Environmental Technology Verification

Supplement to Test/QA Plan for Biological and Aerosol Testing of General Ventilation Air Cleaners

Bioaerosol Inactivation Efficiency by HVAC In-Duct Ultraviolet Light Air Cleaners

EPA Cooperative Agreement R-83191101
Research Triangle Institute Project 029309

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Paul Groff
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<thead>
<tr>
<th>Acronym/Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADQs</td>
<td>audits of data quality</td>
</tr>
<tr>
<td>ANSI</td>
<td>American National Standards Institute</td>
</tr>
<tr>
<td>APCT center</td>
<td>Air Pollution Control Technology verification center</td>
</tr>
<tr>
<td>ARTI</td>
<td>Air-Conditioning and Refrigeration Technology Institute, Inc.</td>
</tr>
<tr>
<td>ASHRAE</td>
<td>American Society of Heating, Refrigerating, and Air-conditioning Engineers</td>
</tr>
<tr>
<td>ASME</td>
<td>American Society of Mechanical Engineers</td>
</tr>
<tr>
<td>Bg</td>
<td>Bacillus atrophaeus [formerly B. subtilis var niger, or Bacillus globigii]</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>cm³</td>
<td>cubic centimeter(s)</td>
</tr>
<tr>
<td>CFU</td>
<td>colony forming unit</td>
</tr>
<tr>
<td>cfm</td>
<td>cubic feet per minute</td>
</tr>
<tr>
<td>cm</td>
<td>centimeter(s)</td>
</tr>
<tr>
<td>culturable</td>
<td>able to be grown on microbiological media</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variance</td>
</tr>
<tr>
<td>d₅₀</td>
<td>50% cut point on AGI sampler</td>
</tr>
<tr>
<td>DQO</td>
<td>data quality objective</td>
</tr>
<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Eeff</td>
<td>effective (germicidal) irradiation</td>
</tr>
<tr>
<td>EPA</td>
<td>U.S. Environmental Protection Agency</td>
</tr>
<tr>
<td>Eq.</td>
<td>equation</td>
</tr>
<tr>
<td>ETV</td>
<td>environmental technology verification</td>
</tr>
<tr>
<td>°F</td>
<td>degrees Fahrenheit</td>
</tr>
<tr>
<td>g</td>
<td>gram(s)</td>
</tr>
<tr>
<td>HEPA</td>
<td>high efficiency particulate air</td>
</tr>
<tr>
<td>HVAC</td>
<td>heating, ventilation and air conditioning</td>
</tr>
<tr>
<td>in.</td>
<td>inch(es)</td>
</tr>
<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
</tr>
<tr>
<td>KCl</td>
<td>potassium chloride</td>
</tr>
<tr>
<td>Kr</td>
<td>krypton</td>
</tr>
<tr>
<td>L</td>
<td>liter(s)</td>
</tr>
<tr>
<td>m</td>
<td>meter(s)</td>
</tr>
<tr>
<td>m³</td>
<td>cubic meter(s)</td>
</tr>
<tr>
<td>MERV</td>
<td>minimum efficiency reporting value of ASHRAE Standard 52.2</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>min</td>
<td>minimum</td>
</tr>
<tr>
<td>min.</td>
<td>minute(s)</td>
</tr>
<tr>
<td>ML</td>
<td>microbiology laboratories</td>
</tr>
<tr>
<td>mL</td>
<td>milliliter(s)</td>
</tr>
<tr>
<td>mm</td>
<td>millimeter(s)</td>
</tr>
<tr>
<td>MSDS</td>
<td>material safety data sheet</td>
</tr>
<tr>
<td>MS2</td>
<td>bacterial virus or bacteriophage</td>
</tr>
<tr>
<td>NIST</td>
<td>National Institute of Standards and Technology</td>
</tr>
<tr>
<td>n</td>
<td>count, number of microorganisms</td>
</tr>
<tr>
<td>nm</td>
<td>nanometer</td>
</tr>
<tr>
<td>OPC</td>
<td>optical particle counter</td>
</tr>
<tr>
<td>PEA</td>
<td>performance evaluation assessment</td>
</tr>
<tr>
<td>PFU</td>
<td>plaque forming unit</td>
</tr>
<tr>
<td>PSE</td>
<td>particle size (removal) efficiency</td>
</tr>
<tr>
<td>psig</td>
<td>pounds per square inch gauge</td>
</tr>
<tr>
<td>PSL</td>
<td>polystyrene-latex</td>
</tr>
<tr>
<td>QA</td>
<td>quality assurance</td>
</tr>
<tr>
<td>QC</td>
<td>quality control</td>
</tr>
<tr>
<td>QMP</td>
<td>quality management plan</td>
</tr>
<tr>
<td>RH</td>
<td>relative humidity</td>
</tr>
<tr>
<td>RTI</td>
<td>Research Triangle Institute, RTI International*</td>
</tr>
<tr>
<td>sec</td>
<td>second(s)</td>
</tr>
<tr>
<td>SMPS</td>
<td>scanning mobility particle sizer</td>
</tr>
<tr>
<td>SOP</td>
<td>standard operating procedure</td>
</tr>
<tr>
<td>t</td>
<td>temperature</td>
</tr>
<tr>
<td>TOP</td>
<td>technical operating procedure</td>
</tr>
<tr>
<td>TSA</td>
<td>technical system audit</td>
</tr>
<tr>
<td>TTEP</td>
<td>technology testing and evaluation program</td>
</tr>
<tr>
<td>μm</td>
<td>micrometer</td>
</tr>
<tr>
<td>μW</td>
<td>micro watt</td>
</tr>
<tr>
<td>UV</td>
<td>ultra-violet light</td>
</tr>
<tr>
<td>UVC</td>
<td>ultra-violet light in the C (shortwave) band, wavelength 290 nm - 100 nm</td>
</tr>
<tr>
<td>UVG</td>
<td>ultra-violet light that is germicidal, wavelength of 253.7 nm</td>
</tr>
</tbody>
</table>

* RTI International is a trademark of the Research Triangle Institute
SECTION A: PROJECT MANAGEMENT

A4: Project/Task Organization
See Section A4 of the test/QA plan.

A5: Problem Definition/Background Information
RTI's Air Pollution Control Technology Verification Center (APCT Center) has selected general ventilation air cleaners as a technology area. Information can be found in the *Generic Verification Protocol for Biological and Aerosol Testing of General Ventilation Air Cleaners*\(^1\) on the US EPA's Environmental Technology Verification (ETV) website. This supplement is for the *Test/QA Plan for Biological and Aerosol Testing of General Ventilation Air Cleaners*\(^2\) that corresponds to the generic protocol. The supplement describes the test and quality assurance (QA) procedures that will be used to evaluate the performance of in-duct ultraviolet (UV) light air cleaners to inactivate airborne microorganisms from a heating, ventilation and air-conditioning (HVAC) duct under standard test conditions. In this document, test/QA plan will refer to the test/QA plan defined in this paragraph. Any reference for the test plan will include both the original test/QA plan and the supplement. All work will be done following the APCT Center and ETV quality management plans\(^3,4\) (QMPs).

