

**BSR/ASHRAE Standard 185.5P** 

# **Public Review Draft**

# Method of Testing HVAC-duct mounted Devices and Systems and In-Room devices for Particle and Microorganism Removal or Inactivation in a Chamber with a Recirculating Duct System

# First Public Review (April 2025) (Draft Shows Complete Proposed Standard)

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ASHRAE, 180 Technology Parkway NW, Peachtree Corners, GA 30092

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## FOREWORD

Many test methods exist for single pass testing of HVAC mounted devices that remove contaminants in the unit. There are also tests (AHAM, ASHRAE) for many in-room air cleaners. However, there were no standard test methods for air cleaners that are mounted in a duct but perform most or all of their function in the occupied spaces in a building. To address this issue, ASHRAE convened SPC 185.5 to develop tests for bioaerosol and for particle challenges in a chamber with recirculating duct test facility as Standard 185.5P. At around the same time SSPC 145 formed a subcommittee to write Standard 145.4P to address the same issues for gas-phase challenges.

For both possible types of challenge in Standard 185.5, there are chamber and recirculating duct specifications, temperature and humidity requirements, maximum background levels for the challenge and other possible contaminants, quality assurance tests and requirements, sampling and analyses requirements, data analysis including statistical requirements, and reporting specifications. The main output of each test is an efficacy measurement in the form of a VACS for each contaminant. The VACS is essentially the same as a clean air delivery rate (CADR) (from the AHAM AC tests) and is named using the term recently put forth in ASHRAE Standard 241. A test for either MS2 or particles is considered a Standard 185.5 test; reporting will include which challenge was used.

Test specifications are included that are intended to simulate real situations to the extent that a lab test can. Lab tests must be more controlled and repeatable than general in-situ use which limits these accommodations. The user must determine how to apply the results to their own application. These specifications include locations allowed for devices, residence time in the recirculating duct after the duct-mounted air cleaners, and allowed ranges for air change rate and air velocities at the air cleaners.

For bioaerosols, the standard requires a test with an MS2 challenge with analysis for MS2, formaldehyde, ozone, TVOC and ultrafine particles. Reporting of the  $V_{ACS}$ , Net Percent Reduction at a certain time, and concentrations of the other species are required. The  $V_{ACS}$  is expected to be acceptable for use in meeting Standard 241 requirements and will be useful for determining air cleaning ability for other uses. Since we do not specify the solution that the bioaerosol is generated from, it is important for users to know that they should only compare results for the same solution as this will change the level of inactivation and capture for some air cleaners.

For the particle challenge test, the challenge is a KCl aerosol measured over at least 20-300 nm. This test sets required equipment, aerosolization technique, and sampling techniques to allow comparison of results across test labs. The VACS for these particles, reported by size range, are intended to meet the need for ultrafine particle removal efficacy.

Reporting requirements include details of the air cleaning system (ACS) that is tested, the test lab, the test itself, the basic data, and calculated values.

#### 1. PURPOSE

This standard provides a method of test for evaluating HVAC-duct mounted devices and in-room devices and systems for particle and microorganism removal or inactivation in a chamber with a recirculating duct system.

# 2. SCOPE

- 2.1 The method of test specific specific particle or selected indicator microorganisms in the test chamber and defines procedures for generating the particles or bioaerosols required for the method of test.
- **2.2** This standard provides a method for counting the number of specific particles or viable microorganisms in the chamber to calculate the elimination efficiency for each specific particle or microorganisms.
- **2.3** This standard establishes minimum performance specifications for the equipment required to conduct the tests, defines test methods as well as the calculation and reporting of results obtained from the test data, and establishes a reporting system to be applied to HVAC-duct mounted devices and in-room devices and systems covered herein.

- 2.4 This method of test requires a chamber with a recirculating duct system.
- **2.5** This standard does not address the health and safety effects of operating devices and systems in an occupied room.

*Informative Note:* Residential air cleaners that are covered by AHAM AC-1 and/or AC-5 are excluded. Air cleaning systems designed to vent into a mechanical exhaust system or directly outside the room are not included in this standard.

# 3. DEFINITIONS, SYMBOLS, AND ACRONYMS

#### 3.1 Definitions

ACS (air cleaning system) test: test performed with the air cleaning system (ACS) installed and running, when applicable.

aerosol: particles (solid or liquid) suspended in air.

*air changes per hour (ACH):* number of times during one hour that a volume of air equal to the volume of the chamber is suppled to, and removed from, the chamber. *Informative Note:* ACH indicates how many times during a one-hour period the air volume in the chamber is replaced by supply air. This definition assumes that all return air is recirculated to become supply air.

air cleaner: device used to remove airborne impurities from air.

air cleaning device: one or more air cleaners in a single container with one airstream flowing through it.

air cleaning system (ACS): one or more air cleaning devices used together to clean the air in a chamber.

*baseline:* concentration of analytes (microorganisms, ions, chemicals, particles) in the chamber air before injection of the test aerosol.

bioaerosol: aerosol containing biologically active bacteria, spores, viruses, toxins, and other similar materials.

*diffuser-mounted air cleaner:* air cleaning device mounted within the duct or diffuser, and within five feet of the supply air discharge, that cleans the air in the duct and/or in the test chamber.

*in-duct air cleaner:* air cleaning device mounted in the duct that cleans the air in the duct and/or in the test chamber.

in-room air cleaner: air cleaning device or system that is placed in a room to clean the air.

microorganism: virus or bacteria.

*natural decay:* test performed, either without the ACS installed or with the ACS installed but not running, for cases where the ACS does not change the results of the test (e.g., due to blocking airflow).

*net percent reduction:* percent reduction in contaminant over time after subtracting the percentage reduction due to natural decay.

particle size distribution: particle counts for an aerosol, separated by particle diameters.

percentage reduction: percent reduction in contaminant over time.

#### 3.2 Acronyms

- ACD air cleaning device
  ACS air cleaning system
  ANSI American National Standards Institute
  ASTM American Society for Testing and Materials
  ATTC American Type Culture Collective
  BMBL Biosafety in Microbiological and Biomedical Laboratories
- BSL biosafety level

CDC	Centers for Disease Control and Prevention
CEU	colony forming unit
CIU	
CV	coefficient of variance
DI	deionized
DMAC	diffuser-mounted air cleaner
GLP	good laboratory practice
HEPA	high efficiency particulate air (filter)
ID	internal diameter
IDAC	in-duct air cleaner
IRAC	in-room air cleaner
KCl	potassium chloride
NIH	National Institutes of Health
NIST	National Institute of Standards and Technology
OSHA	Occupational Safety and Health Administration
PFU	plaque forming unit
QA/QC	quality assurance/quality control
RH	relative humidity
SMPS	scanning mobility particle sizer
SOP	standard operating procedure
TVOC	total volatile organic compounds
UFP	ultrafine particles
UV	ultraviolet

# 4. EQUIPMENT

#### 4.1 Test Chamber

- a. The test chamber Shall, as applicable, comply with government and local agency requirements for handling and testing biological samples, including, but not limited to, OSHA, NIH/CDC, and ANSI/NSF 49.
   Exception to 4.1a: Chambers which will only be used for particle contaminant testing (see Section 6)
- b. For devices that emit UV radiation, in-room air cleaners (IRACs) shall have a UV reflectance of less than 30% at the operational wavelength provided by the manufacturer of the ACS under test. For HVAC-mounted devices, this reflectance requirement shall apply to the duct within 1 m (3.3 ft) of the air cleaner when installed.

*Informative Note:* The lab can use the reflectivity data provided by the manufacturer of the chamber, or a handheld spectroradiometer that is calibrated with a NIST traceable standard.

- c. The test chamber shall be constructed of impervious surfaces, such as stainless steel or glass, be electrically well-grounded or bonded as necessary, be sealed with inert materials, and avoid poisoning materials, such as silicone. The sealed chamber condition shall be verified by aerosol surrogate or gas mixing testing (e.g., CO<sub>2</sub>) as described in Section 8.
- d. The test chamber shall have a viewing port of inert material such as glass or other non-reactive suitable material with limited area.
- e. The test chamber shall include a door capable of opening from both the inside and outside and able to be sealed shut during testing. All sealing materials shall be determined not to emit gases during testing of devices relative to no device tests.
- f. The test chamber shall be at least 22.7 m<sup>3</sup> (800 ft<sup>3</sup>). The height shall be between 2.4 m and 3.0 m (8 ft and 10 ft). The width shall be at least 40% of the length. For IRACs operating above 680 m<sup>3</sup>/h (400 cfm), a chamber larger than the minimum or an alternative test methodology shall be required. Chamber size

consideration shall reflect the measurement instruments, methodology of sample collection, and the air cleaning effectiveness of the IRAC.

