BSR/ASHRAE Standard 185.3P

Public Review Draft

Method of Testing In-Room Devices and Systems for Microorganism Removal or Inactivation in a Chamber

First Public Review (March 2023)
(Complete Draft for Full Review)

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Foreword

The COVID-19 pandemic made it clear that enhancements to standard ventilation practices are required to protect building occupants from diseases spread via infectious aerosols. The use of in-room air cleaners (IRACs) is one quick, simple, and cost-effective method to reduce the concentration of infectious aerosols in indoor spaces. The number of IRACs that are commercially available continues to increase, as does the number of technologies (or combinations of technologies) used to remove infectious aerosols from the air. Many IRACs claim to inactivate or remove microorganisms from the air. Often, there is limited to no scientific data to support those claims.

To assist building owners and operators in making informed decisions, consensus methods of tests are necessary to gather the scientific evidence on which claims of effectiveness can be based. Robust test standards form the foundation for air-cleaner selection and end-user confidence in the ventilation industry. ASHRAE and the Association of Home Appliance Manufacturers (AHAM) have both developed test standards focused on IRAC removal of airborne microorganisms in a room. AHAM Standard AC-5, entitled *Method for Assessing the Reduction Rate of Key Bioaerosols by Portable Air Cleaners Using an Aerobiology Test Chamber* and published in 2022, results in clean air delivery rate for microbes (m-CADR) for residential IRACs. However, AHAM AC-5-2022 does not cover larger commercial and industrial IRACs.

This standard contains test procedures that may be applied to any brand or model IRAC used in commercial or industrial applications. This standard is not meant to replace AHAM AC-5-2022 for residential IRACs. The laboratory test chamber, required equipment, prescribed microorganisms, analytical techniques, and result calculations are designed to limit variability and error between individual tests. Calculated outcomes include the percent reduction, net percent reduction, and clean air delivery rate (CADR) for each of four organisms. Test reporting includes requirements for descriptions of the device, test setup, and the results.

The results of tests performed under this standard may be publicly stated.

Considerable capital may be required to establish a laboratory with the required test chamber and analytical capabilities. However, compliance with the test standard cannot be met with an alternative test chamber or procedures. Modified testing (e.g., using different microorganisms or a chamber with different dimensions) under this standard is possible, but this would be considered a non-standard test. “Non-standard” testing results under this standard should be clearly labeled as such because they should not be compared to testing results obtained under a standard test. Properly interpreting the results of a non-standard test would require a level of understanding and expertise that is not necessary when the standard is followed as written.

This test method covers the efficacy of the IRAC for the removal of bioaerosols from the air, along with associated output measurements. It does not cover the safety aspects of the tested IRACs or conformance with energy regulations. Regarding safety, ASHRAE recommends that products manufactured or marketed in the United States be submitted to an appropriate independent Nationally Recognized Testing Laboratory for inspection and listing in conformance with the safety standards and procedures followed by such laboratories. Specific to ozone emissions from IRACs, UL Standard 2998 (2020) *Environmental*

Claim Validation Procedure (ECVP) for Zero Ozone Emissions from Air Cleaners should be used where required. This standard method does not replace UL 2998 certification.

Regarding energy use, the IRAC manufacturer must follow all state, provincial, and federal requirements for such equipment. It is recommended that products be submitted to an appropriate independent, Nationally Recognized Testing Laboratory (NRTL) for inspection and listing in conformance with the energy regulations.

1. Purpose

This standard establishes a method of test for evaluating in-room air cleaners (IRACs) and systems for commercial or industrial consumers for microorganism removal or inactivation in a test chamber.

2. Scope

2.1 This method of test specifies selected indicator microorganisms in the test chamber and defines procedures for generating the bioaerosols required for the method of test.

2.2 This standard provides a method for counting the number of viable microorganisms in the test chamber to calculate the elimination efficiency for each microorganism.