UV lamps have been used to inactivate airborne microorganisms for many years. Much of the early work was directed at the control of very infectious microorganisms (particularly *Mycobacterium tuberculosis*, the causative agent of tuberculosis), often in medical facilities. Wavelengths within the short wave, or C band of UV light (UVC), were found to be the most effective germicidal light wavelength. UVC is generated by electrical discharge through low-pressure mercury vapor enclosed in a glass tube that transmits UVC (primary wavelength of 253.7 nm) light. UVC at 253.7 nm is also referred to as UVG to indicate that it is germicidal. UVC has been shown to inactivate viruses, mycoplasma, bacteria, and fungi, whether suspended in air or deposited on surfaces.

For constant and uniform UVC irradiance, the effect on a single species population of microorganisms can be expressed as (Phillips, 1992\(^5\)):

\[
\frac{N_t}{N_0} = \exp (- k \cdot \text{dose})
\]

(Equation 1)

Where:
- \(N_t\) = the number of microorganisms at any time \(t\),
- \(N_0\) = the number of microorganisms at start, before exposure begins,
- \(k\) = a microorganism-dependent rate constant, in \(\text{cm}^2/\mu\text{W}\cdot\text{s}\),
- \(\text{dose} = E_{\text{eff}} \cdot \Delta t\), in \(\mu\text{W}\cdot\text{s}/\text{cm}^2\),
- \(E_{\text{eff}}\) = the effective (germicidal) irradiance received by the microorganism, in \(\mu\text{W/cm}^2\), and
- \(\Delta t\) = exposure duration, s, between the start and time \(t\).
The equation describes an exponential decay in the number of living organisms as a constant level of UVC exposure continues. The same type of equation is used to describe the effect of disinfectants on a population of microorganisms, with the dose in that case being a concentration-time product. The fractional inactivation after time $t$ is \((1-N_t/N_0)\).

Numerous studies of UVC to inactivate microorganisms have been conducted, for a variety of purposes and with a variety of methods. While the overall result (UVC destroys microorganisms) has been the same, the studies have differed with regard to some of the secondary parameters. No standard method exists for evaluating culturable bioaerosol inactivation by these devices. As part of the project entitled, “Defining the Effectiveness of UV Lamps Installed in Circulating Air Ductwork” funded by the Air-Conditioning and Refrigeration Technology Institute (ARTI), RTI developed a test method for measuring culturable bioaerosol inactivation efficiencies or kill by UV lights. This method was derived from earlier bioaerosol air cleaner test methods developed for determining the bioaerosol filtration efficiencies of various air cleaning devices ranging from room air cleaners to duct-mounted ventilation filters to vacuum cleaner filters. RTI’s inert particulate filter testing methodology was the basis of the American National Standards Institute/American Society of Heating, Refrigerating and Air-Conditioning Engineers (ANSI/ASHRAE) Standard 52.2-1999, Method of Testing General Ventilation Air-Cleaning Devices for Removal Efficiency by Particle Size (ASHRAE Standard 52.2). The inert particle efficiency tests described in the ETV protocol are used as a point of comparison for quality assurance/quality control (QA/QC) of the culturable bioaerosol results and will be used as a “self-consistency” check within the same QA framework for these bioaerosol tests.

This test/QA plan supplement focuses on UV air cleaners that are mounted in the HVAC ducting (in-duct air cleaners) and that operate on a “fly-through” basis. That is, they are designed to destroy bioaerosols in the circulating air stream as they pass through the device. This is distinguished from UV devices that are designed to treat specific surfaces within the HVAC system, in particular, the cooling coils and the condensate drain pan, to prevent biological growth on those surfaces. Only airborne inactivation of bioaerosols will be tested. Surface inactivation is beyond the scope of this testing.

To evaluate the in-duct devices on a standard basis, a full-scale module of each UV device to be tested will be installed in one of RTI’s standard full-scale test ducts (meets ASHRAE 52.2 specifications), configured and instrumented to enable verification of the performance of HVAC air cleaning devices. The test devices will be installed in the test duct as directed by the manufacturer, as they would be used in a HVAC duct.

The test duct is discussed in Appendix A. Air flows through this duct on a single-pass basis. The selected challenge bioaerosol is injected into the inlet air stream upstream of a mixing baffle to provide aerosol mixing prior to entering the test device. Bioaerosol concentration is measured both upstream and downstream of the test section to obtain the challenge and surviving concentrations of test organisms, respectively. The test duct required for this method operates at positive pressure to minimize infiltration of room air or external bioaerosol.
The objective is to test the performance of each UV device under standard conditions using the test/QA plan. In some cases, the varying designs of different UV devices may unavoidably result in differences in, such as flow rates, residence times, UV irradiances, and temperatures inside the device. In an effort to address this issue, key parameters regarding device operation will be reported (power input to the lamps, air temperatures entering and leaving the device), so that these differences will be clear to the user of the results. Also, the data analysis (Eq. 6) will help account for differences in irradiance and residence time by calculating the dose.

The applicability of Eq. 1 is well established for a microorganism exposed to a constant UVG irradiance. The results from RTI's project\textsuperscript{6} for ARTI showed that Eq. 1 appears to be adequate for practical use in ducts, where irradiance will vary with time as the airborne organisms pass through different sections of the device and the entrance/exit ducting.

UV light systems will be subjected to a series of tests. Prior to testing, each lamp will be subjected to a standard 100-hour burn in. Single-pass bioaerosol efficiency tests will be performed for each of three test organisms (see Section B1.1.1). The airborne inactivation efficiency will be computed from the ratio of downstream concentration to upstream concentration of culturable organisms (see Section B10.2). The three organisms have been selected to cover the range from those that are easy to kill to those that are difficult to kill. In addition, no-light correction factors for each of the test organisms will be determined by measuring the upstream and downstream airborne culturable colony forming units (CFUs) with the test device in place but without the light(s) turned on. Even with the lights off, losses between the upstream and downstream measurements are expected due to organism deposition in the test apparatus (test duct and device) and a decrease in culturability of the microorganisms, especially the vegetative bacteria, due to injury or death. The no-light control tests are included for quality control purposes. These tests provide a relatively stringent check of the adequacy of the overall duct, sampling, measurement and bioaerosol generation system. They will be performed using the same procedure as normal light tests, except the light will be turned off. The results from the no-light tests will be used during data analysis to correct efficiency measurements obtained during the tests with the light on (light-on tests). For some devices that are more complex, an additional QC no-device test may be required due to substantial organism losses within the device itself. In this case, the entire device will be removed from the duct to more effectively assess the adequacy of the overall duct, sampling, measurement and bioaerosol generation system independent of the test device.

In addition to the required biological tests, inert aerosol tests will provide a further quality control check for the biological tests. An inert no-light test based on the ASHRAE 52.2 test will be performed. If a device tested has a filter or a configuration that results in substantial internal loss, the device will be removed and an additional no-device test will be done.

A6: Task Description and Schedule
A6.1: Task Description
The task consists of the three steps summarized below:

- Acquiring the UV light systems for testing from the manufacturer and installing them per the manufacturer’s instructions,
Performing the biological testing of the UV light air cleaners using the three challenge bioaerosols, and

Preparing verification reports and statements.

A6.1.1: Identification and Acquisition of UV Light Systems
Each manufacturer who requests this testing will provide its own UV light system. A Microbiologist will be the custodian and will be responsible for storage, labeling, etc., of the systems. She is responsible for documentation showing chain of custody. The systems will be stored in Bay 5 at RTI until used and will be retained until the verification report and statement for the system are approved. All systems will be logged in upon receipt and will be checked out for testing, then returned to storage when testing is complete.