- g. The test chamber size shall be large enough to operate at airflows that achieve the expected applied operating conditions of an HVAC system at six to ten air changes per hour (ACH). Recirculating duct airflow shall not operate below 6 ACH or above 10 ACH relative to the combined volume of the chamber and recirculating duct.
- h. The test chamber shall be equipped with a duct for the installation of a recirculation fan and in-duct air cleaner (IDAC) or diffuser-mounted air cleaner (DMAC) that meets the following requirements.
  - 1. Constructed of impervious surfaces, with flex duct permitted only for transitions and not for main duct runs.
  - 2. Able to accommodate air cleaners of the size to be tested.
  - 3. Include a recirculating duct sized to accommodate air velocities between 0.51 and 3.05 m/s (100 and 600 feet per minute [FPM]) and providing a 3 second residence time after the air cleaner. The supply duct shall be at least 10.67 m (35 ft) in length after the recirculating fan. Air velocities shall not operate below 0.51 m/s (100 FPM) or above 3.05 m/s (600 FPM) within the duct or across the ACD. Table 4-1 provides examples of chamber/duct volumes, recirculating duct diameters, and ACR. These examples show some of the options that could be used and highlight the limits on air cleaner airflow or velocity for these configurations. Residence times, calculated assuming the same size duct is used for exactly 10.67 m (35 ft), are also included.
  - 4. Have either round duct with a minimum 254 mm (10 in.) diameter or rectangular duct of equivalent diameter or greater.
  - 5. The quantity and size of inlet and outlet connections to the chamber shall be sufficient to meet the required mixing conditions within the chamber. The discharge velocity into the chamber shall not exceed 2.03 m/s (400 FPM). All air supply inlets must be positioned overhead, at a minimum height of 2 m (6.5 ft) above the chamber floor.
  - 6. Include a multi-directional supply grille at the supply inlet(s) to achieve acceptable mixing in the chamber.
  - 7. Any connecting ductwork between the chamber and main recirculating test duct shall be designed to allow for proper mixing of the air in the chamber.
  - 8. Have capability to run power lines through the duct wall without air leaks.
  - 9. Allow visual or other means to check that the device is operating.
  - 10. Include a process fan sized to provide sufficient airflow and static pressure to meet the requirements of the air cleaning system under test, duct pressure loss, and appurtenances. The fan capacity shall allow for control of the airflow to different values.
  - 11. The chamber and duct shall meet the required level of leakage.
  - 12. Include a properly sized airflow measuring station with acceptable range and accuracy for the characteristics of the duct used.
  - 13. Allow for installation of the air cleaning device (ACD) or system (ACS) in the orientation recommended by the manufacturer's installation and operations manual as represented in Figure 4-1 and include any required ductwork transitions to minimize the effect of non-uniform airflow through the air cleaning device (ACD) being tested.
  - 14. Allow for the device to be located either within five feet of the recirculating fan or, if specified by the manufacturer's installation and operations manual, within five feet of the supply diffuser discharge.
  - 15. Include a diffuser perforated plate device or flow straightener device downstream of the fan for blowthrough devices to establish uniform airflow through the air cleaner. The device shall have impervious surfaces.
  - 16. Include equipment to measure velocity or airflow in the recirculating duct before any branch ducts, and at diffuser discharge.
  - 17. Be able to measure resistance to airflow (pressure drop) across the ACD.
  - 18. Be able to control duct wall temperature (T) to within 5.5°C (10°F) of the chamber temperature.

- i. The test chamber shall include mixing fans in the corners of the chamber positioned upward and not pointed at the IRAC or at the inlet or outlet. These fans shall operate within an airflow range of 50 to 250 cfm and be sufficient to provide well-mixed conditions in the chamber when the fans and duct are operating.
- j. The test chamber shall be equipped with a system to provide an initial chamber temperature of 23°C ± 3°C (73°F ± 5°F) and relative humidity of 38% to 52%. The system shall continuously monitor the chamber temperature and relative humidity.
- k. The test chamber shall be equipped to provide 115 V  $\pm$  1%, 60 Hz  $\pm$  1% to power the devices. When other power levels are used, the value shall be noted in the test reports.
- The test chamber shall have an air change rate with the air outside of the chamber/recirculating duct of less than 0.03 ACH as determined by ASTM E741, *Standard Test Method for Determining Air Change in a Single Zone by Means of a Tracer Gas Dilution* or an equivalent method, and shall allow measurement of pressure difference between inside and outside of duct and chamber system such that pressure change in the system may be measured to within ±1% during natural decay tests.
- m. For bioaerosol tests, the test chamber shall include not less than three sampling ports located in positions representative of the airflow dynamics of the chamber (lacking high turbulence or unidirectional velocity). These probes shall be located at a distance of greater than 0.3 m (1 ft) from each wall, and at a sufficient distance from the supply and return openings of the recirculating duct, mixing fan, and air cleaner(s) such that the sample is not influenced. The sampling locations shall be representative of the chamber dynamics and located 1.0 to 1.8 m (3.3 to 6 ft) above the chamber floor.
- n. For particle challenges, the test chamber shall include a single sampling location with the inlet inside the chamber located not in the immediate effluent location of an IRAC or the inlet from the recirculating duct but in an area shown to be well mixed.

Informative Note: One of the bioaerosol locations is acceptable.

- o. The test chamber shall be capable of mounting and installing the most common in-room air cleaning devices, such as wall-mounted, tabletop, ceiling-mounted, and floor air cleaners, and shall include air-tight ports for power lines and remote controls.
- p. The test chamber shall be equipped with a contaminant injection port that allows the contaminant to be well mixed within the room. Contaminant injection port shall be located so that it does not interfere with any sampling probes and be at least 0.7 m (2 ft) away from the air cleaning system and return air inlet of the duct.
- q. The test chamber shall be equipped to allow decontamination and cleaning of the chamber without humans entering. Each lab shall have a standard operating procedure (SOP) delineating the decontamination methodology.
- r. The test chamber shall be equipped with a system capable of flushing and/or disinfecting the air in the chamber pre-and post-testing. For chamber/duct systems to be used for particle contamination tests, the cleanup system shall be capable of lowering the air concentration to less than 10 particles/cc per Scanning Mobility Particle Sizer (SMPS) size bin.

*Informative Note:* For example, air may be pulled from the chamber through a cleanup section before being returned to the chamber. A HEPA filter can provide effective removal of particles; carbon/sorbent beds can be used to remove other species from the air and should be designed to remove all other gaseous contaminants and by-products. This cleanup system shall be designed to be shut completely off from influencing the chamber during the testing.

- s. The test chamber shall be capable of achieving and maintaining all microbial background contaminant concentrations, which shall be maintained at less than 35 CFU/m<sup>3</sup> or PFU/m<sup>3</sup> (1 CFU/ft<sup>3</sup> or PFU/ft<sup>3</sup>) over multiple sampling periods or a level deemed acceptable through laboratory standard operating procedures, if the system will be used for bioaerosol testing. Particle, VOC, and aldehyde background levels shall not exceed the levels defined in Table 7-1 and shall be measured using the methods listed in Table 7-1. However, to avoid penalizing more sensitive equipment, backgrounds that would not be considered significant relative to expected test challenges shall be considered acceptable.
- t. The test chamber shall meet the QA/QC requirements in Section 8 before testing is performed. A schematic of a possible test chamber and recirculating duct setup is presented in Figure 4-1.