2.3 This standard establishes minimum performance specifications for the equipment required to conduct the tests, defines methods of calculating and reporting results obtained from the test data, and establishes a reporting system to be applied to in-room air cleaners (IRACs) and systems covered herein.

2.4 This standard does not address the health and safety effects of operating IRACs and systems in an occupied room.

3. Definitions and Acronyms

3.1 Definitions

Aerosol: n. small particles (solid or liquid) suspended in air.

Air changes per hour (ACH): the clean air delivery rate divided by Chamber Volume expressed in compatible units.

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

Bioaerosol: an aerosol containing biologically active bacteria, spores, viruses, toxins, and other similar material.

Breathing zone*:* n. the region within an occupied space between planes 48 and 60 in. (120 and 150 cm) above the floor and more than 2 ft (60 cm) from the walls or fixed air-conditioning equipment.

Byproduct: n. species or analyte added to the air in the test chamber during the test other than the intended challenge species. In this method, the byproduct is considered analytes and includes species intentionally generated by the device and species created by the reactions or off-gassing within the chamber during a test.

Equivalent Air Changes per Hour: air changes per hour of air that is free of infectious aerosols.

In-room air cleaner: n. a device that is placed in a room to clean the air. This may include standalone, portable, wall-mounted units.

Leq or Lavg: best described as the Average Sound Level over the period of the measurement. Usually measured with A-weighting, the Leq has no time constant applied, but the Lavg is usually Slow time weighted. As it is an average, it will settle to a steady value, making it much easier to read accurately than with a simple instantaneous Sound Level. Being an average, it is also showing the total energy of the noise being measured, so it is a better indicator of potential hearing damage or the likelihood that the noise will generate complaints.

Limit of Blank (LOB): the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested. $LOB = mean_{blank} + 1.645(SD_{blank})$

Limit of detection (LOD): the lowest analyte concentration is likely to be reliably distinguished from the LOB and at which detection is feasible. LOD is determined by utilizing both the measured LOB and test replicates of a sample known to contain a low concentration of the analyte. $LOD = LOB + 1.645(SD_{\text{low concentration sample}})$

Limit of quantification (LOQ): the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met. The LOQ may be equivalent to the LOD, or it could be at a much higher concentration. 10 times the standard error of the calibration graph divided by the slope of the calibration curve.

Net % Removal/Inactivation: n. % Removal/Inactivation in contaminant over time accounting for % Removal/Inactivation due to natural decay.

% Removal/Inactivation: n. percentage reduction in contaminant over time.

Reflectance: n. the ratio of the light reflected by a surface to the light incident upon it.

Statistically valid CFU/PFU count: one generally considers 25-250 (some say 30-300) per nominal 100-mm Petri plate. This can also be described as 1-13 CFU/cm^2 or 1-13 PFU/cm^2 for plates regardless of diameter.

Triage: preliminary evaluation and or stabilization of test conditions.

Well-mixed chamber: n. a chamber or room in which the various species in the air are considered to be evenly spaced out.

3.2 Acronyms

ACH Air Changes per Hour
AIHA American Industrial Hygiene Association
AIHA LAP AIHA Laboratory Accreditation Programs
ANSI American National Standards Institute
ASTM American Society for Testing and Materials
ATCC American Type Culture Collective
BMBL Biosafety in Microbiological and Biomedical Laboratories
BSL Biosafety Level
CADR Clean Air Delivery Rate
CDC Center for Disease Control and Prevention
CFM Cubic Feet per Minute
CFU Colony Forming Units
CV Coefficient of Variance
EPA Environmental Protection Agency
FDA Food and Drug Administration (USA)
FPM Feet per Minute
GLP Good Laboratory Practice
IRAC In-Room Air Cleaner
ISO International Standards Organization
LOB Limit of Blank
LOD Limits of Detection
LOQ Limit of Quantitation
NIH National Institutes of Health (USA)
NIOSH National Institute of Occupational Safety and Health (USA)
NIST National Institute of Science and Technology
NSF National Science Foundation
OSHA Occupational Safety and Health Administration
PEL Permissible exposure limit
PFU Plaque Forming Unit
QA Quality Assurance
QC Quality Control
RH Relative humidity
SOP Standard operating procedure
T Temperature
TLV Threshold Limit Value
UV Ultraviolet

4. Test Apparatus and Materials

4.1 Test Chamber. The test chamber shall:

4.1.1 Comply as appropriate with government and local agency requirements for handling and testing biological samples, for example not limited to OSHA, NIH/CDC, and ANSI/NSF 49.