A6.1.2: Performance of ASHRAE 52.2-1999 Test
Section deleted. Not applicable.

A6.1.3: Performance of Culturable Bioaerosol Testing
Biological testing will be performed using three different bioaerosols on the UV light system. Light and no-light tests will be performed with all three bioaerosols after a standard initial 100-hour burn-in. In some cases, a no-device test may be needed. The specifics of the testing are discussed in further detail in Section B1.1.

A6.1.4: Performance of Inert Efficiency Test
Inert no-light testing will be performed based on the ASHRAE Standard 52.2 and will establish baseline losses within the test duct with the test device in place. As with the bioaerosol, in some cases a no-device test may be needed. The inert efficiency test will cover the particle size range of 0.3-10 μm and will be performed as a quality check against the no-light efficiency test with the bioaerosols and to confirm the bioaerosol removal efficiency of the UV light systems due to deposition and losses within the device or test duct (with no UV airborne inactivation of the organisms). The specifics of the testing are discussed in further detail in Section B1.2.

A6.1.5: Preparation of Report
Comply with Section A6.1.5 of the test/QA plan.

A6.2: Schedule
Comply with Section A6.2 of the test/QA plan.

A7: Data Quality Objective and Criteria for Measurement Data
Data quality objectives (DQOs) are qualitative and quantitative statements designed to ensure that the type, quality, and quantity of data used are appropriate for the intended application. The DQO for the three critical measurements, bioaerosol challenges, are found in Table 1. The test specifications are found in Appendix A.
Table 1. DQOs for Biological Aerosols

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Frequency and description</th>
<th>Control Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum upstream counts for samplers</td>
<td>Each efficiency test.</td>
<td>Minimum of 10 CFU(^a)/plate or PFU(^b)/plate</td>
</tr>
<tr>
<td>Maximum counts for samplers</td>
<td>Each efficiency test.</td>
<td>Maximum of 500 CFU/plate or 800 PFU(^b)/plate</td>
</tr>
<tr>
<td>100% Penetration (no light) (correlation test)</td>
<td>Performed at least once per test sequence per organism.</td>
<td>Test Organism Penetration Range</td>
</tr>
<tr>
<td>Upstream CFUs</td>
<td>Each test. Statistical check of data quality.</td>
<td></td>
</tr>
<tr>
<td>Upstream PFUs</td>
<td>Each test. Statistical check of data quality.</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) CFU = colony forming units  
\(^b\) PFU = plaque forming unit  
\(^c\) CV = coefficient of variance

All data will be reviewed for accuracy (correctness) and reasonableness. If the results are deemed unreasonable (e.g., internally inconsistent), they will be discarded, the procedures reviewed, and the test repeated if necessary. Data points that are analyzed and determined to be obvious outliers will be discarded without requiring the entire test to be repeated. Any discarded data will be documented.

A8: Special Training Requirements/Certification  
Comply with Section A8 of test/QA plan.

A9: Documentation and Records  
Comply with Section A9 of test/QA plan.

A9.1: Laboratory Documentation  
A9.1.1: ASHRAE 52.2-1999 and Inert Aerosol Tests  
Comply with Section A9.1.1 in the test/QA plan for Inert Aerosol Test. ASHRAE 52.2-1999 will not be performed.

A9.1.2: Bioaerosol Tests  
Comply with Section A9.1.2 in the test/QA plan. In addition, the following will be documented:  
- Power consumption will be measured. If there is only one power cord for the system, then a total consumption will be measured. If there are separate cords, then, if possible, the UV
bulbs and ballast will be measured separately from any other components required by the vendor (e.g., for bulb cooling);

- Other measurements as discussed in Section A9.4 of this document.

A9.2: QA Reports
Comply with Section A9.2 in the test/QA plan.

A9.3: Reporting
Comply with Section A9.3 in the test/QA plan.

A9.4: Verification Reports and Statements
Verification reports and statements will be prepared by RTI staff and reviewed by the APCT Center director and the APCT Center quality manager prior to submittal to the EPA project officer and EPA QA manager for approval. Procedures for the preparation, review and dissemination of verification reports and statements are described in the APCT Center and ETV QMPs.

The verification reports and statements will include the airborne inactivation efficiencies of each of the three bioaerosol challenges for the tested device. The reports will also include the calculated UV radiation dose experienced by the bioaerosol in the tested device as discussed in Section B10.2.2, Eq. 6.

The following additional information will be noted in the verification reports:

- The no-light (UV-off) penetration over the 0.3 to 10 µm size range (and the no-device penetration, if performed),
- The test air flow rate (normally the flow specified by the vendor) and the inlet and outlet temperature,
- The system or device design, installation and operating specifications as supplied by the manufacturer. This is particularly important because many UV systems are configured for a specific application/HVAC system. Among the specifications to be recorded are: the wattage and rated output of the bulbs, other bulb characteristics that could impact $E_{\text{eff}}$, the characteristics of the ballast (root mean square voltage and current), the dimensions and configuration of the bulbs and device, and the specified (or apparent) UV reflectivity and the configuration of any reflective material incorporated into the device. Notations and photographs will be used to document the configuration.
- Calculated mean dosage calculated from Equation 6 and range resulting from standard deviation of the k value,
- A single irradiance measurement at 254 nm will be taken using an International Light IL 1700 Research Radiometer detector. The location of the measurement will be device or system dependent but will be described in the test report. The measurement will not be used to calculate irradiance, but to demonstrate that the lamps were functioning and to provide a test reference value for the laboratory for documentation purposes.
- Measures of energy consumption by the unit, including the pressure drop across the device, air temperature rise through the device, and the power consumed by the bulbs/ballasts and by any ancillary equipment required by the vendor.
- A description and photograph of the UV system/device tested.
SECTION B: MEASUREMENT/DATA ACQUISITION

B1: Test Design
The test program will measure the culturable bioaerosol inactivation efficiency of general ventilation in-duct UV light systems, together with data regarding the energy consumption required to achieve this efficiency (pressure drop, power consumption by bulbs/ballasts, temperature rise through the device). To accomplish this goal, biological testing will be performed using three different bioaerosols on the UV light system. Light and no-light tests will be performed with all three bioaerosols. As described earlier, in some cases, a no-device bioaerosol test may be needed. Inert no-light testing will be performed based on ASHRAE Standard 52.210 and will confirm the bioaerosol baseline losses within the test duct. As with the bioaerosol, in some cases a no-device test may be needed. The inert efficiency test will cover the particle size range of 0.3-10 μm and will be performed as a quality check against the no-light efficiency test with the bioaerosols and to confirm the bioaerosol non-kill removal efficiency of the UV light systems. Lamps will have a 100-hour burn in before the first round of testing.

B1.1: Culturable Bioaerosol Testing
The bioaerosol testing is based on the inert methodology, and uses the same test duct as has been used previously to test the removal of inert aerosols by, e.g., general media filters. The methodology is described in ML TOP #037 (Technical Operating Procedure for Determining Inactivation of Bioaerosols Using Ultraviolet Lights). In summary, the TOP describes how to measure the inactivation efficiency of ultraviolet (UV) light or UV light-containing device by aerosolizing a suspension of a known quantity of microorganism into a test duct. Samples are collected upstream and downstream of the UV light or other device being tested. The details are described in the rest of Section B.