		254 mm Diameter Duct (min. allowable)		305 mm × 305 mm Duct		610 mm × 610 mm Duct	
Chamber + Duct Volume (m <sup>3</sup> )	Supply Airflow for 6 ACH (m <sup>3</sup> /s)	Air Velocity (m/s)	Residence Time, 10.67 m of duct (s)	Air Velocity (m/s)	Residence Time, 10.67 m of duct (s)	Air Velocity (m/s)	Residence Time, 10.67 m of duct (s)
31.2	0.05	1.03	10	0.56	19	N/A	N/A
59.5	0.10	1.96	5	1.07	10	N/A	N/A
116.1	0.19	N/A	N/A	2.08	5	0.52	20
		254 mm Di (min. al	ameter Duct lowable)	305 mm × 3	05 mm Duct	610 mm × 6	510 mm Duct
Chamber + Duct Volume (ft <sup>3</sup> )	Supply Airflow for 10 ACH (m <sup>3</sup> /s)	Air Velocity (m/s)	Residence Time, 10.67 m of duct (s)	Air Velocity (m/s)	Residence Time, 10.67 m of duct (s)	Air Velocity (m/s)	Residence Time, 10.67 m of duct (s)
31.2	0.09	1.71	6	0.93	11	N/A	N/A
59.5	0.17	N/A	N/A	1.78	6	N/A	N/A
116.1	0.32	N/A	N/A	N/A	N/A	0.87	12

 Table 4-1
 Example Allowable Chamber, Duct, Airflow, and Residence Times (SI)

 Table 4-1
 Example Allowable Chamber, Duct, Airflow, and Residence Times (I-P)

		10" Diameter Duct (min. allowable)		1 ft × 1 ft Duct		2 ft × 2 ft Duct	
Chamber + Duct Volume (ft <sup>3</sup> )	Supply Airflow for 6 ACH (cfm)	Air Velocity (FPM)	Residence Time, 35 ft. of duct (s)	Air Velocity (FPM)	Residence Time, 35 ft. of duct (s)	Air Velocity (FPM)	Residence Time, 35 ft. of duct (s)
1100	110	202	10	110	19	N/A	N/A
2100	210	385	5	210	10	N/A	N/A
4100	410	N/A	N/A	410	5	102.5	20
		10" Diamete allov	er Duct (min. vable)	1 ft × 1	ft Duct	2 ft × 2	ft Duct
Chamber + Duct Volume (ft <sup>3</sup> )	Supply Airflow for 10 ACH (cfm)	10" Diamete allov Air Velocity (FPM)	er Duct (min. vable) Residence Time, 35 ft. of duct (s)	1 ft × 1 Air Velocity (FPM)	ft Duct Residence Time, 35 ft. of duct (s)	2 ft × 2 Air Velocity (FPM)	t ft Duct Residence Time, 35 ft. of duct (s)
Chamber + Duct Volume (ft <sup>3</sup> ) 1100	Supply Airflow for 10 ACH (cfm) 183	10" Diamete allow Air Velocity (FPM) 336	er Duct (min. vable) Residence Time, 35 ft. of duct (s) 6	1 ft × 1 Air Velocity (FPM) 183	ft Duct Residence Time, 35 ft. of duct (s) 11	2 ft × 2 Air Velocity (FPM) N/A	2 ft Duct Residence Time, 35 ft. of duct (s) N/A
Chamber + Duct Volume (ft <sup>3</sup> ) <u>1100</u> 2100	Supply Airflow for 10 ACH (cfm) 183 350	10" Diamete allow Air Velocity (FPM) 336 N/A	er Duct (min. vable) Residence Time, 35 ft. of duct (s) 6 N/A	1 ft × 1 Air Velocity (FPM) 183 350	ft Duct Residence Time, 35 ft. of duct (s) 11 6	2 ft × 2 Air Velocity (FPM) N/A N/A	e ft Duct Residence Time, 35 ft. of duct (s) N/A N/A



#### Figure 4-1 Schematic of one possible configuration of the chamber with recirculating duct.

#### 4.2 Air Cleaners

- **4.2.1** Air cleaners shall be tested based on their intended use and shall be installed according to the manufacturer's installation and operations manual. Devices shall be installed either within five feet of the chamber supply fan or, if specified in the manufacturer's installation and operations manual, within five feet of the supply diffuser discharge.
- **4.2.2** If there are no location instructions, the device shall be placed closest to the middle of the room for in-room devices.
- 4.2.3 Air cleaners that require power shall have an available means to turn the device on from outside the chamber. *Informative Note:* Plug-in cords and remotes are acceptable, as are externally mounted switches for induct or diffuser-mounted devices.
- **4.2.4** IRACs shall be installed where they are not in the airflow of the mixing fan. For air cleaners that discharge air in a specific direction, the air discharge shall not be directed toward a sampling port or toward a wall.
- **4.2.5** For IRACs designed for volumes smaller than the test chamber, multiple IRACs may be located in the chamber during testing. All IRACs used for this purpose shall be identical. Sizing and placement shall be consistent with the manufacturer's specifications and shall be validated by the testing laboratory.
- **4.2.6** In-duct and diffuser-mounted air cleaners and IRACs may include multiple stages and/or multiple technologies. These shall be tested simultaneously with a standard test. An IRAC may be tested with an induct or diffuser-mounted device if it is to be sold in that configuration. If stages are tested separately, the test report shall be labeled to show that the test was not of the complete ACS.

# 5. BIOAEROSOL GENERATION, SAMPLING, AND ANALYSIS

#### 5.1 Nebulizers for Bioaerosol Generation

**5.1.1** Nebulizers shall be capable of nebulizing microbial suspensions into particles containing no more than one bacterium, fungal spore, or virion.

- 5.1.2 Compressed air shall be filtered to remove contaminants, including oil, from the air.
- **5.1.3** The generated aerosol shall be dried down to a stable condition using a drying tower or other dehumidifier, or by allowing sufficient time in the chamber during the required mixing time after the aerosol injection. The lab shall ensure that their nebulizing procedure does not increase the RH in the chamber above acceptable levels.
- **5.1.4** Collison 6-jet or 24-jet nebulizers, ultrasonic nebulizers, or other bioaerosol-generating devices, driven by purified filtered air supply or equivalent shall be chosen to allow correct operation with the organism of a specific test. Given that the organisms are different sizes, the nebulizer shall allow the organisms to pass through the nozzle. Nebulizers shall be chosen to allow organisms to survive generation.
- **5.1.5** Biological agents shall be suspended in an appropriate maintenance and delivery solution of salt buffer, artificial sputum, or other buffer solution that maintains viability or infectivity of the biological agent prior to and immediately following nebulization. The lab shall determine the appropriate suspension buffer based on the test organism and the air cleaning device being tested.

#### 5.2 Bioaerosol Samplers

- **5.2.1** Air sampling devices shall be placed inside or outside the chamber based on the lab's SOP. Samplers placed outside the chamber shall be connected to the chamber via sampling ports and the appropriate size tubing.
- **5.2.2** Air sampler(s) or sampling port(s) shall be located along the centerline of the room in the lengthwise direction. These probes shall be located at a distance of more than 0.3 m (1 ft) from each wall and at a distance sufficient from the mixing fan, IRAC(s), and duct exit such that the sample is not influenced. The probes shall be equally spaced on the long wall and shall be at a height of  $1.5 \text{ m} \pm 0.1 \text{ m}$  (4.9 ft  $\pm 0.3 \text{ ft}$ ) above the floor of the chamber.
- **5.2.3** Sampling shall be done with impingers, impactors, or other samplers with similar performance. Sampling times and volumes shall be determined to give sufficient and not excessive counts and minimum concentrations in air to obtain statistically valid counts; see Section 10. Calculated concentrations shall be corrected to account for differing sample times and volumes when they are not the same. When using impactors, the correction hole factor shall be applied before other calculations and shall be required for reporting. When using impingers or gelatin filters, triplicate plates are required for each.
- **5.2.4** The sampling system shall include a method for rapid changeout to allow samples to be taken as quickly as needed for each test.
- **5.2.5** Samplers shall be confirmed leak free and the associated sampling areas sanitized following each use to minimize any potential carryover between tests and for personnel safety.
- **5.2.6** Laboratory SOPs shall include validation of generation and sampling methodologies, including concentrations generated, the efficiency of collection methodologies, and particle size distribution. Sample preparation and analysis shall be based on the specific organism, sampler, and air cleaner.

## 5.3 Microorganisms

- **5.3.1** Microorganisms selected for the challenge bioaerosol shall be handled with appropriate primary containment, secondary containment, and following good laboratory practice (GLP) guidelines in accordance with the current version of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL). Where applicable, the lab shall use American Type Culture Collection (ATCC)-traceable microorganisms.
- 5.3.2 Testing shall be performed with the non-enveloped bacteriophage MS2 (host *Escherichia coli*).

*Informative Note:* See Informative Appendix B for information on other possible organisms that may be tested in addition to MS2.

**5.3.3** Background levels for the total of all microorganisms in the chamber air shall be less than 35 CFU/m<sup>3</sup> or less than 35 PFU/m<sup>3</sup> (<1 CFU/ft<sup>3</sup> or <1 PFU/ft<sup>3</sup>). A background sample shall be collected prior to the initiation of each nebulization period to confirm acceptable microorganism background levels during each individual test.

#### 5.4 Chamber Biosafety Level (BSL)

**5.4.1** The chamber and recirculating duct system configuration and testing parameters shall meet the minimum ACS testing requirements for any bioaerosols, whether requiring a BSL-1 setting (e.g., MS2), or for

bioaerosols requiring BSL2 and/or BSL-3 settings.

