQuant®-test is designed to provide a rapid method to estimate total bacteria in water samples. With BactiQuant®-test, bacteria are concentrated from water

samples by passing the sample through a membrane filter. Enzyme substrate is added to the filter unit and left to react over a period of time based on temperature. Bacterial enzyme reacts with the substrate releasing a fluorescent product. The amount of bacteria in the water sample is determined by measuring the concentration of the fluorescent product released into the solution during the reaction period. This technology is designed for application to a range of liquid samples including: potable water, processed water, CIP (cleaning in place), wastewater and recreational water. BactiQuant®-test can also be applied to surface and air samples.

A7 VERIFICATION TEST DESCRIPTIONS AND SCHEDULE

The purpose of these verification tests is to generate performance data on Mycometer®-test and BactiQuant®-test rapid fungi and bacteria detection technologies. The data generated from these verification tests are intended to provide organizations and users interested in detecting and monitoring bacteria and fungi with information on the potential use of these technologies. The Mycometer®-test and BactiQuant®-test technologies will be verified for repeatability and inter-assay reproducibility by detecting fungi in air samples and bacteria in water samples, respectively. Linearity will be assessed for both technologies using dilutions of stock cultures in tap water.

A7.1 Verification Tests Description

Battelle testing staff will conduct the test between April and June, 2011. There will be two verifications conducted with separate testing to verify the analytical performance under controlled laboratory conditions for each technology, Mycometer®-test to detect fungi in air and BactiQuant®-test to detect bacteria in water. As noted above, linearity will be assessed for both technologies using dilutions of stock cultures in tap water. The linearity test for fungi will be a modification of test procedures in place for air and surface samples.

The Mycometer®-test and BactiQuant®-test will be verified for the following performance parameters (attributes):

- Linearity,
- Repeatability,
- Inter-assay reproducibility,

- Data completeness, and
- Operational and sustainability factors.

Mycometer®-test for Fungi

Linearity of the Mycometer®-test will be demonstrated using two fungal cultures from American Type Culture Collection (ATCC), *Cladosporium herbarum* ATCC 58927 and *Aspergillus flavus* ATCC 58870. The two types of fungal cultures (Genera) were chosen based on their presence in indoor fungal isolates as reported in the literature[8,9,10,11]. The specific strains (species designation and ATCC number) were selected based on isolation from air samples as indicated by ATCC. The ATCC cultures will be confirmed based on a Certificate of Analysis (COA) provided by ATCC. The stock concentrations will be estimated (N = 5) for each fungal culture using a hemocytometer (American Society for Testing and Materials (ASTM) D4300-01 Annex[12]) to determine the number of spores per milliliter (spores/mL). The volume of the fungal spore stocks may need to be adjusted based on volume and concentration requirements for testing. A dilution series for each of the fungal cultures (based on the spore counts) will be performed using dechlorinated tap water. The dilution series will target a test range of approximately 500 to 50,000 spores/mL since these concentrations are expected to generate fluorescence covering the range fluorescence that might typically be encountered by a user. Since 100 µL of the stock dilutions will be used per reaction and will be added to 2 mL of enzyme substrate for a total volume of 2.1 mL, the stock dilutions will be approximately 21X the target test solution concentration (range from 1.1×10^4 to 1.1×10^6 spores/mL). Preliminary testing prior to initiation of the performance tests will be conducted to check the target concentration of fungi spiked into tap water. In addition, the resulting fluorescence of the stocks will be checked to determine if proposed concentrations will need to be adjusted. Dechlorinated tap water will be used for the dilutions. Each of the four test concentrations in the dilution series will be sub-sampled five times, processed identically with the same volume of sample, enzyme substrate and developer using the Mycometer®-test technology, and the fluorescence measured as shown in Table 1. The Mycometer®-test fluorescence values will be plotted against test solution concentrations based on the estimated spore count to generate linearity data.

Table 1. Solutions Used to Generate Mycometer®-test Linearity Data

Estimated Solution Concentration of Stock Dilutions (spores/mL)	Volume (mL) of Stock Dilution added to 2 mL Enzyme Substrate to create Test Solution	Final volume of Test Solution (mL)	Target Test Solution Concentration (spores/mL)	Number of Aliquots Processed	Equivalent Concentration in Air * (spores/m^3)
1.1×10^4	0.1	2.1	5.2×10^2	5	3.5×10^3
5.3×10^4	0.1	2.1	2.5×10^3	5	1.6×10^4
1.1×10^5	0.1	2.1	5.2×10^3	5	3.5×10^4
1.1×10^6	0.1	2.1	5.2×10^4	5	3.5×10^5

*Calculated as the air concentration necessary to generate a Test Solution Concentration the same as the Target Test Solution when collected for 15 min. at 20 LPM and processed with 2 mL Enzyme Substrate.

Tap water used for dilutions will be collected and dechlorinated with sodium thiosulfate as described in SM 9060A[6]. The tap water will be characterized for pH, free chlorine, and total chlorine by Battelle as detailed in Section B2.2 to ensure that the water has been sufficiently dechlorinated. The tap water will also be characterized by a subcontract laboratory, Pace Analytical, for turbidity, total organic carbon, specific conductivity, alkalinity, hardness, and dissolved oxygen as noted in Table 2. The characterization parameters in Table 2 are for informational purposes only, since tap water can vary from location to location. These data will not be used in evaluating the technologies.

Repeatability and inter-assay reproducibility tests will be conducted in Battelle's Aerosol Research and Component Assessment (ARCA) chamber (Figures 3 and 4) to produce controlled air samples. One fungal stock will be used to determine repeatability and inter-assay reproducibility. Selection of the fungal stock will be based on the fluorescence response observed during linearity testing and the consistency of linearity data.

The starting spore stock concentration (spores/mL) for generation of the aerosol will be determined by calculations based on the generation chamber flow rate (liters per minute [LPM]), aerosol generation rate (mL/minute), total concentration generated, expected sample concentration (total spores), sampling flow rate (20 LPM), expected aerosol concentration (spores/liter [L]), and sampling time (15 minutes).

Table 2. Water Quality Parameters* for Characterizing Dechlorinated Columbus, Ohio Tap Water for Testing

Analysis	Method
Turbidity	EPA Method 180.1[7]
Total Organic Carbon	SM 5310-C[6]
Specific Conductivity	SM 2510[6]
Alkalinity	SM 2320-B[6]
Hardness	SM 2340-B[6]
Dissolved Oxygen	SM 4500-O[6]

*Analyses to be provided by Pace Analytical

Figure 3. ARCA Chamber

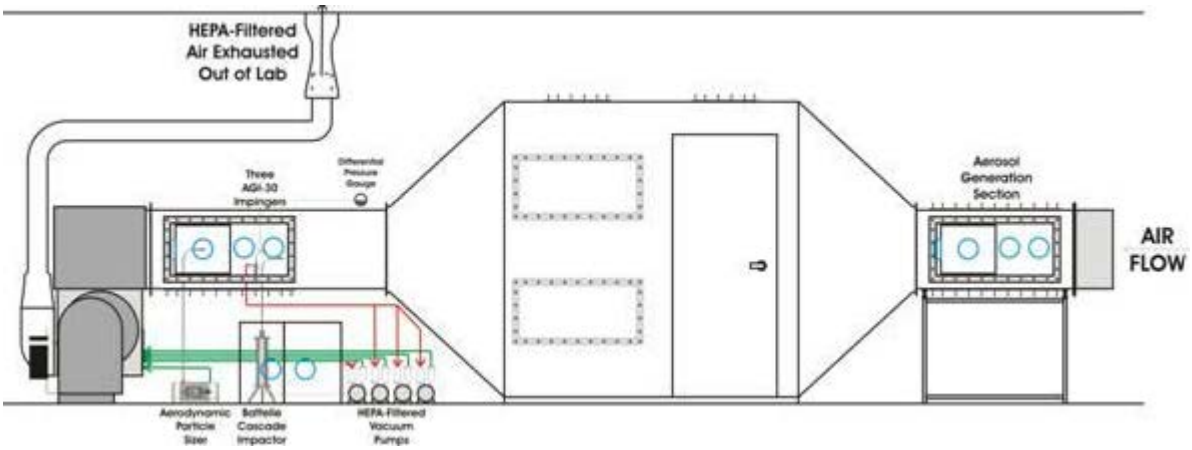

Figure 4. Schematic Diagram of the ARCA Chamber

The goal will be to produce an aerosol sample which results in a fluorescence signal at least 300 fluorescence units above the level of a blank, as the vendor indicates this level is often seen in buildings. Linearity data from the diluted spore stock will be used to select the target sample concentration for collection with the cartridges based on fluorescence measurements. An initial characterization run will be performed to check the test parameters to ensure sample fluorescence is measured by the unit and adjust the test system or stock concentration accordingly. For repeatability and inter-assay reproducibility, a total of eight air pumps (GAST Model 1532 Pumps with IAQ option) supplied by the vendor will be used. The pumps will be placed outside of the chamber. The flow rate for each pump will be adjusted using a calibrated flow meter. Two tripod stands, holding four Mycometer®-test sampling cartridges each, will be placed in the chamber in close proximity (Figures 5 and 6). The selected fungal culture will be released into the chamber using an aerosol generator at one target concentration. Once the system has reached steady state, sampling will follow the vendor's operating instructions which include collecting 300 L of air at a sampling rate of 20 LPM for 15 minutes[13].