Bioaerosol is generated from a suspension of the test organism and the sampling is achieved using bioaerosol samplers. The use of microorganisms as the challenge aerosol requires that a number of technical issues be addressed. These include:
- Measuring the survivability and culturability of the organisms through the aerosol generation and collection process;
- Determining whether the test organisms are being aerosolized as singlets with a narrow size distribution when appropriate;
- Generating the bioaerosol challenge in sufficient concentration to maintain the sampling duration within the sample time limits of the bioaerosol sampler; and
- Establishing the generation protocol for the test organisms.

All of these issues have been addressed in earlier RTI work, but they will be revisited for the specific organisms used in the APCT Center verification.

B1.1.1: Test Organisms
Because the organisms naturally vary in both their sizes and shapes, there is a need to select organisms that reflect that natural diversity. Additionally, because we are evaluating the efficacy of UV light to inactivate the organisms, it is necessary to select organisms that range from

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All SOPs and TOPs are proprietary and are maintained on file at RTI. Access to these files is permitted on-site at RTI.
readily inactivated to more difficult to kill. Generally, vegetative bacteria are readily killed and bacterial spores, more difficult. As shown in Section A5, the susceptibility of a microorganism to UVC is defined as the k value and is expressed as cm²/µW-s. The magnitude of the k value defines the susceptibility.

The bioaerosol tests will be conducted using three organisms, covering a range of sizes and shapes, and susceptibility to UVC. *Bacillus atrophaeus* (formerly *B. subtilis* var *niger* and *Bacillus globigii* or Bg). The Bg spore is elliptically shaped with dimensions of 0.7 to 0.8 by 1 to 1.5 µm. The second organism, *Serratia marcescens*, is a rod-shaped gram-negative bacteria. *S. marcescens* is 0.5 to 0.8 by 0.9 to 2.0 µm. The third, MS2 bacteriophage, a virus that infects the bacteria *E. coli*, is being used as a surrogate for human viruses. These test organisms were used as simulants in prior testing of general media filters under the Environmental Technology Verification (ETV) program (Biological Testing of General Ventilation Filters¹¹, under EPA Contract GS-10F-0283K-BPA-01) and Technology Testing and Evaluation Program (TTEP) Bioaerosol Inactivation Efficiency by HVAC In-Duct Ultraviolet Light Air Cleaners¹² (EPA Contract No. GS-23F-0011L, Task Order 1112 with Battelle Memorial Institute) Research Triangle Institute Project 09367.

**B1.1.2: Bioaerosol Preparation and Generation**

Bacteria suspension preparation for the aerosolization process requires that the specific test organism be grown in the laboratory and the suspension prepared for aerosol generation in the test duct following ML SOP #012 (SOP for Quantitation of Viable Microorganisms in Suspension). The microbial challenge suspensions are prepared by inoculating the test organism onto solid or into liquid media, incubating the culture until mature, wiping organisms from the surface of the pure culture (if solid media), and eluting them into sterile fluid to a known concentration to serve as a stock solution. The organism preparation is then diluted into sterile nebulizing fluid. The nebulizing fluid is composed of salts (buffering), peptone and antifoam (as needed). The composition of the nebulizing fluid should provide a protective effect similar to organic matter (dirt, debris, etc.) for the *S. marcescens* and possibly the MS2 against the inactivation of the UVC. Based on the ARTI⁶ study, little or no effect is anticipated for the *B. atrophaeus* as spores were found to be relatively unaffected by protective factors. The nebulizing fluid is quantified on trypticase soy agar to enumerate the bacteria.

The phage challenge will be prepared by inoculating a logarithmic phase broth culture of the host bacteria (*E. coli*) with phage and allowing it to multiply overnight or until the majority of the host bacteria are lysed. The mixture is processed to collect and concentrate the phage. Then, the phage stock is filter sterilized (0.2 µm) to remove the bacteria. The phage stock will be used as the challenge aerosol. The concentration of the phage stock will be approximately 1 x 10⁹ or higher plaque forming units (PFU)/mL, and will be quantified following TOP # 034, TOP For Bacterial Virus (Bacteriophage) Plaque Assay. The virus assay uses a standard double agar layer plaque assay.¹¹ In the assay, the host cell *Escherichia coli* C3000 (ATCC 15597) in the log phase of growth and serial dilutions of the MS2 virus stock (ATCC 15597-B1) are combined and top agar added and then poured onto bottom agar plates. After incubation, at least overnight, at 37 °C plaques (loci of infection) are counted against an opaque lawn of host cell *E. coli* C3000.
The challenge organism suspensions will be aerosolized using a Collison nebulizer (BGI, Waltham, MA) at 15 psi air pressure. The Collison nebulizer generates droplets with an approximate volume mean diameter of 2 \( \mu \text{m} \). The particle diameter after the water evaporates depends on the solids content of the suspension. Particle size is determined by the size of the suspended particles (if singlets). Prior experience has shown that the bacterial organism aerosols will exist largely as singlets.

Human viruses are thought to be spread by airborne or droplet transmission. Because human viruses can be expensive and cumbersome to work with, the bacterial virus (bacteriophage) MS2 (0.02 – 0.03 \( \mu \text{m} \)), having approximately the same aerosol characteristics as a human virus, will serve as a surrogate for the viruses of similar and larger size and shape.

Although the individual virus particles are in the 0.02 – 0.03 \( \mu \text{m} \) size range, the test particle size planned for the virus tests will span a range of sizes (polydispersed bioaerosol) in the micron range. This test is not designed to study the removal/inactivation efficiencies for single individual virus particles; rather, it is designed to determine the removal efficiencies for virus particles as they are commonly found indoors. A representative challenge would be a polydispersed aerosol containing the phage because:

- The aerosols created from sneezing and coughing vary in size from < 1 to 20 \( \mu \text{m} \); but the largest particles settle out and only the smaller sizes remain in the air for extended periods for potential removal by an air cleaner;
- For some viruses (i.e., Coxsackie virus), few virions have been found associated with the smallest particles; and
- Nearly all 1 – 2 \( \mu \text{m} \) particles are deposited in the respiratory tract, while larger particles may not be respired.

**B1.1.3: Bioaerosol Samplers**

All the bioaerosols will be collected in liquid impingers, AGI-4 (Ace Glass Inc., Vineland, NJ). Because exposure to UV radiation is a common environmental hazard, cells have developed a number of repair mechanisms to counteract UV-induced damage that must be considered when experimentally measuring UV effects. Collecting in impinger fluid will maximize the collection of damaged organisms.

After sampling, the impinger fluid will be plated. To quantify the microbial counts, the plates are incubated at the appropriate temperature and time for the test organism (overnight to a week). Colonies or plaques are counted.