**5.4.2** Compliance for all BSL testing to meet the specific biosafety requirements shall be the responsibility of the testing laboratory.

# 6. PARTICLE (NON-BIO) GENERATION, SAMPLING, AND ANALYSIS

### 6.1 Particle Generation

- 6.1.1 Particles for particle challenge tests shall be generated using a 4B Laskin nozzle (ATI #T4B0-1155) operated at 20 psi and 25 cfm with three nozzles open. The particles shall be generated from 0.01% KCl solution in DI water.
- **6.1.2** The relative humidity in the chamber shall be verified to be less than 45%, then the generator shall be operated for exactly ten minutes.
- **6.1.3** Particles shall be dried before the test begins. After the generator is turned off, the chamber shall be mixed for five minutes, or per the duration determined in the particle stability test per Section 8.
- **6.2** Particle Sampling and Analysis. A SMPS shall be used with settings to sample from at least 20 to 300 nm. Samples shall be taken at least every five minutes. Sampling shall continue for one hour or until the counts per size bin are less than 10 particles/cc.

# 7. AIR SAMPLING BEYOND THE CHALLENGE CONTAMINATION

- 7.1 For the analytes listed in Table 7-1, baseline monitoring shall be conducted before challenge generation for all tests. It is essential to record and summarize this data in the final test report, ensuring comprehensive documentation of baseline conditions.
- 7.2 Throughout all testing, the test chamber shall be monitored, either continuously or with samples taken for external analysis at regular intervals for analytes specified in Table 7-1, except for UFP, which shall be monitored continuously. The data logging of analyte concentrations shall occur at intervals not exceeding ten minutes. In cases where data is recorded at intervals shorter than ten minutes, it shall be averaged to ten-minute intervals for consistency. The final report shall present this data, in tabular format with optional graphs, to facilitate clear interpretation and analysis. Non-continuous samples shall be taken at least four times during the test in addition to the background sample.
- **7.3** The specific requirements for detection limits of analytes are detailed in Table 7-1, which outlines the minimum detection ranges and resolution for each analyte to ensure accurate and reliable monitoring. For continuous monitors, these specifications apply to the analyzer used during the test; for non-continuous samples, these specifications apply to the combination of sample taken and the analyzer used for later analysis. Analyzers that speciate are allowed for TVOCs; the sum of the compounds from C4 to C10 shall be reported as TVOC. Individual compounds may be reported but are not required for the test.
- 7.4 In addition to the analytes required for all tests, tests for ACS that state that a specific level of a species is needed in the air for their device to work as expected or well, shall test for that analyte during all tests. These analytes include, but may not be limited to, positive and negative ions and hydrogen peroxide. The detection limit of the analyzer shall be less than 10% of the required or otherwise noted value for the analyte. The sampling for these analytes shall be performed in both the Natural Decay and ACS tests and shall meet the requirements for sampling in Table 7-1.
- 7.5 All devices used for recording analyte concentrations per Table 8-1 shall be calibrated and operated according to manufacturer specifications. Adherence to these guidelines ensures the reliability and accuracy of the data collected.
- **7.6** Key environmental variables, including temperature, barometric pressure, and relative humidity, shall be recorded and reported as described in the Procedure section. All devices used for environmental monitoring shall be calibrated according to manufacturer specifications and should be traceable to NIST standards where applicable.

#### Table 7-1 Specific Analytes that Require Monitoring for Chamber Baseline and Testing Trials<sup>1</sup>

	Abbreviation	Minimum Range	Resolution	Detection Limit	Accuracy	Allowed Bkg Conc.
Formaldehyde	CH <sub>2</sub> O	10 - 125 (8 - 100)	1 (1)	5 (4)	5%	8 (6)
Ozone	O3	0 - 200 (0 - 100)	2 (1)	1 (1)	2%	10 (5)
Total Volatile Organic Compounds <sup>b</sup>	TVOC	0 - 2100 (0 - 500)	4 (1)	21 (5)	3%	20 (5)
Ultrafine Particles <sup>c</sup>	UFP	10 - 1000	1	$3/cm^3$	<30%	100/cm <sup>3</sup> for bio test <sup>d</sup>

a. Units are  $\mu g/m^3$  (ppb) except for ultrafine particles, which are particle/cm<sup>3</sup>

b. MW assumed to be 100 for conversion between units

c. Ultrafine particles, for this test, are defined as those ≤100 nm diameter

d. See Section 6 for particle test requirements

# 8. QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

Qualification tests shall verify quantitatively that the chamber and recirculating duct system, sampling procedures, and equipment are capable of providing reliable challenge aerosol concentration and air-cleaner drawdown measurements. Qualification tests shall be performed as required by Table 8-1. System qualification shall be done before initial testing, after major changes to the test system and on the schedule in Table 8-2.

Table 8-1	<b>Chamber and Duct S</b>	vstem and Instrument (	<b>Dualification</b> Re	quirements
1		Joe and and another and the		

Section Number	Parameter	Specific to Challenge	Requirement
8.1.1	Background level check	Yes	Meet requirements of Table 7-1
8.1.2	Clean-up system check	No	Meet requirements of Table 7-1
8.2	Chamber air change rate (total allowable leakage)	No	<0.3%/min. of chamber/duct volume
8.3.1	Airflow measurement	No	6 to 10 ACH
8.3.2	Air velocity measurements	No	100 to 600 FPM in duct; ≤400 FPM at diffuser
8.4	Chamber mixing and uniformity	No	ASTM D6670 or equivalent (Section 8.4.1)
8.5	Particle stability	Yes	Stability shall be shown
8.6.1	Analyzer response time	Yes	<20% sample line transit
8.6.2	Sample transit time	No	Determine correction factor
8.6.3	Analyzers and sampling systems zeroes	Yes	Below detection limit
8.6.4	Gas sampling pump flow rate check	No	NIST traceable flow device
8.6.5	Gas analyzer calibration	Yes	Use traceable gas standards Shall be done for gas analyzers
0.7		N	Curve fit $R^2 \ge 0.95$
8./	PLI accounting device	INO N-	Factory calibrated
8.8	Kri measuring device	INO	Factory calibrated
8.9	Airflow through recirculating duct	No	At least three values

Maintenance Item (Subsection Reference)	Each Test	Each Testing Day	Challenge Gas Change	Biannually or After Duct Modification	Other
System Purge (Section 8.1.2)					
Air Change Rate (Total Allowable Leakage) (Section 8.2)					
Airflow and Velocity Measurements (Section 8.3)					
Chamber Mixing and Uniformity (Section 8.4)					
Particle Stability (Section 8.5)					
Analyzer Response Time (Section 8.6.1)					
Gas Analyzer Calibration Checks at 0%, 10%, 50%, and 100%					Within two weeks prior to testing and per
Gas Analyzers Zero and Span (Section 8.6)					
Particle Analyzer Calibration (Section 8.6)					Annually
Sample Transit Time (Section 8.6)					When changing sample lines
Analyzer and Sampling System Zero (Section 8.6)					
Gas Sampling pump flow rate check (Section 8.6)					
Temperature (Section 8.7)					
Relative humidity (Section 8.7)					Every six months
Resistance to airflow across empty test section (Section 8.8)					
Airflow through Recirculating Duct (Section 8.9)					

 Table 8-2
 Qualification Maintenance Items and Schedules

#### 8.1 Meeting Background Requirements

- **8.1.1** The test laboratory shall demonstrate and document that the experimental setup can meet the background levels for the challenges and other analytes that will be sampled for as listed in Table 7-1.
- **8.1.2** The test laboratory shall use a cleanup system to remove all contaminants (particles and gases) to levels below the background concentration limits as shown in Table 7-1.
- **8.2** Chamber Air Change Rate Test (Total Allowable Leakage). The test chamber shall be sufficiently airtight during testing (leakage of less than 0.03 ACH) as determined in accordance with ASTM E741, ASTM D6670 (Section 8.2.1), or an equivalent method, with the caveat that the recirculating duct shall be considered part of the single zone with the chamber and shall be operated as usual for tests except that a non-reactive device to add resistance to airflow (e.g., an orifice plate) shall be installed in the recirculating duct during the test.

Informative Note: See Informative Appendix D for an example of an equivalent method.

# 8.3 Airflow and Velocity Measurements

**8.3.1** The recirculating duct airflow shall be tested to verify that the total airflow is maintained between 6 and 10 air changes per hour (ACH), relative to the combined volume of the chamber and the recirculating duct and to verify that the total airflow rate is maintained within 10% of the planned test rate throughout all quality checks and tests.