Figure 5. Sampling Stand and Pump

For repeatability, one vendor-trained analyst will perform sampling and analysis of air samples collected from all eight pumps from one chamber test using one fluorometer unit. For inter-assay reproducibility, two vendor-trained analysts will each perform sampling and analysis of air samples collected from four pumps during one chamber test. The repeatability and inter-assay

reproducibility test scheme is described further in Table 3. Each analyst will use a separate fluorometer unit for analyzing samples. Eight background air samples will be collected in the chamber prior to release of the fungal culture and will be used as the measured fluorescence basis of comparison for repeatability and inter-assay reproducibility.

Table 3. Repeatability and Inter-assay Reproducibility Test Scheme for Mycometer®-test

Analyst	Fluorometer Unit	Number of Repeatability Samples	Number of Inter-assay Reproducibility Samples
Analyst 1	A	8	4
Analyst 2	B	none	4

An example chamber test schedule is listed below:

- Decontaminate the ARCA test chamber (prior to test),
- Set up,
- Characterization run to check test parameters and stock concentration,
- Perform an air wash of the chamber,
- Collect background samples (Mycometer®-test analysis),
- Run one test run with an aerosolized fungal stock to collect repeatability data (Mycometer®-test analysis),
- Perform an air wash of the chamber,
- Surface decontaminate the tripod stands,
- Collect background samples (Mycometer®-test analysis),
- Run one test with an aerosolized fungal stock to collect inter-assay reproducibility data (Mycometer®-test analysis),
- Perform an air wash of the chamber,
- Remove tubing and surface decontaminate the tripod stands, and
- Decontaminate the chamber (after test day).

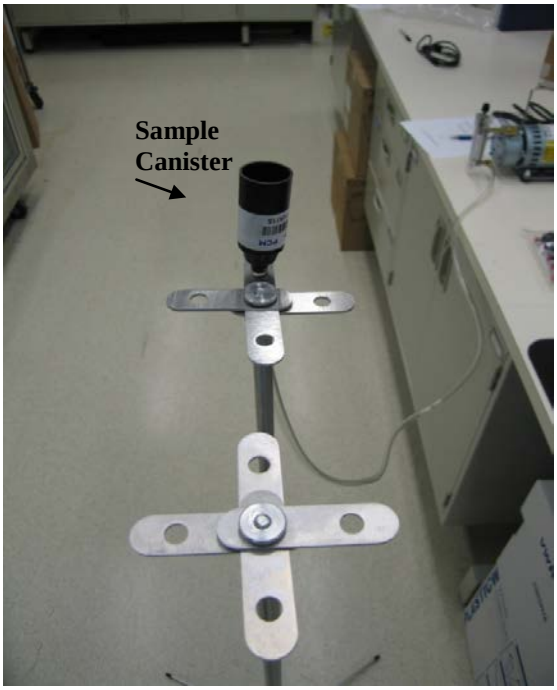

Figure 6. Sampling Stand Set-Up for Collection of Four Samples per Stand

BactiQuant®-test for Bacteria

For the BactiQuant®-test, linearity will be determined using Columbus, Ohio tap water spiked with two types of bacterial stock cultures: a QC organism originating from ATCC and a consortium of indigenous flora from a local environmental water source (e.g., river water or lake). Tap water will be collected and dechlorinated as described above for the Mycometer®-test.

The spiked water samples will be serially diluted with dechlorinated tap water to target a concentration range from approximately 50 to 50,000 colony forming units per milliliter (CFU/mL). The enzymatic activity and resulting fluorescence of the bacterial stocks will be checked to determine if proposed concentrations will need to be adjusted. The spiked water samples for each of four test concentrations will be plated to determine the concentration of viable bacteria in the spiked water using heterotrophic plate counts (SM 9215[6]). Each of the four test concentrations will be sub-sampled five times and processed using the BactiQuant®-test reagents following the procedure for water samples as shown in Table 4. The fluorescence data

generated using BactiQuant®-test will be plotted against the average heterotrophic plate counts for each of four test solution concentrations to generate linearity data.

Table 4. Solutions Used to Generate BactiQuant®-test Linearity Data

Estimated Solution Concentration of Stock Dilutions (CFU/mL)	Volume (mL) of Stock Dilution to be filtered	Final volume of Test Solution after addition of Enzyme Substrate (mL)	Target Test Solution Concentration (CFU/mL)	Number of Aliquots Processed
5.0×10^1	250	2.5	5.0×10^3	5
5.0×10^2	250	2.5	5.0×10^4	5
5.0×10^3	250	2.5	5.0×10^5	5
5.0×10^4	250	2.5	5.0×10^6	5

Inter-assay reproducibility and repeatability will be determined by having two vendor-trained analysts each perform sampling and analysis of four sub-samples taken from one concentration of tap water spiked with indigenous flora and four sub-samples from one concentration of tap water spiked with the control organism. Each analyst will use a separate fluorometer unit. This testing scheme is further described in Table 5. Repeatability will evaluate variability within each analyst's operation of the technology and inter-assay reproducibility will evaluate variability between analysts and fluorometer units.

Table 5. Repeatability and Inter-assay Reproducibility Test Scheme for BactiQuant®-test

Analyst	Fluorometer Unit	Number of Repeatability* Samples-Indigenous Flora	Number of Repeatability* Samples-Control Organism
Analyst 1	A	4	4
Analyst 2	B	4	4

*Repeatability sample results will also be used to generate inter-assay reproducibility data.

For both technologies, data completeness will be determined from a review of the valid data (i.e., data which meet all measurement quality objectives [MQO]) collected during the verification testing period against the expected amount of total data to be generated. Operational performance parameters such as maintenance requirements, ease of use, sustainability factors, and portability will be determined from observations by the Battelle testing staff. Information on costs will be provided by the technology vendor.

Subsequent to the verification tests, Battelle will draft one verification report which will describe the results for both the Mycometer®-test and BactiQuant®-test and two verification statements (one for each Mycometer®-test and BactiQuant®-test). The report will be reviewed by the technology vendor and by peer reviewers, revised, and submitted to EPA for review and approval. In performing the verification tests, Battelle will follow the technical and QA procedures specified in this QAPP and will comply with the data quality requirements in the AMS Center QMP.[1]

A7.2 Proposed Testing Schedule

Table 6 shows the planned schedule of testing and data analysis/reporting activities to be conducted in this verification. Testing is planned during the April to June 2011 timeframe. The verification process is planned to be completed in September, 2011. Data from the fungi and bacteria detection technologies will be immediately checked by the testing staff. For each technology, data will be compiled, recorded, and transmitted to the VTC on the first day of testing and on a weekly basis thereafter so that any data quality issues can be rapidly identified. The VTC will post the first day of testing data for QA and EPA review within five days of test initiation and the remaining data every two weeks thereafter. Unaudited data will include the disclaimer "has not been reviewed by Battelle QM."

A7.3 Battelle Laboratory Testing Facilities

All verification testing will be conducted in Battelle laboratories in Columbus, Ohio. Battelle staff will conduct testing in laboratories that are fully equipped for handling bacteria and fungi cultures and contain the ARCA chamber to be used for generating air samples.