The AGI-4 is a high-velocity liquid impinger operating at a flow rate of 12.3 – 12.6 L/min. The \( d_{50} \) is approximately 0.3 \( \mu \text{m} \). The AGI-4 is relatively efficient at collecting particles in the size range of the bacterial aerosolized for the testing. The AGI-4 (containing collection fluid) will be used for the sampling. The collection fluid will be processed following SOP #009, SOP for Quantitative Evaluation of Microorganisms.
B1.2 Inert Testing
B1.2.1: Particle Counters
For the inert aerosol filtration efficiency measurements, the particle sizing measurements will be
made using a Climet Model 500 spectrometer/ optical particle counter (OPC) covering the
particle diameter size range from 0.3 - 10 μm in 12 particle sizing channels, consistent with
ASHRAE 52.2 Section 4.6. The OPC uses a laser-light illumination source and has a wide
collection angle for the scattered light. The OPC’s sampling rate is 7.1 L/min. (0.25 cfm). The
OPC is equipped to provide a contact closure at the end of each sample and also provides a 15
sec delay in particle counting after each sample. The contact closure is used to control the
operation of electromechanical valve actuators in the upstream and downstream sample lines.
The 15 sec delay allows time for the new sample to be acquired.

B1.2.2: Inert Aerosol Generation
In accordance with ASHRAE 52.2 Section 4.3, the inert aerosol used in these tests are fine
particles of potassium chloride salt (KCl). The KCl solution will be nebulized using a two-fluid
(air and liquid) atomizing nozzle (Spraying Systems 1/4J siphon spray nozzle). The nozzle is
positioned at the top of a 0.30 m (12 in.) diameter, 1.3 m (51 in.) tall transparent acrylic spray
tower. The tower serves two purposes. It allows the salt droplets to dry by providing an
approximately 40 sec mean residence time, and it allows larger particles to fall out from the
aerosol. After generation, the aerosol passes through a TSI Model 3054 aerosol neutralizer
(Krypton-85 radioactive source) or the TSI Ionizer (part of TSI 8108 Large Particle Aerosol
Generator) to neutralize any electrostatic charge on the aerosol prior to its injection into the test
duct upstream of the test device. (Electrostatic charging is an unavoidable consequence of most
aerosol-generation methods).

The KCl solution is fed to the atomizing nozzle atop the spray tower at 1.2 mL/min. by a pump.
Varying the operating air pressure in the nozzle allows control of the output aerosol
concentration.

B2: Sampling Methods Requirements
Inert aerosol sampling method requirements and critical dimensions and configurations of the
test apparatus to draw the samples for the Climet OPCs are specified in ASHRAE Standard
52.2\textsuperscript{10} Section 4.4. Bioaerosol sampling methodology will comply where appropriate.
Bioaerosol samplers (AGI-4 impingers) are operated according to each manufacturer’s
specifications. The vacuum pumps required for operating the samplers are calibrated following
ML SOP #029 (SOP for Calibrating Pump Flows Using a Dry Gas Meter).

B3: Sample Handling and Custody Requirements
Comply with Section B3 in the test/QA plan.

Upon receipt of the test devices, each will be serially numbered using a permanent marker (or
other means as appropriate). They will be stored in a single indoor, air conditioned common area
(Bay 1).

B4: Analytical Methods Requirements
Comply with Section B4 of the test/QA plan.
**B5: Quality Control Requirements**
Comply with Section B5 of the test/QA plan.

**B6: Instrument/Equipment Testing, Inspection, and Maintenance Requirements**
Comply with Section B6 of the test/QA plan.

**B7: Instrument Calibration and Frequency**
Comply with Section B7 of the test/QA plan.

**B8: Inspection/Acceptance Requirements for Supplies and Consumables**
Comply with Section B8 of the test/QA plan.

**B9: Data Acquisition Requirements (Non-direct measurements)**
Comply with Section B9 of the test/QA plan.

**B10: Data Management**
Comply with Section B10 of the test/QA plan.

**B10.1: Data Recording**
Comply with Section B10.1 of the test/QA plan.

**B10.2: Data Analysis**

**B10.2.1: Bioaerosol Data**
Data analysis will be performed using commercially available software (Microsoft Excel\textsuperscript{14}) to enter the raw data into a spreadsheet and calculate results from a series of equations.

The term \( N_t/N_0 \) from Equation 1 is the survival rate of the test microorganism after exposure to UV. Due to the complexity of bioaerosol testing, an accurate determination of the airborne survival rate requires a series of tests and the determination of the corrected survival rate as shown in Equation 4. Therefore, \( N_t/N_0 \) is the Survival Rate\textsubscript{corrected} which will be calculated as described below.

Samples will be collected simultaneously using multiple samplers. A minimum of five, usually ten, replicates will be collected for each inactivation efficiency determination.

When the total of the summed downstream counts is less than 50, Table 2 (95% confidence limits for the mean values of a Poisson variable) from section 2.9, Abramowitz and Stegun\textsuperscript{17}, will be used. Section 2.9 gives the 95% confidence limits on a single observed particle count from 0 to 50. For a single observed particle count \((n)\), there is a 95% confidence that the true mean count is between the upper and lower limits given in the table. The true mean count is the average count that would be obtained if the tests were repeated indefinitely. For larger values of \(n\), the Poisson distribution tends toward normality with mean \(n\) and variance \(n\).

Using the table, the upper confidence limit will be used as the CFU downstream to calculate the total CFU/ft\textsuperscript{3} downstream. For example, if there are no counts (0) collected when the replicates
are summed, the value 3.7 will be used as the number of downstream CFU to determine the total CFU/ft$^3$ downstream.

Table 2. 95% confidence limits for the mean value of a Poisson variable

<table>
<thead>
<tr>
<th>Observed Count (n)</th>
<th>Lower</th>
<th>Upper</th>
<th>Observed Count (n)</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>3.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.1</td>
<td>5.6</td>
<td>26</td>
<td>17.0</td>
<td>38.0</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>7.2</td>
<td>27</td>
<td>17.8</td>
<td>39.2</td>
</tr>
<tr>
<td>3</td>
<td>0.6</td>
<td>8.8</td>
<td>28</td>
<td>18.6</td>
<td>40.4</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>10.2</td>
<td>29</td>
<td>19.4</td>
<td>41.6</td>
</tr>
<tr>
<td>5</td>
<td>1.6</td>
<td>11.7</td>
<td>30</td>
<td>20.2</td>
<td>42.8</td>
</tr>
<tr>
<td>6</td>
<td>2.2</td>
<td>13.1</td>
<td>31</td>
<td>21.0</td>
<td>44.0</td>
</tr>
<tr>
<td>7</td>
<td>2.8</td>
<td>14.4</td>
<td>32</td>
<td>21.8</td>
<td>45.1</td>
</tr>
<tr>
<td>8</td>
<td>3.4</td>
<td>15.8</td>
<td>33</td>
<td>22.7</td>
<td>46.3</td>
</tr>
<tr>
<td>9</td>
<td>4.0</td>
<td>17.1</td>
<td>34</td>
<td>23.5</td>
<td>47.5</td>
</tr>
<tr>
<td>10</td>
<td>4.7</td>
<td>18.4</td>
<td>35</td>
<td>24.3</td>
<td>48.7</td>
</tr>
<tr>
<td>11</td>
<td>5.4</td>
<td>19.7</td>
<td>36</td>
<td>25.1</td>
<td>49.8</td>
</tr>
<tr>
<td>12</td>
<td>6.2</td>
<td>21.0</td>
<td>37</td>
<td>26.0</td>
<td>51.0</td>
</tr>
<tr>
<td>13</td>
<td>6.9</td>
<td>22.3</td>
<td>38</td>
<td>26.8</td>
<td>52.2</td>
</tr>
<tr>
<td>14</td>
<td>7.7</td>
<td>23.5</td>
<td>39</td>
<td>27.7</td>
<td>53.3</td>
</tr>
<tr>
<td>15</td>
<td>8.4</td>
<td>24.8</td>
<td>40</td>
<td>28.6</td>
<td>54.5</td>
</tr>
<tr>
<td>16</td>
<td>9.2</td>
<td>26.0</td>
<td>41</td>
<td>29.4</td>
<td>55.6</td>
</tr>
<tr>
<td>17</td>
<td>9.9</td>
<td>27.2</td>
<td>42</td>
<td>30.3</td>
<td>56.8</td>
</tr>
<tr>
<td>18</td>
<td>10.7</td>
<td>28.4</td>
<td>43</td>
<td>31.1</td>
<td>57.9</td>
</tr>
<tr>
<td>19</td>
<td>11.5</td>
<td>29.6</td>
<td>44</td>
<td>32.0</td>
<td>59.0</td>
</tr>
<tr>
<td>20</td>
<td>12.2</td>
<td>30.8</td>
<td>45</td>
<td>32.8</td>
<td>60.2</td>
</tr>
<tr>
<td>21</td>
<td>13.0</td>
<td>32.0</td>
<td>46</td>
<td>33.6</td>
<td>61.3</td>
</tr>
<tr>
<td>22</td>
<td>13.8</td>
<td>33.2</td>
<td>47</td>
<td>34.5</td>
<td>62.5</td>
</tr>
<tr>
<td>23</td>
<td>14.6</td>
<td>34.4</td>
<td>48</td>
<td>35.3</td>
<td>63.6</td>
</tr>
<tr>
<td>24</td>
<td>15.4</td>
<td>35.6</td>
<td>49</td>
<td>36.1</td>
<td>64.8</td>
</tr>
<tr>
<td>25</td>
<td>16.2</td>
<td>36.8</td>
<td>50</td>
<td>37.0</td>
<td>65.9</td>
</tr>
</tbody>
</table>