- **8.3.2** Air velocities in the recirculating duct shall be tested to confirm they remain between 0.51 and 3.05 m/s (100 and 600 FPM). Additionally, discharge velocities into the chamber shall be tested to ensure that they do not exceed 2.03 m/s (400 FPM). Air velocities shall remain within 10% of the planned test rate throughout all quality checks and tests
- **8.4** Chamber Mixing and Uniformity. The chamber shall be evaluated to demonstrate that the challenge gas is well mixed during a test. This evaluation shall be done either using the method ASTM D6670 (Section 8.4.1) modified to include the recirculating duct, or by using one the following procedures using CO<sub>2</sub> as a tracer gas.
- **8.4.1** For multiple analyzers/samplers, the test shall be performed as described below:
  - a. Position at least four analyzers (or the inlet ends of the sampling tubes) in the chamber.
  - b. Turn on recirculating duct to typical airflow.
  - c. Run cleanup system for required length of time per lab SOP, then turn off.
  - d. With mixing fans operating as usual, generate challenge from standard location, and allow to mix your standard length of time.
  - e. Take simultaneous samples with all analyzers/samplers. Repeat at least three times.
  - f. Determine the mean for each point and for the whole data set using at least three data sets.
  - g. The criterion for a well-mixed chamber and recirculating duct system is that all of the sample points' means are within 10% of the overall mean.

*Informative Note:* If the test does not pass, examine the data for trends indicating that the mixing improved over time. This may indicate that the lab needs to increase their pretest missing time. In this case, later samples may be used to determine acceptable mixing. Adjust the required mixing time by adding the time required to meet the acceptance goal.

- **8.4.2** For a single analyzer or sampler with multiple sample locations, the test shall be performed as described below:
  - a. Set up the chamber for sampling.
  - b. Turn on recirculating duct to typical airflow (the same air flow used in the leakage test).
  - c. Run cleanup system for required length of time per lab SOP, then turn off.
  - d. With mixing fans operating as usual, generate challenge from standard location, and allow to mix your standard length of time.
  - e. Take at least four samples from each point changing between locations between samples.
  - f. Determine the mean for each point and for the whole data set using at least four data sets.
  - g. The criterion for a well-mixed chamber and recirculating duct system is that all of the sample points' means are within 10% of the overall mean.

*Informative Note:* This setup may mean that the analyzer can be moved from outside the chamber, that multiple sample lines are set up with the analyzer moved between them outside the chamber or some other means.

- 8.5 **Particle Stability.** The particle stability test shall be performed as described below:
  - a. Use the aerosol required for the Particle Challenge Test
  - b. Set up the chamber for particle sampling including the sampling probe, sample lines, and SMPS.
  - c. Seal the chamber as usual.
  - d. Run the cleanup system per the lab SOP.
  - e. After turning off the cleanup system, turn on the fans in the chamber as usual for a test. Turn on the duct to an average airflow rate for that chamber/duct system.
  - f. Turn on the SMPS and take at least two samples to show that the chamber is cleaned of particles.
  - g. Continue running the SMPS.
  - h. Generate the KCl aerosol as used for the tests.
  - i. Monitor the aerosol particle size distribution for at least 20 minutes.

- j. Turn of the SMPS.
- k. Graph the data to show if the aerosol size distribution became stable and at what point this occurred. Set the time when this occurred plus one minute as the point where a particle challenge test may begin.
- 1. If the distribution does not stabilize, repeat the test with longer sampling times until the distribution does stabilize.
- **8.6** Analyzer and Sampling Systems. To report the time in the chamber when the sample was removed, it is important to know both the transit times for any sample lines and the analyzer response time for each analyzer. If the airflow through the lines is known (e.g., by calibrated pumps pulling the air), the internal diameter (ID) can be used to calculate the transit time.
  - **8.6.1** Analyzer Response. With the analyzer operating per manufacturer's instructions, expose the analyzer to air that meets the clean air requirements in Section 7 long enough to show a steady reading, then turn on a stable concentration of the appropriate gas. Record the time to a stable and correct reading; use this time to correct the time for the sample.
  - **8.6.2** Sample Line Transit Time. Set up sample lines as they will be used. Connect to an analyzer with known response time; run clean air through the sample lines and analyzer until a stable reading is obtained. Turn on a stable concentration of the appropriate contaminant. Record the time to a stable and correct reading. Subtract the analyzer response time from the combined time; use this time to correct the time for the sample whenever this sample line is used at the same sample flowrate. Sample line transit time shall be determined when sample lines are changed in length and diameter.
  - **8.6.3** Analyzers and sampling systems zeroes shall be performed per manufacturer's specifications.
  - **8.6.4** Sampling pump flow rate check (for air samples/grab samples), if used, shall be checked regularly prior to use per the laboratory SOP.
  - **8.6.5** Gas Analyzer Calibration. Zero, or check zero as appropriate to the analyzer, and calibrate at approximately 10%, 50%, and 100% of challenge concentration. Zero air shall have concentration levels below the reporting limit of the analyte. Use traceable gas standards. Calibrations shall be done for all analyzers, and the curve fit shall have  $R^2 \ge 0.95$ .
  - **8.6.6** Calibrate particle analyzer per manufacturer's instructions.
  - **8.6.7** Other Calibrations. Measuring instruments used to measure air flow, air velocity, and resistance to air flow shall be calibrated per the manufacturer's instructions. Any measuring instrument not specified in another section shall be calculated according to the manufacturer's instructions.
- **8.7** Maintaining Temperature and Relative Humidity Levels. Measuring instruments shall determine temperature to within ±1°C at 23°C (±1.8°F at 73.4°F) and relative humidity to within ±5% at 50% RH.
- 8.8 Measure the resistance to airflow across the empty duct section using the technique to be used in regular testing.
- **8.9** Airflow Through the Recirculating Duct. Using a NIST traceable technique or currently calibrated AMCA-approved flow measurement system, determine that the airflow in the recirculating duct matches that measured with the test technique across the range of values expected in the testing. At least three values shall be tested unless the test lab will not use any others. This test shall be repeated if significant airflow resistance is added to the test duct to reconfiguration for different devices.

# 9. PROCEDURES

Tests with the ACS and natural decay (usually without the air cleaner, but in some cases done with the device installed but OFF as described in Section 9.1.7) shall be performed identically, except for the ACS presence and operation. This is critical so that the natural decay test correctly reflects the change in concentrations due to the ACS itself. The order of the tests is not specified. A natural decay test run with the same batch of microorganisms, recirculating duct airflow, and environmental conditions within three days of, and with the same sampling time points as the ACS test, shall be acceptable as the paired test for more than one ACS test.

# 9.1 Before Testing–Setup

- **9.1.1** Take pictures of the ACS, both separately and as mounted for the test, with identifying markings and labels included.
- 9.1.2 Determine if the ACS identifying markings and labels agree with the manufacturer's information provided

by the test requestor.

- 9.1.3 Measure IRAC airflow.
- 9.1.4 Take a lab or chamber barometric pressure reading sometime during either test or setup.
- 9.1.5 Make sure that proper decontamination SOP has been followed and completed.
- **9.1.6** If the chamber has been decontaminated using chemicals, ensure that the air in the chamber has no chemical or other residue from the decontamination process following their SOP.
- **9.1.7** Determine the coordinated chamber test and sampling plan, including chamber setup, airflows, air velocities, air change rate (ACR), sampling locations, environmental conditions, contaminant generation and needed initial concentration, sample times, lengths of samples, which species/contaminants need to be sampled for, and other relevant details.
- **9.1.8** Prepare for sampling (e.g., plates ready, space in the incubator prepped, instruments that need daily calibration calibrated, data loggers up and running, and other details for running the test).
- 9.1.9 Set up in-chamber mixing fans, sampling probes, and all needed equipment in the chamber.
- **9.1.10** Configure the recirculating duct appropriately to maintain required air velocities for main duct, device installation location, branch ducts, diffuser discharge velocity, supply air inlet height, and total duct length.

## 9.1.11 Installation of the ACS

a. For duct-mounted ACSs, the ACS shall not be installed during the natural decay test unless the device (when off) will not influence the test. For ACS tests, the ACS shall be installed in the duct.

*Informative Note:* During the natural decay test, a media filter should not be installed; however, a UV lamp may be installed.