4.1.2 Have ultraviolet (UV) reflectance of <30% at the operational wavelength provided by the manufacturer of the in-room air cleaner (IRAC) under test. This only applies to IRACs that emit UV. The test report should declare the reflectance value. The lab can use the reflectivity data provided by the manufacturer of the chamber. The lab can also use a handheld spectroradiometer that is calibrated with a NIST traceable standard.

4.1.3 Be constructed of impervious surfaces such as stainless steel, epoxy, or glass, and be well grounded. The chamber shall be rectangular with "height," "width," and "length" referring to the dimensions shown in Figure **A**. Be at least 27 m^3 (954 ft^3). The height shall be between 2.4 m and 3.1 m (7.7 ft and 10.2 ft). The width shall be between 85% and 100% of the length. For IRACs operating above 680 m^3/h (400 cfm), a chamber larger than the minimum or an alternative test methodology may be required.

4.1.4 Figure A: drawing w/ sampling ports, an example of mixing fans, example location(s) of IRACs, etc.

4.1.5 Be equipped to create and maintain a starting temperature (T) of 23 +/- 3°C (73 +/- 5°F) and relative humidity (RH) of 50% ±10% and to continuously monitor them. These values shall be recorded during testing and reported for each test in the test report.

4.1.6 Be equipped with not less than three sampling locations for biological air sampling. These sampling locations will be positioned to be representative of the airflow dynamics of the chamber (lacking high turbulence or unidirectional velocity). These probes shall be located at a distance of $\geq$0.3 m (1 ft) from each wall and at a distance sufficient from the mixing fan and the IRAC(s) such that the sample is not influenced. The sampling locations shall be representative of the chamber dynamics and 1.0-1.8 meters above the floor.

4.1.7 Be equipped with not less than three sampling locations for nonbiological air sampling. Ideally, the probes in 4.1.6 & 4.1.7 should be in the general vicinity of each other but not interfere with the collection efficiency of either probe. These probes shall be located at a distance of $\geq$0.3 m (1 ft) from each wall and at a distance sufficient from the mixing fan and the IRAC(s) such that the sample is not influenced. The probe shall be capable of continuous sampling. The sampling locations shall be representative of the chamber dynamics and 1.0-1.8 meters above the floor.

4.1.8 Provide well-mixed conditions in the chamber when the fans are operating. The well-mixed conditions shall be verified by aerosol surrogate testing. See AIHA Mathematical Modeling in the reference section. For example, a ceiling paddle fan providing 0.03-0.05 m^3/s (64-100 CFM) of volumetric airflow rate has been shown to promote a well-mixed chamber resulting in an average vertical velocity of 0.1-0.2 m/s (20-40 feet per minute [fpm]).

4.1.9 Be capable of mounting and installing the most common IRACs, wall-mounted IRACs, tabletop IRACs, ceiling-mounted, and floor IRACs in a central location in the test chamber. This includes air-tight ports for power lines and remote controls. IRACs shall be installed according to the manufacturer's published installation instructions for proper operation. IRACs designed to vent into a mechanical exhaust system or directly outside the room are not included in this standard. See Informative Appendix D: Mounting and installation of IRAC.

4.1.10 Be equipped with a nebulizer injection port that is between 1.5 m and 1.8 m (5 ft and 6 ft) above the floor such that the bioaerosols are mixed within the room.

4.1.11 Be equipped to allow decontamination of the chamber without humans entering. Each lab shall have a standard operating procedure (SOP) delineating the decontamination methodology.