The upstream and downstream culturable organism counts will be summed and the total CFU or PFU/ft$^3$ calculated based on the combined sampler volumes and sampling times for all the AGIs.

The calculation of the test organism survival rate (culturable transmission) is based on the ratio of the downstream to upstream culturable organisms. The survival rate with the UV light system installed and turned on in the test duct is shown in the following equation:

\[
\text{Survival Rate} = \frac{D}{U}
\]  
(Equation 2)

where:
\[ D = \text{Total downstream CFUs or PFUs/ft}^3 \text{ with the UV light turned on and,} \\
U = \text{Total upstream CFUs or PFUs/ft}^3 \text{ with the UV light turned on.} \]

The no-light transmission rate (light is not turned on in the test duct) is calculated as the survival rate test but using the culturable organism results of the no-light tests.

\[ \text{No Light Transmission Rate} = \frac{D_{\text{no light}}}{U_{\text{no light}}} \quad \text{(Equation 3)} \]

where:

\[ D_{\text{no light}} = \text{Total downstream CFUs or PFUs/ft}^3 \text{ with the light off in the test duct and} \]
\[ U_{\text{no light}} = \text{Total upstream CFUs or PFUs/ft}^3 \text{ with the light off in the test duct.} \]

To remove system bias, the Survival Rate is corrected by the results of the blank no-light transmission test.

\[ \text{Survival Rate}_{\text{corrected}} = \frac{\text{Survival Rate}}{\text{No Light Transmission Rate}} \quad \text{(Equation 4)} \]

[Note: \( \text{Survival Rate}_{\text{corrected}} = N_t/N_0 \)]

If a no-device test is required, the no-device transmission will be calculated as the no-light transmission.

The airborne inactivation efficiency is then calculated as:

\[ \text{Airborne Inactivation Efficiency} (%) = 100 \left( 1 - \text{Survival Rate}_{\text{corrected}} \right) \quad \text{(Equation 5)} \]

**B10.2.2: Dosage Calculation**

As stated in Section A5, Eq. 1 is well established for organisms exposed to constant irradiance and was found to be applicable for practical use in a duct. This is advantageous because measurement of actual dose experienced by an average particle while passing through a specific device can be extremely complex. Estimating the average \( E_{\text{eff}} \) as well as the \( \Delta t \) experienced by the particles, as required by Eq. 1, would involve a complex model, requiring extensive information regarding the irradiance pattern throughout the device and the flow trajectories of all of the particles.
However, Equation 1 can be rearranged so that we can calculate dose without measuring or estimating $E_{eff}$ and $\Delta t$. Since dose = $E_{eff} \cdot \Delta t$,

$$Dose = \frac{\ln\left(1 - \frac{N_t}{N_0}\right)}{k} \quad \text{(Equation 6)}$$

The value of $\frac{N_t}{N_0}$ ($Survival \ Rate_{corrected} = \frac{N_t}{N_0}$) will be computed as described in Equation 5. Thus, the average dose received by the average particle within the device can be computed if the $k$ value (the microorganism-dependent rate constant) is known.

We have rigorously determined the $k$ value of $1.6 \times 10^{-4} \pm 0.3 \times 10^{-4}$ cm$^2$/µW·s for *Bacillus atrophaeus* as part of a separate project [Biocontamination Research and Indoor Air Quality task of EPA Cooperative Agreement CR-828936-01 (RTI Project No. 93U-8174)]. A rigorous $k$ value should always include the standard deviation because we are not dealing with a single microorganism, but a population. The resistance to UV is variable within a population.

Accordingly, the average dose received by *B. atrophaeus* in a given UV device will be determined using the $Survival \ Rate_{corrected}$ calculated during the testing of this organism in that device, together with this given $k$ value. The average UV dosage for the other two organisms (*S. marcescens* and MS2) will be assumed to be the same as that received by the *Bg* bioaerosol.

**B10.2.3: Inert Aerosol Data**

As a QC check to assess system bias, the transmission of inert particles is measured during a blank control test for which the UV light is turned off in the duct. The computation of inert aerosol transmission is based on the ratio of the downstream-to-upstream particle concentrations corrected on a channel-by-channel basis for background counts (i.e., upstream and downstream counts observed when the aerosol generator is off).

A minimum of two background and six upstream and six downstream counts will be taken. These data will be used for determining transmission efficiency of the inert aerosol through the test duct with the UV light turned off.

$$Transmission = \frac{(D - D_b)}{(U - U_b)} \quad \text{(Equation 7)}$$

where:

- $D$ = Downstream particle count,
- $D_b$ = Downstream background count,
- $U$ = Upstream count, and
- $U_b$ = Upstream background count.

If a no-device test is required, the no-device transmission will be calculated as the no-light transmission.
B10.3: Data Storage and Retrieval
Comply with Section B10.3 of the test/QA plan.

SECTION C: ASSESSMENT/OVERSIGHT
Comply with Section C of the test/QA plan.

SECTION D: DATA VALIDATION AND USABILITY
Comply with Section D of the test/QA plan.
References


12. RTI. 2005. Test/QA Plan for Biological Inactivation Efficiency by HVAC In-Duct Ultraviolet


RTI Operating Procedures Referenced in the Supplement

1. ML SOP #009 (SOP for Quantitative Evaluation of Microorganisms)
2. ML SOP #012 (SOP for Quantitation of Viable Microorganisms in Suspension)
3. ML SOP #013 (SOP for Pipet Calibration)
4. ML SOP #029 (SOP for Calibrating Pump Flows Using a Dry Gas Meter)
5. ML TOP #034 (TOP For Bacterial Virus (Bacteriophage) Plaque Assay)
6. ML TOP #037 (TOP for Determining Inactivation of Bioaerosols Using Ultraviolet Lights)
Appendix A: Test Specifications

Test specifications for the inert aerosol tests are defined in ASHRAE Standard 52.2\textsuperscript{7} and shown in Table A1. Table A2 shows the test specifications for the bioaerosol test. The test duct performance specifications applicable to all testing are found in Table A3.