- b. In-room ACS shall be installed in the chamber prior to initiation of the natural decay test.
- c. Devices shall be installed according to the manufacturer's installation and operations manual.
- d. Devices shall be located either within five feet of the chamber supply fan or, if specified in the manufacturer's installation and operations manual, within five feet of the supply diffuser discharge.
- e. Devices shall be installed so that they can be turned on and off once the chamber/duct system is closed, and in such a manner that the devices do not interfere with each other.
- **9.1.12** Seal chamber and duct, then turn on the recirculating duct fan to the planned test air velocities, airflow, and air change rate (ACR).
  - a. The recirculating duct airflow shall be maintained between 6 and 10 ACH, relative to the combined volume of the chamber. The recirculating duct airflow shall be maintained within 10% of the planned test rate throughout all quality checks and tests. All results shall be documented to confirm compliance with the specified airflow range.
  - b. Air velocities in the recirculating duct and across the ACD shall be maintained between 100 FPM and 600 FPM. Additionally, discharge velocities into the chamber shall not exceed 400 FPM. Air velocities shall be maintained within 10% of the planned test rate throughout all quality checks and tests.
- **9.1.13** At least every five minutes, take measurements required to verify that all velocities, airflows, and ACH are maintained within specified limits.
- **9.1.14** Operate chamber/duct cleanup system as per lab SOP or established procedure to achieve required background levels. At the start of sampling, the temperature shall be  $21.1^{\circ}C \pm 5.5^{\circ}C$  (70.0°F  $\pm 10.0^{\circ}F$ ), and RH shall be between 40% and 60%.
- 9.1.15 Turn off the cleanup system and then the recirculating duct before either the ACS or natural decay test.
- 9.2 Background and Baseline Sample Collection. Each ACS and natural decay test shall include the following.
  - **9.2.1** Begin monitoring temperature (T) and relative humidity (RH), then continue monitoring and recording throughout the test for at least four values of each. During the test, the temperature shall not exceed 27°C (80°F). For bioaerosol tests, RH shall be maintained between 40% and 60%; for particle tests, RH shall be maintained below 55%.
  - 9.2.2 Turn on the chamber fans.
  - 9.2.3 Turn on all continuous, direct-read analyzers and record the start time for each analyzer. Analyzer

instructions shall be followed for needed equilibration times before official test data is collected.

**9.2.4** Take all background samples required for all microorganisms, including viruses, bacteria, mold, mildew (as appropriate), and non-biological species, such as ions, relevant gases, and particles as described in Section 8. Record time and sampling rates for the beginning and end of each sample collected.

*Informative Note:* For particle contaminant tests with no bioaerosols, microorganism samples are not required.

- **9.2.5** If the background results exceed the limits specified in Section 5.3.3 and Table 7-1, the test shall be considered invalid.
- **9.2.6** Turn on the recirculating duct fan. While the recirculating duct fan is on, measure and record the resistance to airflow across the in-duct or diffuser-mounted device or the location where the device will be installed for the ACS test.
- **9.2.7** At least every five minutes, take measurements required to verify that all velocities, airflows, and ACH are maintained within specified limits.
- 9.2.8 Immediately begin an ACS or natural decay test.

#### 9.3 ACS or Natural Decay Test

- **9.3.1** Begin contaminant generation per Section 5 for bioaerosol generation and Section 6 for particle generation. Continue operation until the desired concentration is achieved, then stop the generation.
- **9.3.2** Mix the room air to ensure generated contaminants are uniformly distributed and have had time to dry down to a stable size distribution (if not dry when generated) before the test samples are taken. The duration of initial mixing after generation is stopped, shall be at least five minutes with the results of the mixing test (see Section 8) used to determine the actual length. As soon as the initial mixing is done, the recirculating duct shall be turned on and run for two minutes before moving to Section 9.3.3.
- **9.3.3** Take the time zero samples.
- **9.3.4** As soon as the initial sample is completed, for the ACS test, turn the device on. Record the time on. *Informative Note:* For the Natural Decay test, leave the Device Off.
- **9.3.5** At least every five minutes, take measurements required to verify that all velocities, airflows, and ACH are maintained within specified limits.
- **9.3.6** For bioaerosol challenge tests, take bioaerosol samples during at least five-time points over a period of up to three hours. A minimum of three samples shall be taken at each time point (i.e., triplicate) during each test run. These samples shall include enough quantity/volume to result in acceptable counts to meet the statistical requirements in Section 6.
- **9.3.7** For particle-only tests, sample for at least five minutes after the mixing period and before the start of the test (this is the ACS ON point for the ACS test). Sample particles at least every five minutes during the test period of 1 hour or until the counts per size bin are less than 10 particles/cc, whichever is sooner.
- **9.3.8** Take samples and/or record data for all other required species at least three times during the test, where required species includes both direct and indirect measure of the ACS active agent (e.g., ions, hydrogen peroxide, byproducts, etc.).
- 9.3.9 Turn off the ACS, if it was ON.
- **9.3.10** Turn off the airflow in the recirculating duct.
- **9.3.11** Operate the cleanup system per lab SOP or established procedure to achieve needed background levels. If personnel enter the chamber, appropriate PPE shall be worn.
- **9.3.12** Repeat the procedure from the beginning of Section 9.2, continuing into the test section for the remaining test(s).
- **9.3.13** Once all tests are done, clean out the chamber with the ventilation system. After this, follow the lab procedure to decontaminate the lab, if needed.

# **10. CALCULATIONS AND GRAPHING REQUIREMENTS**

#### 10.1 Challenge Contaminant Sample Calculations

10.1.1 Determining Contaminant Concentration for Each Sample

- **10.1.1.1** For impactors, the hole correction procedure shall be performed first (see Macher, JM. 1989). For any other samples, any required adjustments to the data shall be performed. If the samplers were overloaded or underloaded, the test shall be repeated.
- 10.1.1.2 For samples with counted plates, convert counts to concentrations. The exact equation will depend on the sampling device and dilutions (if used). The basis form of the equation is as shown in Equation 10-1.

**10.1.1.3** For samples reporting particle mass, the concentration based on mass shall be determined using Equation 10-2.

**10.1.1.4** For samples reporting particle counts, the concentration for each size range for each sample (including background), and adjustment of test samples for background levels, shall be determined using Equations 10-3 and 10-4.

$$Concentration = C_t - C_b \tag{10-4}$$

where

 $C_{\rm t}$  = concentration at time point (t)

- $C_{\rm b}$  = concentration of background sample
- **10.1.2** For non-continuous sampling, calculate the average and coefficient of variance (CV) for each time point. If the CV of any point exceeds 25%, repeat the test or discard that time point's data.
- 10.1.3 Net percent reduction over time (only required for bio test) shall be calculated using Equations 10-5 and 10-6. The calculation shall be performed for both ACS and natural decay data.

% Reduction = 
$$100 \times (1 - (C_t/C_0))$$
 (10-5)

where

 $C_t$  = concentration at time point (t)

 $C_0$  = average concentration at time zero

Net % Reduction = % Reduction<sub>ACS</sub> 
$$-$$
 % Reduction<sub>natural decay</sub> (10-6)

All valid time points shall be reported. Data at a given time point for both ACS and natural decay are required for the time point to be considered valid.

- 10.1.4 For bioaerosols, data for time points above or below appropriate concentration levels for the sample shall be excluded. Samples that are below the limit of detection (l.d.) shall either be assigned the value for that l.d., or excluded from the calculations. Data for time points that do not meet the CV requirement shall also be excluded. All concentration data values for each of the remaining time intervals (at least five, including
  - Ct) shall be used. Calculate the natural logarithm of each remaining concentration value.

For particles, all particles samples above three times the background level or greater than or equal to five counts per sample (whichever is greater) shall be used.

#### 10.1.4.1 Decay Rates

- **10.1.4.1.1** Calculate the slope of the linear fit to ln(concentration) vs. time relationship for both the ACS and natural decay tests. This gives the decay rate for each test.
- **10.1.4.1.2** Determine the  $R^2$  value for each fitted line. If  $R^2 < 0.85$ , either repeat the test or consider removing higher CV time periods and check the  $R^2$ . At least five remaining good time period sample sets are required for the test to be considered valid.

*Informative Note:* It is not required to use the  $C_t$  in the  $V_{ACS}$  calculation.

**10.1.4.2** Calculate  $V_{ACS}$  per Equation 10-7.

$$V_{ACS} = (k_{ACS} - k_{natural \ decay}) \times V \tag{10-7}$$

where

VACS	=	clean air delivery rate for microorganisms inactivated or particles removed during the test, $m^3/h$ (CFM)
V	=	volume of the chamber, m <sup>3</sup> (ft <sup>3</sup> )
<i>kACS</i>	=	ACS test decay rate, particles/h (particles/minute)
knatural decay	=	natural decay test rate, particles/h (particles/minute)

**10.2** For other analytes, calculate the concentrations at the time intervals of each microbiological sample or at least every 10 minutes for continuous samples. It is acceptable to calculate concentrations for each time value, if desired. Subtract the background concentration of the same analyte and tabulate the results.