Table 6. Planned Verification Test Schedule for Testing at Battelle (Columbus, OH)

Approximate Date(s)	Testing Activities	Data Analysis and Reporting
May 2010 - April 2011	Testing preparation Technology operation training/installation Growth of cultures	
April-June 2011	Mycometer®-test fungi BactiQuant®-test bacteria	Review and summarize testing staff observations Technical Systems Audit (within the first week of testing) Compile data from testing Begin data analysis Audit of Data Quality Begin preparation of report template
June 2011		Complete draft report
July 2011		Complete vendor and peer review of draft report
August-September 2011		Revise draft report Submit final report for EPA review and approval

A8 QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA

The objective of these verification tests is to verify the performance of the Mycometer®-test and BactiQuant®-test under potential scenarios relevant to monitoring fungi and bacteria in the environment. These verifications will assess the capabilities of the Mycometer®-test to detect common fungi in air and BactiQuant®-test to detect common bacteria in water under controlled and repeatable test conditions. The verification tests will also rely upon operator observations to assess other performance characteristics of the Mycometer®-test and BactiQuant®-test including data completeness, ease of use, and maintenance requirements.

Data quality indicators (DQIs) ensure that these verification tests provide suitable data for a robust evaluation of performance. DQIs have been established for pump flow rate accuracy and the analytical means to determine concentrations of fungal and bacterial cultures. The DQIs were established to ensure that data used to support the quantitative performance evaluations of the Mycometer®-test and BactiQuant®-test are of sufficient quality. The DQI for these supporting measurements are quantitatively defined in Table 7 along with the acceptance criteria.

Quantitative performance parameters for vendor technology performance are discussed in Section B.

Additionally, the verification tests rely in part on observations of the Battelle testing staff for assessment of the performance of the Mycometer®-test and BactiQuant®-test being tested. The requirements for these observations are described in the discussion of documentation requirements and data review, verification, and validation requirements for these verification tests.

The Battelle QM or designee will perform a technical systems audit (TSA) of laboratory testing activities to augment these QA/QC requirements. A TSA will be performed within the first week of the start of verification testing for each technology. The EPA QM 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 locations. The Battelle QM may verify the presence of appropriate training records prior to the start of testing. 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 by the vendor, which states which Battelle technical staff have been trained to use their test kits 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. Battelle technical staff supporting these verification tests have a minimum of a Bachelor's degree in a scientific field or equivalent work experience.

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

Table 7. DQI and Criteria for Critical Supporting Measurements

Technology	DQI	Method of Assessment	Frequency	Measurement Quality Objectives (MQO)	Corrective Action
Mycometer®-test	Sampling pump flow rate accuracy	The flow rate for each pump will be set at 20 LPM using a calibrated flow meter	Prior to each sample collection and throughout the test run	±10%	Investigate discrepancy. Inspect pump and connections and adjust accordingly.
Mycometer®-test	Spore count precision	Hemocytometer	One test, 5 replicates per fungal spore stock	%RSD of replicate analysis ± 30%	Repeat test
Mycometer®-test	Chamber air flow accuracy	Flow rate check at exit of chamber	Monitored during set up to achieve steady state and throughout the test run	±10%	Check and adjust system
BactiQuant®-test	Standard plate count of the *P. aeruginosa* strain Precision	Replicate samples for the QC strain working stock in buffer	Each test day for bacteria	%RSD of ±30%	Re-check control calculations and raw data for control. Re-check analyst technique.
BactiQuant®-test	Positive media controls	Check for growth of stocks inoculated onto agar media	Each test day media is used; each lot of media	Growth	Re-check control data and associated data forms. Re-test if no error is identified
BactiQuant®-test	Negative media control	Check for no growth of organisms on media incubated with samples	Each test day media is used; each lot of media	No growth	Re-check control data and associated data forms. Re-test if no error is identified or assess the impact on data and include discussion in report
BactiQuant®-test	Sterile water or diluent blank negative controls	Check dilution water or buffer for the presence of contaminants	Each test day buffer or water is used is used; each lot of buffer	No growth	Re-check control data and associated data forms. Re-test if no error is identified

A10 DOCUMENTATION AND RECORDS

The documents for these verification tests will include the QAPP, vendor instructions, reference methods, the verification report, verification statements, and audit reports. The project records will include certificates of analysis (COA), chain-of-custody forms, laboratory record books (LRB), data collection forms, electronic files (both raw data and spreadsheets), and QA audit files. All of these documents and records will be maintained in Battelle's microbiology laboratories or the VTC's office during the tests and will be transferred to permanent storage at Battelle's Records Management Office (RMO) at the conclusion of the verification tests. Electronic documents and records will also be uploaded to a SharePoint site designated for these tests and will be provided to EPA upon request. All Battelle LRBs are stored indefinitely by Battelle's RMO. EPA will be notified before disposal of any files. Section B10 further details the data recording 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 QM for review prior to use so that appropriate changes, if any, can be made.

SECTION B

MEASUREMENT AND DATA ACQUISITION

B1 EXPERIMENTAL DESIGN

The verification tests described in this QAPP will specifically address verification of Mycometer®-test and BactiQuant®-test by evaluating the following performance factors:

- Linearity,
- Repeatability,
- Inter-assay reproducibility,
- Data completeness, and
- Operational and sustainability factors.

The verification tests will be conducted during the April to June, 2011 timeframe with several weeks needed to grow and confirm the fungal cultures and approximately 4 weeks to carry out the tests. The Mycometer®-test and BactiQuant®-test undergoing verification will report fluorescence units for all testing activities. Vendor provided spreadsheets will be used for data recording and calculations of the fluorescence (Appendix 1). In addition, a number of Battelle standard operating procedures (SOPs) will be utilized (Appendix 2). There will be two verifications:

- Mycometer®-test – Battelle will verify the analytical performance of the Mycometer®-test to detect fungi in air under controlled laboratory conditions.
- BactiQuant®-test –Battelle will verify the analytical performance of the BactiQuant®-test to detect bacteria in water under controlled laboratory conditions.

Table 8 presents a summary of the tests to be performed.

Throughout the verification tests, each Mycometer®-test and BactiQuant®-test test will be conducted by Battelle staff that have been trained by the vendor.

Table 8. Summary of Tests and Testing Frequency (see Tables 11 and 13 for QC)

Technology	Performance Parameter	Objective	Comparison Based On	Testing Frequency	Number of Data Points
Spore Counts for Mycometer®-test	Verification of stock solution used for linearity	Determine linearity of response over a range of fungi concentrations for two types of fungi	Spore counts	Five replicate measurements of each spore stock for 2 fungal cultures (5 replicates x 2 fungal cultures)	**10**
Mycometer®-test	Linearity	Determine linearity of response over a range of fungi concentrations for two types of fungi	Mycometer®-test fluorescence measurements	Four concentrations of spores for 2 fungal cultures; 5 replicates of each concentration) (4 concentrations x 5 replicates x 2 fungal cultures)	**40**
Mycometer®-test	Repeatability	Determine repeatability of eight successive measurements of one fungal culture from one test in ARCA chamber	Repetitive measurements under constant facility conditions measured	Eight successive measurements of one fungal culture at one concentration by one analyst	**8**
Mycometer®-test	Inter-assay reproducibility	Determine reproducibility of four successive measurements of one fungal culture from one test in ARCA chamber between two analysts and two Mycometer®-test units	Repetitive measurements with two analysts and two Mycometer®-test units	Four successive measurements of one fungal culture at one concentration by two separate analysts	**8**
Heterotrophic Plate Counts for BactiQuant®-test	Verification of stock solution used for linearity	Determine linearity of response over a range of bacterial concentrations	Heterotrophic plate counts of culturable bacteria	Three plate count determinations for each stock solution and four concentrations of spiked tap water for one control organism and one indigenous flora (3 plate counts x 5 solutions x 2 bacterial cultures)	**30**