Test Duct /System

A schematic of the test duct is shown in Figure A-1. The drawing is approximate to scale. The test duct cross-section is a 610 mm (24 in.) x 610 mm (24 in.) square. The locations of the major components, including the sampling probes, test section (UV test device holder), the aerosol generator (site of aerosol injection) are shown.

There are presently no standards available to directly “calibrate” the test system for penetration. However, a number of parameters can be checked to verify proper performance. 0\% and 100\% penetration measurements are made by using a high efficiency particulate air (HEPA) filter and an empty (no air cleaner) test section, respectively, using the optical particle counter (OPC) and KCl as the inert particulate matter. Separate tests with the bioaerosol will be done using the test bioaerosol and the bioaerosol samplers. A wooden light baffle with slats and a metal mesh is inserted to the test rig between the biological samplers and the device section. The baffle is located 18 in. downstream of the upstream probes. The light baffle is used to mix the aerosol flowing through to the device and to block the UVC from inactivating the upstream challenge prior to collection when the irradiance at the upstream probes is calculated to exceed 900 µW/cm\textsuperscript{2}.

![Figure A-1. Schematic of Test Duct (Top View) Used for Air Cleaner Testing.](image)

The flow rate will be measured using the pressure drop across an American Society of Mechanical Engineers (ASME) long radius flow nozzle (i.e., nozzle size 8½ in.) mounted in the center of the duct downstream of the air cleaner. It will be the primary standard for the
laboratory. Prior to use, the nozzle is visually inspected to be free from defects. The installation of the nozzle in the duct will be inspected to confirm that it is seated in place.

The pressure drop across the UV air cleaner will be measured with an inclined manometer and/or a digital micromanometer. The zero and level of the manometer will be confirmed and connecting tubing will be inspected for integrity.

Measurements of the in-duct air temperature upstream and downstream of the air cleaner, the in-duct relative humidity (RH), and room atmospheric pressure will be made. A wet and dry bulb psychrometer will be used for determination of temperature and relative humidity and an aneroid barometer for atmospheric pressure (periodically compared to a mercury barometer in an adjoining lab). For the bioaerosol, the in-duct RH goal is 20 - 70%. No specific quality control checks on these instruments are planned other than an inspection of the instruments for mechanical faults (e.g., mercury separation in the thermometers, poor tubing connections), and inspection of the data for reasonableness.
Table A1. Quality Control Parameters For Inert Aerosol Tests

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Frequency and description</th>
<th>Control Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum counts per OPC channel for challenge aerosol</td>
<td>For each efficiency test, the total number of particles counted per OPC sizing channel for the upstream challenge aerosol is computed.</td>
<td>Minimum total of 500 particle counts per channel.</td>
</tr>
<tr>
<td>Maximum total OPC count per sample</td>
<td>Each efficiency test.</td>
<td>Not to exceed maximum challenge aerosol concentration determined in the OPC upper concentration limit test referenced in Table A3.</td>
</tr>
<tr>
<td>100% Efficiency test (0% Penetration)</td>
<td>Monthly. A HEPA^ filter is used for the test device.</td>
<td>Measured penetration must be &lt;0.01.</td>
</tr>
<tr>
<td>100% Penetration (correlation test)</td>
<td>A 100% penetration test performed at least once per week during testing.</td>
<td>Particle Acceptable Size range Penetration Range:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3 to 1 μm: 0.90 to 1.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 to 3 μm: 0.80 to 1.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 to 10 μm: 0.70 to 1.30</td>
</tr>
<tr>
<td>Penetration error limit for OPC data</td>
<td>Each test. Statistical check of data quality. Expected to be achieved on tests of clean air cleaners. May not always be achieved with dust-loaded air cleaners if the air cleaner sheds a significant amount of the collected dust.</td>
<td>Per definitions and procedures of ASHRAE 52.2 Section 10.6.4^7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[ T \leq \frac{0.07 \cdot P \ or \ 0.05 \cdot \text{whichever is greater}}{\sqrt{n}} ] for 0.3 – 3 μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[ T \leq \frac{0.15 \cdot P \ or \ 0.05 \cdot \text{whichever is greater}}{\sqrt{n}} ] for 3 – 5.5 μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[ T \leq \frac{0.20 \cdot P \ 0.05 \ or \ \text{whichever is greater}}{\sqrt{n}} ] for 5.5 – 10 μm</td>
</tr>
<tr>
<td>[ T = T\text{-distribution variable, } n = \text{number of samples, } P = \text{penetration (fraction), } \sigma = \text{standard deviation} ]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPC calibration: primary calibration</td>
<td>Primary calibration performed by manufacturer at manufacturer-specified intervals; but at least annually.</td>
<td>Manufacturer provides certificate of calibration.</td>
</tr>
<tr>
<td>OPC sizing accuracy check: polystyrene latex spheres (PSL)</td>
<td>Daily. Sample aerosolized PSL spheres.</td>
<td>Peak of distribution should be in correct OPC channel.</td>
</tr>
<tr>
<td>OPC reference filter check</td>
<td>A filtration efficiency test is performed on a reference filter monthly during testing.</td>
<td>Efficiency must be consistent with reference filter measurements made after OPC’s primary calibration; efficiency within +/- 10 percentage points.</td>
</tr>
<tr>
<td>OPC zero count</td>
<td>Each correlation and initial efficiency test.</td>
<td>Less than 10 counts per sample.</td>
</tr>
<tr>
<td>Background count rate</td>
<td>Measured during correlation and clean filter tests.</td>
<td>Upper 95% confidence limit on background counts must be less than 5% of challenge counts.</td>
</tr>
<tr>
<td>Pressure drop across empty test section</td>
<td>Each correlation test.</td>
<td>Measured pressure drop must be &lt; 0.03 in. H₂O.</td>
</tr>
<tr>
<td>Pressure drop across the air cleaner</td>
<td>Annual. Compare to reference manometer.</td>
<td>Inclined fluid manometer or digital manometer readable to within ±0.01 in. H₂O. 10% or better accuracy.</td>
</tr>
<tr>
<td>Pressure drop across the ASME flow nozzle used for measurement of airflow</td>
<td>Annual. Compare to reference manometer.</td>
<td>Inclined fluid manometer or digital manometer readable to within ±0.01 in. H₂O. 10% or better accuracy.</td>
</tr>
</tbody>
</table>

^1 OPC = optical particle counter
^2 HEPA = high efficiency particulate air
### Table A2. Quality Control Parameters for the Test Duct