# **11. REPORTING RESULTS**

The following information shall be included in the test report:

- a. Name and location of testing laboratory
- b. Test date
- c. Laboratory test operators' names
- d. Air cleaner manufacturer's name, the company submitting the air cleaner for testing, how the air cleaner was obtained, and who requested the test.
- e. Description of the air cleaner tested, including the following:
  - 1. Brand and model number (or description of a prototype)
  - 2. Physical description of the air cleaner including technology-specific details
  - 3. State whether air cleaner components comply with the manufacturer's specifications
  - 4. If the air cleaner has options, those used in the test shall be specified. These may include output settings, fan speed, filters, or shielding
  - 5. For IRAC(s), measured airflow rate and stated airflow as determined by the manufacturer
  - 6. Photos or drawings of the air cleaner as positioned or located during the test
  - 7. Number of air cleaners in the test chamber and recirculating duct
  - 8. Location of air cleaner(s) in the test chamber/recirculating duct, as well as the type of air cleaner: induct air cleaner, diffuser-mounted air cleaner, or in-room air cleaner
- f. Operation information as stated by the manufacturer, including recommended installation location and orientation. List procedures that were followed to conduct testing that came from the manufacturer's installation and operation instructions.
- g. Operating conditions for reporting purposes during the test: measured airflow rate of IRAC in m<sup>3</sup>/s (cfm), the temperature in degrees Celsius (degrees Fahrenheit), relative humidity, static pressure relative to space external to the chamber
- h. Chamber/equipment description (as required by Section 4)
  - 1. Actual chamber dimensions, volume in m<sup>3</sup> (ft<sup>3</sup>), type of test chamber (e.g., BSL-1, BSL-2, BSL-3).
  - 2. Mixing fans (airflow, make/model, rotation speed, location)
  - 3. Description of the duct system, specifying the total length of the recirculating duct and measurements of each respective duct section, including the distance between the chamber floor and the supply air inlets, the distance between the supply fan and supply air inlets, airflow rate, air velocity in the main duct, branch ducts, diffuser discharge velocity, velocity across the ACD, attachment points to the chamber,

and the air change rate (ACR) during both the natural decay and ACS tests

- 4. Ultraviolet (UV) reflectance (if required by Section 4.1.2)
- 5. Temperature/relative humidity control system
- 6. Air and/or surface cleanup method(s) used
- 7. Generator used
- 8. Sampler/sampling description(s) and location(s)
- 9. Analysis methods used
- 10. Detection limits of test methods for each analyte
- 11. For microorganisms, cite source, aerosolization media, and quantification of sampling/assay uncertainty
- i. Test Data
  - 1. Diagram of sampling points, mixing fan location(s), and temperature/RH control device
  - 2. Test air temperature and relative humidity (average and range)
  - 3. Lab barometric pressure, ACS resistance to airflow, face velocity, and device power consumption
  - 4. For baseline ACS and natural decay test(s)
    - i. Challenge organism(s) and suspension used, or Particle Challenge Description
    - ii. Duration of each test
    - iii. For bioaerosol tests, organism test data expressed in CFU/m<sup>3</sup> or PFU/m<sup>3</sup> (CFU/ft<sup>3</sup> or PFU/ft<sup>3</sup>), both tables and graphs, with respect to time. Report the  $V_{ACS}$  (including the time range used in the calculation)
    - iv. For particle tests, report the concentrations or particle counts per channel for all samples in tabulated formats. Graph the data for at least the largest and smallest channels. Report the  $V_{ACS}$  for each size channel. Graphs including trend lines shall include axes names, equations, and R-values.
    - v. All analyte testing results, including tabulated and graphed concentrations
  - 5. Required disclaimer on the cover or Page 1 of the report

The data presented in this report represent conditions existing in this test chamber. Caution shall be exercised in drawing conclusions from the data contained in this report as to the efficacy of the air cleaner under different circumstances. If changes are made to any aspect of the design that may change or alter the performance of the system, the system will need to be retested. This includes electronics, reflectors, sources or delivery methods, wavelength, mechanical designs, materials, and design configurations as examples.

# **12. NORMATIVE REFERENCES**

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- 8. Macher, JM. 1989. Positive-hole correction of multiple-jet impactors for collecting viable microorganisms. Am

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# **INFORMATIVE APPENDIX A—BIBLIOGRAPHY**

- 1. NIOSH. 2020. *NIOSH Manual of Analytical Methods (NMAM)*, 5<sup>th</sup> ed. Atlanta, GA: Centers for Disease Control and Prevention. www.cdc.gov/niosh/nmam/pdf/NMAM\_5thEd\_EBook-508-final.pdf.
- 2. ANSI. 2020. NSF/ANSI 49-2020, *Biosafety Cabinetry: Design, Construction, Performance, And Field Certification*. Washington, D.C.: American National Standards Institute.
- 3. Hinds, W.C. 1999. Aerosol Technology, Properties, Behaviour, and Measurement of Airborne Particles. New York, NY: John Wiley & Sons Inc.
- 4. ASHRAE. 2025. ANSI/ASHRAE Standard 145.2, Laboratory Test Method for Assessing the Performance of Gas-Phase Air Cleaning Systems: Air Cleaning Devices. Peachtree Corners, GA: ASHRAE.

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# **INFORMATIVE APPENDIX B—POSSIBLE ADDITIONAL ORGANISMS**

This test currently only requires one organism for a standard test. However, there are many organisms that are found in the air and manufacturers and users may wish to have specific data for them. This section shows some examples of organisms that would be appropriate for this test. These are low Biosafety Level organisms that are used as surrogates for organisms that are more likely to infect humans. Other organisms are acceptable, including higher level ones with the caveat that the laboratory is responsible for using appropriate safety measures and equipment for the organisms they use.

ANSI/ASHRAE Standard 185.3 requires four types of organisms per complete test. Table B-1, reproduced from ANSI/ASHRAE Standard 185.3-2024, Table A-1, shows examples for each of these four categories.

For the purposes of this standard, a complete test requires that one organism of each type be tested, with the option to do additional organisms.

Different types of organisms have different levels of resistance to different killing mechanisms. Testing organisms of different types should give a realistic expectation of how a device works and allows reasonable comparisons across devices. If an organism other than MS2 is tested with the same procedures, the test report should be labeled "modified" and the organism clearly indicated on the cover, summary results, and first page if there is no cover.

All pathogens used in testing should have an ATCC or BEI number, and ideally, a Certificate of Authenticity (COA).

The rationale for using different organisms is supported by two figures showing relative resistance for organisms from Favero and Bond, 2001 (Figure B-1) and from McDonnell and Russell, 1999 (Figure B-2).

		ATCC	Biosafety	
	Organism(s)	Category #	Level	Comment(s)
Gram Positive Bacteria	Staphylococcus aureus	ATCC 6538	2	Organisms found on BEI, but
	Staphylococcus epidermidis	ATCC 12228	1	not exact strain
	Bacillus atrophaeus	ATCC 9372	1	BEI NR-687
	Bacillus subtilis (vegetative & endospores)	ATCC 6633	1	BEI NR-604
Gram Negative Bacteria	Escherichia coli	ATCC 8739	1	Organisms found on BEI, but
	Klebsiella pneumoniae	ATCC 4352	2	not exact strain
	Pseudomonas aeruginosa	ATCC 9027	2	
	Serratia marcescens	N/A		Only available on ATCC
Bacteriophages	Phi X174	ATCC	1	Only available on ATCC
		13706-B1		
	MS2	ATCC 15597-B1	1	
	Phi 6	ATCC 21781-B1		Unable to find on ATCC
Fungi	Penicillium citrinum	ATCC 9849	1	Only available on ATCC
	Aspergillus fumigatus	ATCC 204305	2	
	Penicillium chrysogenum	ATCC 10106	1	
	Penicillium rubens	ATCC 11709	1	
	Stachybotrys chartarum	N/A	1	
	Aspergillus brasiliensis	ATCC 16404	1	

Table B-1 Possible Test Organisms for An ASHRAE Standard 185.5 Test



#### Figure B-1 Relative Resistance to Inactivation

Source: Modified from Favero, M.S. and W.W. Bond. 2001. Chemical Disinfection of Medical and surgical materials. In: Disinfection, Sterilization, and Preservation, 5th Ed., Phila: Lippincott, Williams, & Wilkins, pp. 881-917.