Technology	Performance Parameter	Objective	Comparison Based On	Testing Frequency	Number of Data Points
BactiQuant®-test	Linearity	Determine linearity of response over a range of bacterial concentrations	BactiQuant®-test fluorescence measurements	Four concentrations of bacteria for two bacterial cultures; 5 replicates of each concentration (4 concentrations x 5 replicates x 2 bacterial cultures)	**40**
BactiQuant®-test	Repeatability	Determine repeatability of four successive measurements of one solution with a control organism and one solution with indigenous flora	Repetitive measurements under constant facility conditions measured	Four successive measurements of one concentration for a control organism and one concentration of an indigenous flora for each of two analysts (4 replicates x 1 concentration x 2 bacterial cultures x 2 analysts)	**16**
BactiQuant®-test	Inter-assay reproducibility	Determine reproducibility of four successive measurements of one solution with a control organism and one solution with indigenous flora between two analysts and two BactiQuant®-test units	Repetitive measurements with two analysts and two BactiQuant®-test units	Four successive measurements of one concentration of a control organism and indigenous flora by two separate analysts; both sets will also be used for repeatability (4 replicates x 1 concentration x 2 bacterial cultures x 2 analysts)	**16** **-same as for repeatability**

B1.1 Test Procedures

The Mycometer®-test and BactiQuant®-test will be set up in Battelle's laboratories in Columbus, Ohio. Linearity will be evaluated using a range of concentrations of two fungal cultures for the Mycometer®-test, and one control bacteria and one indigenous flora bacterial culture for the BactiQuant®-test. A single fungal culture will be used to establish repeatability and inter-assay reproducibility for the Mycometer®-test in air samples and one control bacteria and indigenous

flora bacterial culture will be used to establish repeatability and inter-assay reproducibility for the BactiQuant®-test in water samples. These tests will be conducted at room temperature. Room temperature is defined as 13 – 25 °C per Battelle SOP GEN.V-001 (see Appendix 2). The room temperature will be recorded at the beginning and end of each testing day.

B1.1.1 Linearity

The Mycometer®-test and BactiQuant®-test will be used to analyze four concentrations of two fungal and two bacterial cultures in tap water. The dilutions of each stock required to achieve the target sample concentrations will be determined from the bacterial and fungal stock counts. The Mycometer®-test will be evaluated with *Cladosporium herbarum* ATCC 58927 and *Aspergillus flavus* ATCC 58870. The BactiQuant®-test will be evaluated with a QC strain, *Pseudomonas aeruginosa (P. aeruginosa)* ATCC 27853, and indigenous bacteria from a natural water source with (e.g., river or lake water). The target bacterial and fungal culture concentrations to be used are included in Table 9. Bacterial target concentrations are based on data included in the BactiQuant®-test manual.

Table 9. Approximate Target Concentrations for Linearity Testing

Sample Set	*Cladosporium herbarum* ATCC 58927	*Aspergillus flavus* ATCC 58870	*Pseudomonas aeruginosa* ATCC™ 27853	Indigenous bacteria
Sample #	**Spores/mL Water Sample**		**CFU/mL Water Sample**	
1	5.2×10^2	5.2×10^2	5.0×10^3	5.0×10^3
2	2.5×10^3	2.5×10^3	5.0×10^4	5.0×10^4
3	5.2×10^3	5.2×10^3	5.0×10^5	5.0×10^5
4	5.2×10^4	5.2×10^4	5.0×10^6	5.0×10^6

Five replicates of each concentration will be evaluated for each organism.

B1.1.2 Repeatability

Mycometer®-test repeatability will be determined by having one analyst collect and analyze eight air samples containing one fungal culture at one concentration in a controlled chamber test using the ARCA chamber. The target concentration for a 300 L air sample will be selected for analysis based on preliminary fluorescence data generated during development using the spore stocks. The analyst will analyze all eight samples using one Mycometer®-test unit. This test will be conducted at room temperature. The temperature inside the ARCA chamber will be recorded

daily using a calibrated thermometer. The average, range, standard deviation, and %RSD will be determined.

BactiQuant®-test repeatability will be determined by having two analysts perform sampling and analysis of four sub-samples taken from the stock solution of tap water spiked with indigenous flora and four sub-samples from the stock solution of tap water spiked with the control organism, *P. aeruginosa*. Each analyst will use a separate fluorometer unit and the results will be blind to the two analysts. These tests will be conducted at room temperature. The room temperature will be recorded daily using a calibrated thermometer. The average, range, standard deviation, and percent relative standard deviation (%RSD) will be determined for each of the two bacterial cultures for each analyst.

B1.1.3 Inter-assay Reproducibility

Mycometer®-test inter-assay reproducibility will be determined by having two analysts each perform sampling and analysis of air samples collected from four pumps during one chamber test. Each analyst will use a separate fluorometer unit for analyzing samples. As a result, the inter-assay reproducibility tests will evaluate the variability from using multiple sampling pumps, two operators, and different fluorometer units. This test will be conducted at room temperature. The room temperature inside the ARCA chamber will be recorded daily using a calibrated thermometer. The average, range, standard deviation, and %RSD for data generated by each analyst will be determined and the RPD between analyst's results will be calculated.

BactiQuant®-test inter-assay reproducibility will be determined from the same analyses used for repeatability (two analysts each perform sampling and analysis of four sub-samples taken from the stock solution of tap water spiked with indigenous flora and four sub-samples from the stock solution of tap water spiked with the control organism, *P. aeruginosa*). Each analyst will use a separate fluorometer unit. As a result, the inter-assay reproducibility tests will evaluate the variability from using two operators and different fluorometer units. These tests will be conducted at room temperature. The room temperature will be recorded daily using a calibrated thermometer. The average, range, standard deviation, and %RSD will be determined for the four samples analyzed by each analyst as part of the repeatability determination and the relative

percent difference (RPD) between the analyst's results will be calculated for inter-assay reproducibility.

B1.1.4 Data Completeness

No additional test procedures will be carried out specifically to address data completeness. This parameter will be assessed based on the overall data return achieved.

B1.1.5 Operational and Sustainability Factors

Operational and sustainability factors such as waste generated, maintenance needs, calibration frequency, data output, consumables used, power requirements, hazardous components, ease of use, repair requirements, and sample throughput will be evaluated based on operator observations. Battelle testing staff will document observations in a LRB or data sheets. Examples of information to be recorded include the daily status of diagnostic indicators for the technology, use or replacement of any consumables, the duration and causes of any technology down time or data acquisition failure, operator observations about technology startup, ease of use, clarity of the vendor's instruction manual, user-friendliness of any needed software, overall convenience of the technologies and accessories/consumables, or the number of samples that could be processed per hour or per day. Battelle will summarize these observations to aid in describing the technology performance in the verification report on each technology.

B1.2 Statistical Evaluation

The statistical methods and calculations used for evaluation of the quantitative performance parameters are described in the following sections.

B1.2.1 Linearity

Linearity with respect to concentration (determined as heterotrophic plate counts for bacteria and as spore counts for fungi) will be assessed by a linear regression analysis of the Mycometer®-test and BactiQuant®-test fluorescence units using the spore counts or heterotrophic plate counts as appropriate as the independent variable and the Mycometer®-test and BactiQuant®-test results as the dependent variable. The results will be plotted and linearity will be expressed in terms of slope, intercept, and coefficient of determination (R^2). Both the plots and linearity parameters

will be included in the report. Results are expected to be linear on a log scale based on linearity information included in the BactiQuant®-test manual; however, if this is not the case, other curve fitting options will be evaluated.

B1.2.2 Repeatability

Repeatability will be defined as %RSD of the replicate measurements of fungal and bacterial cultures taken with the Mycometer®-test and BactiQuant®-test, respectively. Equations 1 and 2 will be used to calculate repeatability:

$$S = \left[\frac{1}{n-1} \sum_{k=1}^{n} \left(M_k - \overline{M} \right)^2 \right]^{1/2} \quad (1)$$

where S is the standard deviation, n is the number of replicate samples, M_k is the technology fluorescence measurement for the k^{th} sample, and M is the average technology fluorescence measurement of the replicate samples.

$$RSD(\%) = \left| \frac{S}{\overline{M}} \right| \times 100 \quad (2)$$

B1.2.3 Inter-assay Reproducibility

Inter-assay reproducibility will be evaluated from four measurements of one concentration of each bacterial or fungal culture by two separate analysts using two separate fluorometer units. The average and %RSD of each analyst's measurements will be calculated. Inter-assay reproducibility will be expressed as RPD of the average measurements as noted in Equation 3:

$$RPD(\%) = \frac{\left| M_1 - M_2 \right|}{M_1 + M_2} \times 200 \quad (3)$$

where M_1 is the average of replicate measurements made by the first unit of the technology and analyst 1 and M_2 is the average of replicate measurement made by the second unit of the technology and analyst 2.