4.1.12 Be capable of flushing and/or disinfecting the air in the chamber pre-and post-testing. 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 including microorganisms; carbon beds can be used to remove other species from the air. This cleanup system shall be designed to be shut completely off from influencing the chamber during the testing.

4.1.13 Be capable of achieving and maintaining a microbial background concentration which shall be maintained at <35 CFU/m^3 or PFU/m^3 (<1 CFU/ft^3 or PFU/ft^3) over multiple sampling periods or a level deemed acceptable through laboratory standard operating procedures.

4.1.14 The test chamber shall meet the QA/QC requirements in Appendix B before testing may be performed.

4.2 IRACs

4.2.1 IRACS shall be intended for use in a room.

4.2.2 IRACs shall be installed according to the manufacturer's published installation instructions for proper operation.

4.2.3 IRACs shall have an available means to turn the device on from outside the chamber. Plug in cords and remotes are acceptable.

4.2.4 IRAC(s) under test designed for volumes smaller than the test chamber size may have multiple IRACs in the room during testing. All IRACs must be identical. Sizing and spacing must be according to the manufacturers' specifications and will be validated by the test laboratory.

4.2.5 IRACs venting into a mechanical exhaust system or directly outside the room are not included in this standard. See Informative Appendix D: Mounting and installation of IRAC.

4.3 Nebulizers

4.3.1 The nebulizers shall be capable of nebulizing microbial suspensions into particles containing no more than one bacterium, fungal spore, or virion.

4.3.2 The compressed air shall be filtered to remove contaminants including oil from the air.

4.3.3 The generated aerosol shall be dried down to a stable condition. A drying tower or other dehumidifier may be used. The drying may also be done in the chamber through the required mixing time of the chamber. The lab is responsible for determining that their nebulizing procedure does not increase the RH in the chamber above acceptable levels.

4.3.4 Collison 6-jet or 24-jet nebulizer, ultrasonic nebulizer, or another bioaerosol-generating device, driven by purified filtered air supply or equivalent are acceptable. The nebulizer must be chosen to allow correct operation with the organism of a specific test. Given that the organisms are different sizes, the nebulizer must allow the organisms to pass through the nozzle. Nebulizers should also be chosen to allow organisms to survive generation.

4.3.5 Biological agents shall be suspended in an appropriate maintenance and delivery solution.

4.4 Bioaerosol Samplers

4.4.1 Sampling ports will be used with probes attached to pumps to pull air samples from the chamber to the samplers located outside the test chamber.

4.4.2 These probes shall be located along the centerline of the room in the lengthwise direction. These probes shall be located at a distance of ≥0.3 m (1 ft) from each wall and at a distance sufficient from the mixing fan and the IRAC(s) 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 ±0.1 m (4.9 ±0.3 ft) above the floor of the chamber. See Appendix C for limitations on biological samples, direct-read equipment, and other sampling methods.

4.4.3 Impingers and impactors are acceptable samplers. In some circumstances, using both impingers and impactors is beneficial to compare the concentration of bioaerosol based on particle concentration and on microorganism concentration. Other samplers with similar performance are also acceptable. Sampling times and volumes should be determined to give sufficient, but not excessive, counts and minimum concentration in air to obtain statistically valid counts (Section 6). It is preferred that sampling times and volumes be the same for all samples in a given test. Differences in protocols shall be documented and reported. When using impactors, the correction hole factor shall be applied before other calculations and be required for reporting.

4.4.4 Sample preparation and analysis will be organism, sampler, and air cleaner dependent.

4.4.5 The sampling system shall include a method for rapid changeout to allow samples to be taken as quickly as needed for each test.

4.4.6 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.

4.4.7 Laboratory SOPs shall include validation of generation and sampling methodologies, including concentrations generated, the efficiency of collection methodologies, and particle size distribution.