## Descending order of resistance to antiseptics and disinfectants

- Prions (CJD, BSE)
- Coccidia (Cryptosporidium)
- Spores (Bacillus, C. difficile)
- Mycobacteria (M. tuberculosis, M. avium)
- Cysts (Giardia)
- Small non-enveloped viruses (Polio virus)
- Trophozoites (Acanthamoeba)
- Gram-negative bacteria (non-sporulating) (Pseudomonas, Providencia)
- Fungi (Candida, Aspergillus)
- Large non-enveloped viruses (Enteroviruses, Adenovirus)
- Gram-positive bacteria (S. aureus, Enterococcus)
- Lipid enveloped viruses (HIV, HBV)

#### Figure B-2 Descending order of resistance to antiseptics and disinfectants

Source: Modified from McDonnell, Gerald and A. Denver Russell. 1999. Clin. Microbio., doi: 10.1128/CMR. 12.1.147.



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# INFORMATIVE APPENDIX C-EXAMPLE VACS CALCULATIONS

In the examples below, a 1000  $\text{ft}^3$  chamber with 100  $\text{ft}^3$  recirculating duct is used for a test starting at 10000 particles/cm<sup>3</sup>. Note that the example works the same if the concentration is in other units. The important units are the time and chamber volume. If (for example) m<sup>3</sup>/h were to be used, answer could be converted from cfm to m<sup>3</sup>/h, or units of m<sup>3</sup> and h could be used to make the data table and do the calculations.

The natural decay test concentration decreases slowly, while the ACS test concentration decreases more rapidly. Once the data is tabulated, a simple linear graph is recommended for review to ensure that the data is rational. The curves should either fairly stable at or near the initial value or should decrease over time. It is unlikely that the ACS concentration will be higher than the natural decay concentration at the end of the test. If this occurs and the initial concentrations were similar, check the data for errors. Even if the device doesn't work at all, less decrease than the natural decay may indicate an unnoticed leak in the natural decay test that was plugged for the ACS test, or a similar issue. Zigzags in the data are not usual; some variability is likely.

Preparing a log linear chart should show essentially linear data, although for some devices, this may not be true. If there is a distinct change in slope and the early portion of the test has enough data points, the  $V_{ECA}$  may be reported based only on this data. Calculate the natural logarithm (ln) of the concentration, then calculate the slope and R2 of the ln(Conc) vs. time relationship. If the R2 is acceptable, the slope will be the k value.

Perform these steps for both the natural decay and the ACS tests. Then calculate the  $V_{ACS}$  from the total volume of the chamber and recirculating duct multiplied by the difference between the two k-values. Note that if the concentrations decreased faster during the ACS than during the natural decay, the  $V_{ACS}$  value will be a positive number.

The rationality plot is recommended just to show that the data makes sense, but is not required in the test report. Large differences at any time point should be examined. Averages that go up over the test or appear random should also cause enough concern to suggest double-checking the data.

Plotting as ln (Conc) by time (a ln-linear plot) should show linear relationships. The ACS should not have a lower slope than the natural decay. If the device functions properly, the lines should differ significantly. It is not expected for the slopes of a particle test of different sizes or a microorganism test would match.

#### **C1. Particle Test Example**

Use one sample every five minutes

Chamber size: 1000 ft<sup>3</sup> with recirculating duct of 100 ft<sup>3</sup>

This is modeled on a device that is approximately 85% for a 90 CFM airflow on a single pass.

Particle size (nm)	=	200
Chamber volume (ft <sup>3</sup> )	=	1000
Recirculating duct volume (ft <sup>3</sup> )	=	100
Total volume (ft <sup>3</sup> )	=	1100
V <sub>ACS</sub> (CFM)	=	47.2

# **Natural Decay**

Time (min)	Concentration	ln(concentration)
0	10000	9.21
5	9910	9.20
10	9799	9.19
15	9700	9.18
20	9600	9.17
25	9500	9.16
30	9420	9.15
35	9300	9.14
40	9200	9.13
45	9130	9.12
50	9020	9.11
55	8945	9.10
60	8805	9.08

k = 0.0021



## **Natural Decay**

Time (min)	Concentration	ln(concentration)
0	10000	9.21
5	8180	9.01
10	6311	8.75
15	5200	8.56
20	4000	8.29
25	3400	8.13
30	2500	7.82
35	2200	7.70
40	1400	7.24
45	1470	7.29
50	1021	6.93
55	920	6.82
60	650	6.48

k = 0.0450

 $R^2 = 0.994$ 



#### C2. Micro Test Example

This example shows triplicate samples at each time point and includes CV checks and  $R^2$  checks. The intent of this example is to demonstrate a good data set and to provide values for math checks. These are only examples of time intervals; many other time intervals could also be valid.

Chamber volume ( $ft^3$ ) =		1000
Recirculating duct volume (ft <sup>3</sup> )	=	100
Total volume (ft <sup>3</sup> )	=	1100
VACS (CFM)	=	15.3

Time (min)	Concentration	CV	ln(concentration)
0	1000	0.042	6.91
0	920		6.82
0	970		6.88
12	975	0.026	6.88
12	950		6.86
12	925		6.83
24	870	0.033	6.77
24	900		6.80
24	930		6.84
36	875	0.027	6.77
36	845		6.74
36	830		6.72
48	800	0.048	6.68
48	835		6.73
48	758		6.63
60	705	0.042	6.56
60	722		6.58
60	765		6.64

# Natural Decay

k = 0.0047

Time (min)	Concentration	CV	ln(concentration)
0	1000	0.064	6.91
0	880		6.78
0	955		6.86
12	690	0.087	6.54
12	650		6.48
12	580		6.36
24	443	0.111	6.09
24	518		6.25
24	553		6.32
36	505	0.092	6.22
36	472		6.16
36	420		6.04
48	351	0.128	5.86
48	381		5.94
48	295		5.69
60	258	0.115	5.55
60	301		5.71
60	325		5.78

# ACS

k = 0.0186





# Example for an "out of specification" particle test showing a low $\ensuremath{\mathsf{R}}^2$

This example shows what a data set that does not meet the test requirements could look like.

Particle size (nm) =		300
Chamber volume (ft <sup>3</sup> )	=	1000
Recirculating duct volume (ft <sup>3</sup> )	=	100
Total volume (ft <sup>3</sup> )	=	1100
$V_{ACS}$ (CFM)	=	38.1

Time (min)	Concentration	ln(concentration)
0	10000	9.21
5	9910	9.20
10	9799	9.19
15	9700	9.18
20	9600	9.17
25	9500	9.16
30	9420	9.15
35	9300	9.14
40	9200	9.13
45	9130	9.12
50	9020	9.11
55	8945	9.10
60	8805	9.08

## **Natural Decay**

k = 0.0021



A	С	C
Α	U	0

Time (min)	Concentration	ln(concentration)
0	10000	9.21
5	8180	9.01
10	6311	8.75
15	6500	8.78
20	2000	7.60
25	3400	8.13
30	2500	7.82
35	2200	7.70
40	3500	8.16
45	1470	7.29
50	1021	6.93
55	2500	7.82
60	650	6.48



$$R^2 = 0.769$$



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# INFORMATIVE APPENDIX D—EQUIVALENT AIR CHANGE RATE TEST (TOTAL ALLOWABLE LEAKAGE)

One equivalent option to do the air change rate test is detailed below:

- a. Choose easy to work with contaminants that you have a continuous or frequent monitor for (a VOC with PID or 0.5 µm particles are good choices)
- b. Place an air cleaner surrogate with a resistance to airflow of ≥1.0 in H<sub>2</sub>O (use a value equal to or higher than the highest expected resistance to airflow for actual tests). This can be an orifice plate, a media filter or similar device with airflow resistance.
- c. Close up the chamber as you would during the test.
- d. Turn on the airflow through the recirculating duct to a typical value.
- e. Run the cleanup system as you would before a test.
- f. Measure contaminant background levels in the space surrounding the chamber.
- g. Measure chamber backgrounds.
- h. Generate contaminant (even a small spritz of liquid VOC can suffice)
- i. Run mixing fans as you would during an actual test
- j. Wait until the value stabilizes (is not fluctuating indicating lack of mixing or contaminant still evaporating). If it is too high for your normal test needs, you can run the cleanup system to reduce the level. Then make sure the value stabilized.
- k. Monitor for one hour
- 1. Calculate k value or equivalent clean air entering the chamber system
- m. If high, re-gasket, seal openings, make sure the cleanup system is sealed off, etc.
- n. Repeat.
- o. Learn what level is usual for your chamber for ease is noticing that a leak has occurred.
- p. If an air mixing test is done after this that results in changes to the chamber, repeat this test.