B1.2.4 Data Completeness

Data completeness will be assessed based on the overall data return achieved by each Mycometer®-test and BactiQuant®-test analysis during the testing period. For each technology, this calculation will use the total number of apparently valid data points divided by the total number of data points potentially available from all testing. The causes of any incompleteness of data return will be established from operator observations, and noted in the discussion of data completeness results. The goal for data completeness is 100%. Any problems with the data will be brought to the attention of the VTC. The VTC will try to resolve any Mycometer®-test or BactiQuant®-test data issues with the vendor. Data issues which remain will be discussed with the Battelle QM and AMS Center Manager, and EPA Project Officer and QM, as necessary.

B1.3 Reporting

The statistical comparisons described above will be conducted separately for the Mycometer®-test and BactiQuant®-test, and information on the operational performance will be compiled and reported. One verification report will be prepared covering both the Mycometer®-test and BactiQuant®-test. A verification statement will be prepared for each technology. The verification report will present the test procedures and test data, as well as the results of the statistical evaluation of those data.

Operational aspects of the technologies will be recorded by Battelle during and immediately following testing and will be summarized in the verification report. For example, descriptions of the data acquisition procedures, use of vendor-supplied proprietary software, consumables used, repairs and maintenance needed, and the nature of any problems will be presented in the report. The verification report will briefly describe the ETV program, the AMS Center, and the procedures used in verification testing. The results of the verification tests regarding Mycometer®-test and BactiQuant®-test performance will be stated quantitatively. The draft verification report 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, 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 ETV/AMS Center QMP.[1]

B2 SAMPLING METHOD REQUIREMENTS

The *Pseudomonas aeruginosa* QC strain described in section B2.1 will be used as a reference sample for plating. The sampling method requirements for preparation of stock cultures, collection and characterization of tap water, and generation of an aerosol in the ARCA chamber are described below.

B2.1 Preparation of QC and Indigenous Flora

B2.1.1 BactiQuant® Test

The bacterial cultures will include a QC organism and indigenous flora from an environmental water source. *P. aeruginosa* ATCC 27853 (QC strain) will be obtained as a QuantiCult® culture from Remel, Inc. These cultures have <100 CFU per 300 microliter (µL) aliquot. Initial preparation of the QC strain will be in accordance with the manufacturer's directions and the concentration of the rehydrated stock will be determined as described in SOP BIOTECH.III-005. The primary plate will be examined for purity and typical colony morphology (as described by ATCC reference documents). If the agar plate appears pure and exhibits the expected morphology, the agar plate is acceptable for use as the QC primary stock culture. The primary QC stock culture will be sealed with parafilm and held at 2-8 °C for use as a primary stock plate for up to one month. The QC primary stock plate will be streaked for isolation onto an agar plate (e.g., low nutrient growth agar such as R2A agar) and incubated for 18-24 hours, or longer, at the manufacturer's designated temperature (±2 °C) to prepare a working stock for spiking tap water. The working stock plate will be held at 2-8 °C for up to one week. The working stock plate will be removed from refrigerated storage, if necessary, and held at room temperature for at least 15 minutes to acclimate to room temperature. Isolated colonies will be aseptically transferred to a sterile tube containing a diluent (sterile water or buffer) to create a working stock with turbidity equivalent to a 0.5 McFarland standard using a nephelometer (approximately 10^8 CFU/mL) or similar device. The *P. aeruginosa* working stock will be serially diluted with phosphate buffered saline (PBS) and the plate count will be determined in triplicate. Each plate count determination will involve up to three dilutions with at least three plates per dilution using Battelle method MICRO MD2009-01, *Method for Enumeration of Bacteria in Liquid Samples* to determine the stock concentration. Dilutions selected for reporting the plate count will have counts from 30-300 CFU. Procedures described in SM 9215[6] will be used for reporting counts outside of this

range. Subsequent dilutions of the working stock will be prepared in tap water to generate four test concentrations of the organism (estimated concentration 50-50,000 CFU/mL). The four test concentrations of bacteria in dechlorinated tap water will be enumerated using the heterotrophic plate count method (SM 9215[6]). Spread plates will be used for all enumerations using disposable sterile spreaders. All dilutions for plating will be conducted using calibrated pipettors.

Approximately one liter of water from a river or lake will be collected in a sterile container to obtain a natural consortium of environmental bacteria. The sample collection will be noted on a chain-of-custody form as described in Section B3. Samples will be transported back to the laboratory at ambient temperature and receipt will be logged into a laboratory inventory form or documented in a laboratory record book. The water containing indigenous bacteria (primary stock) will be quantified for culturable bacteria using the heterotrophic plate count (SM 9215[6]), within eight hours of sample collection. The heterotrophic plate count does not measure viable unculturable bacteria; therefore, the heterotrophic plate count measurements represent a subset of the bacterial population. The water will be stored at room temperature using simulated environmental conditions (cycled light exposure) during storage. The initial heterotrophic plate count will be used to prepare dilutions in tap water to generate four test concentrations of the flora (estimated concentration 50-50,000 CFU/mL). Three plate count determinations will be made for the primary stock and four test concentrations. Each plate count determination will involve up to three dilutions with at least three plates per dilution using the heterotrophic plate count method (SM 9215[6]). Dilutions selected for reporting the plate count for both indigenous flora and the QC strain will have counts from 30-300 CFU. Procedures described in SM 9215[6] will be used for reporting counts outside of this range. Spread plates will be used for all enumerations using disposable sterile spreaders. All dilutions for plating will be conducted using calibrated pipettors.

B2.1.2 Mycometer® Test

Cladosporium herbarum ATCC 58927 and *Aspergillus flavus* ATCC 58870, the two genera chosen for use in testing, were selected based on their presence in indoor fungal isolates as reported in the literature[8,9,10, 11]. The specific strains (species designation and ATCC number) were selected based on isolation from air samples as indicated by ATCC. The fungal cultures will be obtained directly from ATCC and rehydrated according to the manufacturer's instructions. Spore stocks will be prepared by growing *A. flavus* ATCC 58870 and *C. herbarum* ATCC 58927 on Potato Dextrose Agar (PDA) or Malt Agar at 24±2°C. Several agar plates (at least 10) will be prepared and incubated for at least ten days (typically 10-20 days). In the event sporulation does not occur, environmental conditions (humidity increase, reduced oxygen, temperature shift) may be altered to induce sporulation. Spores will be harvested using sterile water (deionized or distilled) containing a wetting agent, clumps will be broken up by shaking with sterile glass beads, and hyphae will be removed by filtration through sterile glass wool or a double layer of cheesecloth. The spore stocks will be washed with sterile water a minimum of three times. The stock concentrations will be estimated (N = 5) for each fungal culture using a hemocytometer (ASTM D4300-01 Annex[11]) to determine the number of spores per mL. The volume of the primary fungal spore stocks may need to be adjusted based on test requirements. Each stock will be stored at 2-8 °C. Based on the spore counts, a dilution series for each of the fungal cultures will be performed to achieve four test concentrations for assessing linearity. The estimated test concentration of each stock will be back calculated based on the original spore counts.

B2.2 Collection, Characterization, and Preparation of Tap Water

At least 20 L of tap water will be collected and dechlorinated for each test day that tap water is used. Tap water will be collected into one or more sterile carboys. The total volume collected will accommodate the preparation of 250 mL aliquots for water testing using the bacterial and fungal stocks. Tap water samples 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 at least 20 L of water from the tap into one or more sterile (autoclaved) carboys equipped with a spigot, and containing large stir bars.

Once collected, Battelle will take a 100 mL aliquot from the carboy's spigot and characterize the tap water by measuring the pH (SOP GEN.V-003) and the concentration (mg/L) of free and total chlorine using a HACH Chlorine kit (HACH Methods 8021[14] and 8167[15], respectively). Additional water will be removed from the carboy's spigot and sent under chain-of-custody (COC) as described in B3 to Pace Analytical (Columbus, Ohio) for characterization analysis (see Table 2). The tap water will be used the same day it is collected and dechlorinated. Results for pH, and free and total chlorine will be available the same day. Characterization tests performed by Pace Analytical (Table 2) will be available within two weeks. The tap water will be collected, characterized, and dechlorinated with sodium thiosulfate as described below:

- Collect water from tap in Battelle laboratory,
- Remove aliquots necessary to send to Pace Analytical for characterization tests (Table 2),
- Measure pH (SOP GEN.V-003), free and total chlorine (HACH Method 8021[14] & 8167[15]),
- Dechlorinate using sodium thiosulfate (SM 9060A[6]),
- Re-measure pH (SOP GEN.V-003), free and total chlorine (HACH Method 8021[14] & 8167[15]),
- Check the level of contaminating organisms using the heterotrophic plate count (SM 9215[6]), and
- Run water blanks during sample analysis to determine the fluorescence associated with the water.