4.5 Material, chemical, and physical testing.

4.5.1 Testing may be conducted prior to or concurrent with biological testing. It is preferred that testing is completed in conjunction with biological testing to ascertain any significant changes in the chamber air composition, as long as the testing can be performed safely, and associated equipment sanitized. If the testing is not conducted concurrently, it can be conducted prior to biological testing for a period of not less than two hours but must meet four air changes of the chamber based on the manufacturer's specifications, with samples collected prior to turning on the IRAC and after the IRAC has been on for a minimum of two hours and at the completion of the test, within the sealed chamber. The chamber shall be returned to background conditions prior to biological testing. Testing can be done by using either real-time monitoring instruments or collecting samples for laboratory analysis. Laboratories may not be willing to accept samples containing biological test agents.

4.5.2 Baseline and analyte testing shall be performed as described below for all tests at the "normal" location of the occupant in the space and shall include:

Ozone (O_3)
Particulate Matter (PM_{10}, $PM_{2.5}$, $PM_{1.0}$)
Carbon dioxide (CO_2)
Hydrogen peroxide (H_2O_2)
Formaldehyde (CH_2O)
Ultraviolet light (UV, wavelength specific)
Ions (positive and negative ions)
Total Volatile Organic Compounds (TVOCs)

Justification is required if any test is not conducted.

4.5.3 The requirements for analyte detection limits are in Appendix C, Table C1.

4.5.4 Analyzers shall have a LOD of no more than 1% of the TLV or other relevant occupant health standard based on occupants and confirm calibration performance according to NIST traceable standards and the respective equipment manufacturer recommendations. LOD test results shall be included in the test report. For analytes without TLVs or permissible exposure limits (PELs), analyzers should be able to measure a minimum of detection of 10% of the IRAC manufacturer's published recommended concentration.

4.5.5 Appendix C includes selected standards, guidelines, and recommendations with occupant (occupational and non-occupational) exposure limits.

4.5.6 Real-time analyzers, associated equipment, and samplers shall be located outside the chamber during the tests unless proven to not influence the test conditions or results. Analyzers and equipment should be confirmed leak-free per the laboratory SOP and sanitized following each use to minimize any potential carryover between tests and for personnel safety.

4.5.7 Real-time analyzers, associated equipment, and samplers will connect to the nonbiological sampling locations in the chamber. Ideally, the sample locations in

4.1.6 & 4.1.7 should be in the general vicinity of each other but not interfere with the collection efficiency of either system.

4.5.8 Real-time analyzers shall be calibrated per manufacturer's instructions and as a minimum, spanned with "clean air" and a validated calibration standard and method prior to each test. Calibrations shall be performed using a NIST traceable standard or equivalent and documented prior to each use. Real-time analyzers shall be run for a period of not less than 15 minutes prior to powering on the IRAC being tested to establish background concentrations of the analytes within the chamber.

4.5.9 Collected air samples shall be analyzed using validated test methods published by nationally or internationally recognized authorities such as US EPA, OSHA, CDC/NIOSH, ANSI, ASTM, ISO, AIHA, etc. The NIOSH Manual of Analytical Methods (NMAM) 2020, (https://www.cdc.gov/niosh/nmam/pdf/NMAM_5thEd_EBook-508-final.pdf) provides examples of some published laboratory testing methods.

4.6 Noise Dosimeter

4.6.1 See Appendix C for equipment suggestions.

4.6.2 This test is NOT a substitute for other acoustic studies required for regulatory compliance or noise conservation.

4.7 Cleanup/Flushing System

4.7.1 This system shall be able to lower microbial background concentration to <35 CFU/m^3 or PFU/m^3 (<1 CFU/ft^3 or PFU/ft^3) over multiple sampling periods or a level deemed acceptable through laboratory SOPs.

4.8 Materials

4.8.1 Microorganisms

4.8.1.1 Microorganisms selected for the challenge bioaerosol shall be handled with appropriate primary containment, secondary containment, and good laboratory practice (GLP) in accordance with the current version of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL). Biosafety level (BSL)-1 microorganisms are generally the safest to aerosolize. BSL-1 organisms are recommended for use in the test method wherever possible. When possible, use American Type Culture Collective (ATCC)-traceable microorganisms. Refs: Appendix A, BMBL, EPA & FDA hierarchy.