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air velocity uniformity</td>
<td>CV&lt;sup&gt;a&lt;/sup&gt; &lt; 10%</td>
</tr>
<tr>
<td>Based on traverse measurements over a nine-point cross-sectional grid at the test flow rate. Performed upstream of the test section using a TSI Model 8345 digital thermal anemometer.</td>
<td></td>
</tr>
<tr>
<td>Inert aerosol uniformity</td>
<td>CV &lt; 15%</td>
</tr>
<tr>
<td>Based on traverse measurements over a nine-point cross-sectional grid at the test flow rate. Performed upstream of the test section.</td>
<td></td>
</tr>
<tr>
<td>Inert downstream mixing</td>
<td>CV &lt; 10%</td>
</tr>
<tr>
<td>Based on nine-point perimeter injection grid at the test section and center-of-duct readings at the downstream probe locations.</td>
<td></td>
</tr>
<tr>
<td>100% Efficiency test</td>
<td>Efficiency &gt; 99%</td>
</tr>
<tr>
<td>Based on HEPA&lt;sup&gt;b&lt;/sup&gt; filter test.</td>
<td></td>
</tr>
<tr>
<td>100% Penetration (correlation test)</td>
<td>Particle Size range Penetration Range:</td>
</tr>
</tbody>
</table>
|                                                          | 0.3 to 1 μm: 0.90 to 1.10  
|                                                          | 1 to 3 μm: 0.80 to 1.20  
|                                                          | 3 to 10 μm: 0.70 to 1.30 |
| OPC<sup>c</sup> upper concentration limit              | No predetermined level, but must be established prior to testing.            |
| Based on limiting the concentration to below the level corresponding to the onset of coincidence error. |
| Aerosol generator response time                         | No predetermined level.                                                     |
| Duct leakage                                           | Ratio < 1.0%                                                                 |
| Ratio of leak rate to test flow rate.                  |                                                                             |
| Determined by sealing the duct at inlet HEPA filter bank and at the ASME<sup>d</sup> flow nozzle locations followed by metering in air to achieve a steady duct pressure. The flow rate of the metering air (equal to the leakage flow) is measured for a range of duct pressures. |
| OPC zero count check                                    | < 10 counts per sample                                                      |
| OPC sizing accuracy check                               | Relative maximum must appear in the appropriate sizing channel.             |
| Based on sampling aerosolized monodisperse PSL<sup>e</sup> spheres of known size. |
| Aerosol neutralizer activity (if radioactive source is used) | Radioactivity must be detected.                                            |

---

<sup>a</sup> CV = coefficient of variance

<sup>b</sup> HEPA = high efficiency particulate air

<sup>c</sup> OPC = optical particle counter

<sup>d</sup> ASME = American Society of Mechanical Engineers

<sup>e</sup> PSL = polystyrene latex
Appendix B: Inert Aerosol Run Sheet (Sample)

Date: __________________    Test Operator: ______________

Physical Description of Test Device:

Test Requested by: __________________        Charge Number: __________________

Manufacturer: ________________________________________________________

Product Name: _________________________________________________________

Model: ________________________________________________________________

Condition: New or from field  No damage or slight frame damage and/or media damage  (Circle all that apply)

Other/describe damage: __________________________________________________

Generic type:

Other: ________________________________________________________________

Other Attributes: _______________________________________________________

<table>
<thead>
<tr>
<th>Height</th>
<th>Width</th>
<th>Thickness</th>
<th>Media type (if known)</th>
<th>Media color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Correlation Test: (use 3/3 - 10/9 - 3/3 sampling)

Date: ______________    Time: ____________________

Flow rate manometer zeroed and level: _____    Test section pressure drop manometer zeroed and level:

OPC clock correct: _________________________ Valve switch on: _________

OPC: (Set for 0.10 ft³ samples with 15 second purge; use 3/3 - 10/9 - 3/3 sampling)

<table>
<thead>
<tr>
<th>20 min. warm up</th>
<th>Flow is 0.25 cfm</th>
<th>Cl-226 switch “Low” or n/a</th>
<th>Zero Check &lt; 10 total / sample enter actual count</th>
<th>Daily PSL check (Enter size when performed or check if done earlier today)</th>
<th>File Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td>c:\climet\rpmmddyyss R P MM DD YY` SS</td>
</tr>
</tbody>
</table>

** Save daily check to disk using file name.

RPMDDYY-HEPA-PSL.TXT

Test Conditions:

must meet <10 criteria at least once per 52.2 test.

Notify project manager if limit is exceeded.
<table>
<thead>
<tr>
<th>Flow rate (cfm)++</th>
<th>Flow Manometer (in. H₂O)</th>
<th>Dry Bulb Temp. (F) Limits = 50-100 °F</th>
<th>Wet Bulb Temp. (°F)</th>
<th>RH Limits = 20-65%</th>
<th>Atm Pressure (in. Hg) xx.xx</th>
</tr>
</thead>
</table>

++ Is flowrate MERV eligible for this size filter? Y or N (see page 3)

**Aerosol Generator:** **“Test Section” Pressure Drop**

<table>
<thead>
<tr>
<th>Aerosol Type</th>
<th>Pump setting</th>
<th>Drying Air</th>
<th>Nozzle air pressure (psi)</th>
<th>Nozzle air flow meter</th>
<th>Upper Concentration target *</th>
<th>Lower Concentration target **</th>
<th>At start of test x.xx(x)</th>
<th>At end of test x.xx(x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCL</td>
<td>1.2 cc/min</td>
<td>4 cfm</td>
<td>240 cfm</td>
<td>enter Ch 1 count</td>
<td>enter Ch 15 count</td>
<td>must be &lt; 0.03&quot;</td>
<td>must be &lt; 0.03&quot;</td>
<td></td>
</tr>
</tbody>
</table>

Channel 1 targets: CI-500 = 3,000; CI-Spectro = 3,000; CI-226 = 5,000

Channel 15 target: 72 - 150 counts per sample desired range. Notify project manager if these targets are not met.

Using "Correlation" graph in spreadsheet, does data look reasonable? ____ yes _____ no (Should be near 1.0)
Appendix C: Bioaerosol Run Sheet (Sample)

Device #: _______________ Run #: __________
Date: ______________________
Test Operator: ________________
Climet Filename: ______________

Test Conditions:

<table>
<thead>
<tr>
<th>Test Flow Rate</th>
<th>ASME Nozzle Pressure Drop</th>
<th>Temperature</th>
<th>RH</th>
<th>Ambient Atm Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFM</td>
<td>in. H₂O</td>
<td>°F</td>
<td>%</td>
<td>in. Hg</td>
</tr>
</tbody>
</table>

Biological Suspension:
Organism: ______________________________________
Suspension Prep: __________________________________
Drying Air: ______________________________________
Nebulizer Pressure: __________________________________
Initial Volume: ____________________________________
Time On: _________________________________________

Biological Sampling:

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Time Run Begins</th>
<th>Sample Length (min.)</th>
<th>Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>U1, U2, U3, U4, U5,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1, D2, D3, D4, D5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U6, U7, U8, U9, U10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D6, D7, D8, D9, D10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ui = upstream sample i
Di = downstream sample i

Rotometer/Vac #1 Reading: ______________________________
Rotometer/Vac #2 Reading: ______________________________
Rotometer/Vac #3 Reading: ______________________________

In-duct temperature upstream _________
In-duct temperature downstream _______
Pressure drop across the air cleaner _______
Power consumption
Amps (measured) ______  Watts (measured) ______  Watts (rated) ______  Power ______
If multiple cords, Power consumption by other required auxiliaries
Amps (measured) ______  Watts (measured) ______  Watts (rated) ______  Power ______