B2.3 Generation of an Aerosol in the ARCA Chamber

The ARCA chamber will be decontaminated prior to testing as described in the SOP ABAT-H-003. The tripods used to hold sample canisters will be set up in the chamber in close proximity to each other. Air sampling pumps will be outside of the chamber with tubing connected to the sample canisters through chamber ports. Generation of the aerosol will be conducted using a Collision nebulizer. The starting spore stock concentration (spores/mL) for generation of the aerosol will be determined by calculations based on the generation chamber flow rate (LPM),

aerosol generation rate (mL/minute), total concentration generated, expected sample concentration (total spores), sampling flow rate (20 LPM), expected aerosol concentration (spores/L of air), and sampling time (15 minutes). The following provides an example of a back-calculation for determining the initial concentration of spores required in a stock solution for a final sample concentration of 25,000 spores/sample collected:

Sampling flow rate = 20 LPM
Sample time = 15 minutes
Generation rate = 0.25 mL/min
Chamber flow rate = 10,000 LPM

25,000 spores = (aerosol concentration in spores/Liter of air)(20 LPM)(15 min.)

$$\text{Aerosol Concentration} = \frac{\text{Generation rate x Material concentration}}{\text{Chamber flow rate}}$$

Therefore:

$$25{,}000 \text{ spores} = \frac{(0.25 \text{ mL/min.})(X \text{ spores/mL})}{10{,}000 \text{ LPM}} \times 20 \text{ LPM} \times 15 \text{ minutes}$$

$X = 3.3 \times 10^6$ spores/mL

The goal will be to produce an aerosol sample which results in a fluorescence signal at least 300 fluorescence units above the level of a blank, as the vendor indicates this level is often seen in buildings. A calibrated Aerodynamic Particle Sizer (APS) will be used to monitor particle size and concentration. Flow through the chamber will be monitored at the outlet using a calibrated hot-wire anemometer.

An initial characterization run will be performed to check the test parameters to ensure sample fluorescence is measured by the Mycometer®-test unit and adjust the test system or stock concentration accordingly. The chamber will be air washed with HEPA-filtered air for a minimum of 15 minutes. Eight background samples will be collected for analysis using Mycometer®-test. One test run will be conducted using an aerosolized fungal stock to collect repeatability data (Mycometer®-test analysis). The chamber will be air washed for a minimum of 15 minutes. The tripods will be surface decontaminated and canisters set up for collection and analysis of background samples. A second test will be run with an aerosolized fungal stock to collect inter-assay reproducibility data (Mycometer®-test analysis). Upon completion of the test

runs, the chamber will be air washed. The equipment and chamber surfaces will be decontaminated in accordance with the SOP ABAT-H-003 for Staff Entry and Exit, and Decontamination of the Aerosol Research and Component Assessment (ARCA) Chamber.

B3 SAMPLE HANDLING AND CUSTODY REQUIREMENTS

Sample custody will be documented for collection and analysis of the tap water and lake or river water for indigenous flora following Battelle SOP BIOTECH.I-032 for the Transmittal of Test Articles, Control Articles, and Specimens. A COC form will include details about the sample such as the time, date, location, and person collecting the sample. COC forms will track sample release from the sampling location to the testing laboratory. COC forms 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. All COC forms will be delivered to the VTC and maintained with the test records.

The receipt of standards used for testing will also be documented following BIOTECH.I-032 for the Transmittal of Test Articles, Control Articles, and Specimens. For long term storage, the standards will be stored as recommended by the vendor. Working stocks will be stored as described in B2.1.

B4 ANALYTICAL METHOD REQUIREMENTS

Analytical methods that Battelle Testing Staff will use in the conduct of these verification tests are listed in Table 10. Methods to be used by Pace Analytical for tap water characterization are listed in Table 2. Battelle Testing Staff will use sterile disposable spreaders in place of glass rods for all spread plate methods. Dilutions of samples for plating will be prepared using calibrated pipettors. Working stock concentrations (N = 4) for linearity testing will be prepared using volumetric pipettes.

Table 10. Analytical Methods for Bacterial and Fungal Testing

Description	Method
pH	SOP GEN.V-003
Total Chlorine	Hach Method 8167[15], equivalent to U.S. EPA Standard Method 4500-CL G for drinking water
Free Chlorine	Hach Method 8021[14], equivalent to U.S. EPA Standard Method 4500-CL G for drinking water
Standard Plate Count	MCRO MD2009-001
Heterotrophic Plate Count	SM 9215[6] Using the spread plate method, R2A agar, incubation at 20°C-28°C for 5-7 days
Fungal Spore Concentration	ASTM D4300-01 Annex

B5 QUALITY CONTROL REQUIREMENTS

Quality control procedures will follow the requirements described in this QAPP, the ETV QMP[2], and any vendor specified requirements for analysis using Mycometer®-test or BactiQuant®-test. All standard values and equipment calibration for this technology will be documented in the study records. Tap water control blanks, positive control organism, and media controls will all be used to support data generated. DQIs and MQOs are defined in Table 7.

B6 INSTRUMENT/ EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

Battelle staff will operate and maintain the Mycometer®-test and BactiQuant®-test units as directed by the vendor during staff training and as noted in the technology operating manuals. The vendor will be consulted if issues with the technologies arise. Temperatures for supporting equipment including refrigerators, freezers, and incubators will be monitored and logged, at a minimum, on a daily basis, during conduct of the verification tests. Critical measurements and MQOs related to operating the Mycometer®-test and BactiQuant®-test are included in Table 11.

B7 INSTRUMENT CALIBRATION AND FREQUENCY

For each day of testing, the fluorometers will be calibrated prior to analysis of each sample set (four samples and a blank) for the BactiQuant®-test and at a minimum, daily, for Mycometer®-test technologies. If the calibration fails, the fluorometers will be recalibrated by Battelle staff as described in the manual. All calibrations performed will be documented by Battelle staff on the analysis form for the test. Repeat calibrations may be recorded on a separate LRB sheet or

calibration form. Supporting instrumentation will be calibrated or certified as noted in Table 12 following their applicable SOPs (see Appendix 2). The supporting equipment will be within calibration before use in these verification tests.

Table 11. Criteria for QC and Critical Measurements for Bacterial and Fungal Testing

Test	Method of Assessment	Frequency	MQO	Corrective Action
Mycometer®-test and BactiQuant®-test	Check fluorescence of sample blank prior to analysis of a sample set	Run with each sample set	Value <300	Repeat measurement
Mycometer®-test	Perform a two point calibration using the kit standard	Each day of use at a minimum	Black cuvette is 0±1 and the standard value should be within 3% of the standard value noted on the sticker with the instrument	Repeat calibration
Mycometer®-test	Measure ambient air temperature	Prior to each set of analysis	18-30 °C	Adjust room temperature
Mycometer®-test	Sampling volume based on time and flow rate	For each sample set	>300 LPM	Use calibrated timer and adjust
BactiQuant®-test	Measure sample temperature (one per sample set)	Prior to each set of analysis	18-30 °C	Continue to allow samples to reach the appropriate temperature
BactiQuant®-test	Perform a two point calibration with the kit standard	Prior to each series of measurements	Black cuvette 0±1 and standard is within 3% of the reading of the standard value <STD VAL> button	Repeat calibration

Table 12. Supporting Equipment Calibration and Certification for Bacterial and Fungal Testing

Instrument	Frequency
Thermometer	Annually
REES monitoring system	Annually
Micro-pipettors	Every six months
Nephelometer	Check with standards daily when used
Analytical balance	Annual calibration, checked daily when used
Hach colorimeter	Check with standards daily when used
Biosafety cabinets	Certified every six months
pH meter	Calibrated annually, checked daily when used
Aerodynamic Particle Sizer	Annually
Anemometer	Annually

B8 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

Upon receipt of any supplies or consumables used for testing, Battelle will visually inspect and ensure that the materials received are those that were ordered and that there are no visual signs of damage that could compromise the suitability of the materials. If damaged or inappropriate goods are received they will be returned or disposed of and arrangements will be made to receive replacement materials. COA or other documentation provided with all reagents, standards, and ATCC or QuantiCult® cultures will be checked to ensure suitability for these verification tests. Unsuitable materials will be returned or disposed of and arrangements for the receipt of replacement materials will be made.