4.8.1.2 Recommended BSL-1 and BSL-2 microorganisms are listed in Appendix A. A minimum of one Gram-negative bacterium, one Gram-positive bacterium, one bacterial phage, and one fungus shall be included in the series of tests to fulfill the requirements of this standard.

5 Procedures

Tests with the in-room air cleaner (**IRAC) ON** and the **IRAC OFF** (Natural Decay) shall be performed identically except for the IRAC operation. This is critical so that the natural decay (no IRAC or IRAC off) test correctly reflects the change in concentrations due to the sampling itself. The order of the tests

is not specified. However, running the natural decay test first may identify problems without requiring a new IRAC for the next test. A natural decay test may apply to more than one **IRAC ON** test as long as the tests are run with the same batch of microorganisms, within three days, and with the same sampling time points. If test variability is a factor, consider running at least three repetitions of the test.

5.1 Before Testing - Setup

5.1.1 If the device has a fan, determine the airflow rate at all speed settings.

5.1.2 Noise measurement shall be taken before (baseline) and after the IRAC is turned on and in full operation at its maximum speed. See Appendix C for guidance.

5.1.3 Identify details of surface material(s) of walls, ceiling, and floor. Determine reflectivity data for the specific surface material in the chamber for the specific wavelength of IRAC(s) being tested, if available. Note other surfaces (carts, tables, etc.), with a total surface area >20% of the wall/ceiling/floor area.

5.1.4 Take a picture(s) of the IRAC device and identifying markings and labels.

5.1.5 Determine the technologies in use, to the extent possible by visual examination. Check to see if this agrees with the manufacturer's information provided by the test requestor.

5.1.6 Determine the sampling plan including sample times, lengths of samples, which species need to be sampled for, and other relevant details.

5.1.7 Prepare for sampling with, for example, 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.

5.1.8 Fans, sampling probes, etc., shall be set up in the chamber.

5.1.9 No electrical devices other than the IRAC being tested and chamber mixing fans described above shall be located in the chamber during testing which might influence the test conditions or results.

5.1.10 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. This may require its own measurement or demonstration that the Laboratory standard operating procedure (SOP) used by the lab for cleaning provides no residuals.

5.1.11 Install the IRAC in the chamber prior to initiation of the natural decay test.

5.1.12 IRACs shall be installed in the chamber per the manufacturer's instructions. Make sure the IRAC can be turned on and off from outside the chamber. Be sure to place the device and probes so that they do not interfere with each other.

5.1.13 Seal chamber.

5.1.14 Operate cleanup system as per lab SOP or established procedure to achieve needed background levels.

5.1.15 Turnoff the cleanup system before beginning the test.

5.2 Background and Baseline Sample Collection

This section is a required part of each Natural Decay and IRAC test.

5.2.1 Perform background samples for all biological and non-biological species that need separate samples. Record time and sampling rates for the beginning and end of each sample collected.

5.2.2 Turn on all continuous, direct-read analyzers. Record the start time for each analyzer. Follow analyzer instructions for needed equilibration times before official test data is collected.

5.2.3 Collect triage samples for O_3, particulates $PM_{10,\ 2.5,\ 1.0}$, CO_2, H_2O_2, formaldehyde, UV (wavelength specific), ions, and TVOCs.

5.2.4 If the triage results exceed the limit of quantitation (LOQ), further investigation is warranted to determine the sources of contamination. Actions may be needed to eliminate sources of contamination.

5.2.4.1 Take noise measurements. Record the time and location for each measurement.

5.2.4.2 Begin monitoring T and relative humidity (RH). Continue monitoring throughout the test. During the test, the temperature shall not exceed 27°C (80°F) and RH shall not exceed 60%.