B9 NON-DIRECT MEASUREMENTS

Non-direct measurements will not be used during these verification tests.

B10 DATA MANAGEMENT

Various types of data will be acquired and recorded electronically or manually by Battelle staff during these verification tests. All manually-recorded data, such as solution preparation records and results from supporting analyses such as heterotrophic plate counts, will be recorded in permanent ink. Corrections to records will be made by drawing a single line through the entry to be corrected and providing a simple explanation for the correction, along with a date and the initials of the person making the correction. BactiQuant®-test and Mycometer®-test fluorescence readings, and calculated Mycometer®-test air fungal concentration values will be recorded electronically by each technology unit and then downloaded to a computer daily. Table 13 summarizes the types of data to be recorded. All maintenance activities, repairs, calibrations, and operator observations relevant to the operation of the monitoring systems being tested will be documented by Battelle staff in the LRB or on data sheets. Report formats will include all necessary data to allow traceability from the raw data to final results.

Records received by or generated by any Battelle staff during testing will be reviewed by a Battelle staff member within five days of receipt or generation, respectively, before the records are used to calculate, evaluate, or report verification results. 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 who 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. Some of the checks that will be performed include:

- QC samples and calibration standards were analyzed according to the QAPP 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 QAPP, and
- Results of QC samples are reported.

In addition, any 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 and concentration calculations described in this QAPP. A dedicated shared folder within the ETV AMS Center SharePoint site will be established for all project records.

Battelle will provide technology test data (including records, data sheets, and notebook records) from the first day of testing within five days of generation to EPA for simultaneous review. Thereafter, the data will be provided to EPA every two weeks. The goal of this data delivery schedule is prompt identification and resolution of any data collection or recording issues. These data will be labeled as preliminary and may not have had a QA review before their release.

Table 13. Summary of Data Recording Process

Data to Be Recorded	Where Recorded	How Often Recorded	By Whom	Disposition of Data
Dates and details of test events	ETV LRBs or data forms	Start/end of test event	Battelle staff	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
BactiQuant®-test and Mycometer®-test analyst, collection and analysis dates, sample volume and/or time, sample description	ETV LRBs or electronically	When performed	Battelle staff	Incorporated in verification report as necessary
BactiQuant®-test and Mycometer®-test calibration and/or standard value information, substrate and calibration batch information, customer identification, percent difference from standard value for instrument	ETV LRBs or electronically	When performed	Battelle staff	Incorporated in verification report as necessary
BactiQuant®-test and Mycometer®-test ambient temperature or room temperature, reaction time, blank values	ETV LRBs or electronically	With each sample set	Battelle staff	Incorporated in verification report as necessary
BactiQuant®-test and Mycometer®-test readings and calculated Mycometer®-test air values	Recorded electronically by each technology unit and then downloaded to computer daily	Each measurement initiated by Battelle staff	Battelle staff	Converted to spreadsheet for statistical analysis and comparisons

SECTION C

ASSESSMENT AND OVERSIGHT

C1 ASSESSMENTS AND RESPONSE ACTIONS

Every effort will be made in these verification tests to anticipate and resolve potential problems before the quality of performance is compromised. One of the major objectives of this QAPP is to establish mechanisms necessary to ensure this. Internal quality control measures described in this QAPP, which is peer reviewed by a panel of outside experts, implemented by the technical staff and monitored by the VTC, 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 VTC, who will contact the Battelle AMS Center Manager, Battelle AMS Center QM, EPA AMS Center Project Officer, and EPA AMS Center QM if any deviations from the QAPP are observed. The VTC 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 VTC. Technical staff and the VTC will work with the Battelle QM to resolve any issues. Action will be taken by the VTC 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 these verification tests.

C1.1 Performance Evaluation Audit

Performance evaluation (PE) audits for plate counts and hemocytometer counts will be conducted by having two analysts independently take a measurement for one dilution of the QuantiCult QC strain (plate counts) and one stock culture (hemocytometer). To be considered acceptable, the two analysts' results should agree within 20% for the plate counts and 25% for the hemocytometer. QC materials will be used to access technology performance. The DQIs and MQOs listed in Table 7 will be used to assess performance for these measurements.

C1.2 Technical Systems Audits

The Battelle QM or designee will perform a TSA during performance of both the BactiQuant®-test and Mycometer®-test laboratory activities. The purpose of these audits is to ensure that the verification tests are being performed in accordance with the AMS Center QMP[1] and this QAPP. The Battelle QM will compare actual test procedures to those specified or referenced in this plan, and review data acquisition and handling procedures. The Battelle QM or designee will prepare a project-specific checklist based on the QAPP requirements to guide the TSA, which will include a review of the test location and general testing conditions; observe the testing activities; and review test documentation. She will also check data acquisition procedures, and confer with Battelle staff. The Battelle QM will prepare an initial TSA report and will submit the report to the EPA QA Manager (with no corrective actions documented) and VTC within 10 business days after completion of the audit. A copy of each final TSA report (with corrective actions documented) will be provided to the EPA AMS Center Project Officer and QM 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 tests. The TSA findings will be communicated to technical staff at the time of the audit and documented in the TSA reports.

C1.3 Data Quality Audits

The Battelle QM, or designee, will audit at least 10% of the sample results data acquired in the verification tests and 100% of the calibration and QC data versus the QAPP requirements. Two ADQs will be conducted for this project: Data collected on the first day of testing for each technology will be audited within 10 business days of receipt and assessed using a project-specific checklist. The remaining data will be audited at the conclusion of testing and will be completed within 10 business days of receipt of all test data. During these audits, the Battelle QM, or designee, will trace the data from initial acquisition (as received from the vendor's technology), through reduction and statistical comparisons, to final reporting. All calculations performed on the data undergoing the audit of data quality (ADQ) will be checked. Data must undergo a 100% validation and verification by technical staff (i.e.VTC, or designee) before it will be assessed as part of the data quality audit. All QC data and all calculations performed on the data undergoing the audit will be checked by the Battelle QM or designee. Results of each ADQ will be documented using the checklist and reported to the VTC and EPA within 10

business days after completion of the audit. A final ADQ that assesses overall data quality, including accuracy and completeness of the technical report, will be prepared as a narrative and distributed to the VTC and EPA within 10 business days of completion of the audit.

C1.4 QA/QC Reporting

Each assessment and audit will be documented in accordance with Section 3.3.4 of the AMS Center QMP.[1] The results of all audits will be submitted to EPA within 10 business days as noted above. 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, and
- Citation of any noteworthy practices that may be of use to others.

C2 REPORTS TO MANAGEMENT

During the laboratory evaluation, any QAPP deviations will be reported immediately to EPA. The Battelle QM and/or VTC, 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. A summary of the required assessments and audits, including a listing of responsibilities and reporting timeframes, is included in Table 14. If serious quality problems exist, the Battelle QM will notify the AMS Center Manager, who is authorized to stop work. Once the assessment reports have been prepared, the VTC 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 QM will ensure that follow-up corrective action has been taken. The QAPP and final report are reviewed by the EPA AMS Center QM and the EPA AMS Center Project Officer. Upon final review and approval, both documents will then be posted on the ETV website (www.epa.gov/etv).

Table 14. Summary of Quality Assessment and Control Reports[1]

Assessment	Prepared By	Report Submission Timeframe	Submitted To
BactiQuant®-test TSA (within the first week of testing)	Battelle	10 business days after TSA is complete[2] TSA response is due to QM within 10 business days TSA responses will be verified by the QM and provided to EPA within 20 business days	EPA ETV AMS Center
Mycometer®-test TSA (within the first week of testing)	Battelle		EPA ETV AMS Center
ADQ (Day 1 data) each technology	Battelle	ADQ will be completed within 10 business days after receipt of first data set	EPA ETV AMS Center
ADQ (Remaining data and verification report)	Battelle	ADQ will be completed within 10 business days after completion of the verification report review	EPA ETV AMS Center

[1] Any QA checklists prepared to guide audits will be provided with the audit report.
[2] A separate TSA report will be prepared for each technology; the report submission timeframe is the same for each.

SECTION D

DATA VALIDATION AND USABILITY

D1 DATA REVIEW, VERIFICATION, AND VALIDATION REQUIREMENTS

The key data review and data verification requirements for these tests are stated in Section B10 of this QAPP. In general, the data review requirements specify that data generated during these tests will be reviewed by a Battelle technical staff member within five days of generation of the data. The reviewer will be familiar with the technical aspects of the verification test but will not be the person who generated the data. This process will serve both as the data review and the data verification, and will ensure that the data have been recorded, transmitted and processed properly. Furthermore, this process will ensure that the monitoring systems data were collected under appropriate testing.

The data validation requirements for these tests involve an assessment of the quality of the data relative to the DQIs and MQOs for these tests referenced in Table 7. Any deficiencies in these data will be flagged and excluded from any statistical comparisons, unless these deviations are accompanied by descriptions of their potential impacts on the data quality.

D2 VERIFICATION AND VALIDATION METHODS

Data verification is conducted as part of the data review as described in Section B10 of this QAPP. A visual inspection of handwritten data will be conducted to ensure that all entries were properly recorded or transcribed, and that any erroneous entries were properly noted (i.e., single line through the entry, with an error code, such as “wn” for wrong number, and the initials of the recorder and date of entry). Electronic data from the Mycometer®-test and BactiQuant®-test and any other analytical equipment used during the test will be inspected to ensure proper transfer from the data logging system. All calculations used to transform the data will be reviewed to ensure the accuracy and the appropriateness of the calculations. Calculations performed manually will be reviewed and repeated using a handheld calculator or commercial software (e.g., Excel). Calculations performed using standard commercial office software (e.g., Excel) will be reviewed by inspection of the equations used for the calculations and verification

of selected calculations by handheld calculator. Calculations performed using specialized commercial software (i.e., for analytical instrumentation) will be reviewed by inspection and, when feasible, verified by handheld calculator, or standard commercial office software.

To ensure that the data generated from these tests meet the goals of the tests, a number of data validation procedures will be performed. Sections B and C of this QAPP provide a description of the validation safeguards employed for these verification tests. Data validation efforts include the completion of QC activities, and the performance of two TSA audits as described in Section C. The data from these tests will be evaluated relative to the MQOs described in Section A8 of this QAPP. Data failing to meet these criteria will be flagged in the data set and not used for evaluation of the Mycometer®-test and BactiQuant®-test, unless these deviations are accompanied by descriptions of their potential impacts on the data quality.

An audit of data quality will be conducted by the Battelle QM to ensure that data review, verification, and validation procedures were completed, and to assure the overall quality of the data.

D3 RECONCILIATION WITH USER REQUIREMENTS

This purpose of these verification tests is to verify the performance of Mycometer®-test and BactiQuant®-test for use in rapid determination of fungi and bacteria, respectively. To meet the requirements of the user community, input on the tests described in this QAPP has been provided by external experts. Additional performance data regarding operational characteristics of the Mycometer®-test and BactiQuant®-test 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 tests. 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 QAPP and the resulting ETV verification report will be subjected to review by the vendor, EPA, and expert peer reviewers. The reviews of this QAPP will help to improve the design of the verification tests and the resulting report such that they better meet the needs of potential users of these technologies.

SECTION E

REFERENCES

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APPENDIX 1

VENDOR DATA SPREADSHEETS

Mycometer®-Air

Quantification of mold/fungi in air

Project Name _____

Location _____

Collected by _____

Analysis date _____ (enter as mm/dd/yy)

Measured Standard Value _____

Instrument Standard Value _____

Ambient temperature for analysis (between 64.4 - 86 F) _____

Reaction time(min:sec) _____

Sample ID/No.	Sample Location	Sample Date mm/dd/yy	Flow Rate (LPM)	Sample Time (Mins)	Sample Volume (m3)	Blank Value (BV)	Sample Analysis (AV)	Mycometer Air Value (MAV/m3)	Result Category

Interpretation Categories:

A

B

C

BDL Below detection limit

Analyst Signature: _____

Print Name and Company: _____

Mycometer Certification Number: _____

Mycometer®-Air Data Summary

Quantification of mold/fungi in Air

Project Name _____

Location _____ Analysis Date _____

Collected by _____

Sample ID/No.	Sample Location & Surface Type	Sample Date	Mycometer Air Value (MAV)	Result Category A	Result Category B	Result Category C

Interpretation Categories:

A	Fungi concentration is not above normal background level.
B	Above normal background level; typically,high dust spore levels, low density or old fungal growth
C	Level of fungi is high, above normal background level, due to fungal growth
BDL	Below detection limit

Analyst Signature: _____

Print Name and Company: _____

Mycometer Certification Number: _____

Substrate batch:	
Room temperature:	
Date:	
Performed by:	
Customer ID:	
Calibration batch:	
STD Value	
" +/- 2 %"	
Calibration value:	

Minimum incubation time	
Volume filtered (ml)	Calculated (minutes)
	#DIV/0!

Minimum incubation time is 10 minutes

Sample ID	Room temperature (^{o}C)	Reaction time (min)	Blank (Fu)	Bottle weight (empty) (gram)	Bottle weight (filled) (gram)	Weight water (gram)	Analysis value (Fu)	BQ value Calculated
						0		#DIV/0!
						0		#DIV/0!
						0		#DIV/0!
						0		#DIV/0!
						0		#DIV/0!
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APPENDIX 2

LIST OF RELEVANT BATTELLE DOCUMENTS

SOP or Method Number	Document Name
ABAT-H-001	SOP for the Operation of the Aerosol Research and Component Assessment (ARCA) Chamber
ABAT-H-002	SOP for Gathering and Transporting Samples from the Aerosol Research and Component Assessment (ARCA) Chamber
ABAT-H-003	SOP for Staff Entry and Exit, and Decontamination of the Aerosol Research and Component Assessment (ARCA) Chamber
BIOTECH. I-010	SOP for the Operation and Maintenance of a Biological Safety Cabinet
BIOTECH. I-017	SOP for Labeling Reagents, Solutions, Test Articles, Specimen and Waste Bottles in GLP Laboratories
BIOTECH. I-018	SOP for Safe Handling and Storage of Etiologic Agents
BIOTECH.I-019	SOP for Use and Maintenance of Incubators
BIOTECH. I-027	SOP for Handling and Distribution of Test Articles, Control Articles, and Specimens
BIOTECH.I-032	SOP for the Transmittal of Test Articles, Control Articles, and Specimens
BIOTECH.II.006	SOP for the Operation and Maintenance of the Getinge/Castle Model 133L Autoclave
BIOTECH. II-024	SOP for the Maintenance of the Getinge/Castle Model M/C 3533 Steam Sterilizer
BIOTECH. II-025	SOP for Fixed and Adjustable Volume Pipettors
BIOTECH. II-037	SOP for the Operation and Maintenance of a Centrifuge
BIOTECH. II-055	SOP for the Use of the REES Centron Automated Environmental Monitoring and Alarm System
BIOTECH. II-089	SOP for the Operation and Maintenance of the Milli-Q UV Plus
BIOTECH.III-005	SOP for the Use of Quanti-Cult® and Quanti-Cult$^{PLUS®}$ Commercial Reference Strains
GEN.I-030	SOP for Labeling Reagents, Solutions, Test and Control Articles and Specimens
GEN.I-042	SOP for Recording, Reviewing, and Correcting Raw Data
GEN.II-001	SOP for Documentation of Training
GEN.III-017	SOP for Qualifying Spreadsheets and Other Data Analysis Application Software
GEN.IV-003	SOP for Numeric Data and Calculations
GEN.V-001	SOP for the Use of Refrigerators, Freezers, and Room Temperature Facilities

SOP or Method Number	Document Name
GEN.V-002	SOP for the Use of Electronic Balances
GEN.V-003	SOP for the Use of pH Meters to Measure pH
GEN.V-009	SOP for the Use of Equipment Logbooks
GEN.V-013	SOP for the Calibration and Maintenance of Thermometers
GEN.VII-005	SOP for Disposal of Laboratory Waste
MICRO MD2009-01	Method for Enumeration of Bacteria in Liquid Samples
	Battelle Building 20 Biosafety Manual