5.2.4.3 Immediately begin a natural decay test or IRAC test

5.3 The Test (either natural decay or IRAC test)

5.3.1 Begin nebulization and operate until the desired concentration is achieved, then stop the nebulization.

5.3.1.1 The minimum initial concentration may vary depending on the microbe.

5.3.1.2 Initially mix air in the room to ensure generated bioaerosols are uniformly distributed and have had time to dry down to a stable size distribution before the natural decay test. The duration of initial mixing after bioaerosol generation is stopped, shall be at least five minutes after generation is stopped. The results of the mixing test (see Appendix B, QA/QC) shall be used to determine this length. If the Mixing Test determined that a different air-mixing protocol is necessary for the actual test to ensure that the airflow patterns/currents do not affect the air sampling results or the performance of the IRAC, switch to this procedure after the aerosol is well-mixed. Whatever procedure is used, continue to mix air in the room to ensure that generated bioaerosols remain uniformly distributed during the natural decay test.

5.3.1.3 Take the time zero samples.

5.3.2 For the IRAC ON test, turn the IRAC on at this point. Record the time on. For the natural decay test, leave the IRAC Off.

5.3.3 Sample bioaerosols during at least five-time points over a period not to exceed four hours. A minimum of three samples shall be taken at each time point (i.e., triplicate).

5.3.4 These samples must include enough quantity/volume to result in acceptable counts to meet the statistical requirements in Section 6.

5.3.5 Turn off the IRAC, if it was ON.

5.3.6 Operate cleanup system as per lab SOP or established procedure to achieve needed background levels. If personnel are entering the chamber, appropriate PPE shall be worn.

5.3.7 Repeat the procedure from the beginning of the baseline section continuing into the test section for the remaining test(s).

5.3.8 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.

6 Calculations and Graphing Requirements

6.1 Variables

C = concentration

t = time

CADR = clean air delivery rate for microorganisms

V = volume of chamber

L_{ave}= average sound pressure

6.2 For bioaerosol samples, there are several metrics to calculate

6.2.1 For impactors, first perform the hole correction procedure (Macher 1989 includes this table). For any other samplers, perform any needed adjustments to the data. If samplers were over or underloaded, repeat the test.

6.2.2 Convert counts to concentrations. The exact equation will depend on your sampling device and your dilutions if used. The basic equation is C=Counts/Sample Volume.

6.2.3 Calculate the average and coefficient of variance (CV) for each time point. If the CV of any point exceeds 25%, repeat the test or do not use that time point's data.

6.2.4 Net % Removal/Inactivation – calculate *% Removal/Inactivation* over time using the time 0 average as the basis. % Removal/Inactivation is calculated as Red% = 100% * (1 – C_t/C_0) for each time point (t). Do this for both IRAC ON and NO DEVICE data. Calculate Net % Removal/Inactivation by subtracting the NO DEVICE (natural decay) *% Removal/Inactivation* from the IRAC On percent reduction. All times must be reported. Data must have been taken at a given time point for both IRAC ON and NO DEVICE to allow reporting at that time point.

6.2.5 Exclude data for time points that were above or below the appropriate concentration levels for your sample. Also, exclude data for time points that did not meet the CV requirement. Use all concentration data values for each of the remaining (at least five) time intervals. Take the natural logarithm of each remaining concentration value.

6.2.5.1 Calculate the slope of the linear fit to ln (concentration) vs time for both tests. This gives the decay rate for each test.

6.2.5.2 Determine the R-square for each fitted line. If the R=square is less than 0.85, either redo the test or consider removing higher CV time periods and checking the R-squared. You must still have five remaining good time period sample sets to consider the test valid.

6.2.5.3 CADR calculation:

$CADR = (\text{decay rate}_{\text{NO DEVICE}} - \text{decay rate}_{\text{IRAC ON}}) * V$

V = the volume of the chamber.

Confirm the calculated values are in compatible units (CFM or m^3/h)

6.2.5.4 ACH calculation (note that this method defines ACH as a cleaned air change not as simply air moved)

$ACH = CADR / V$

6.3 For other analytes, calculate the concentrations at the time intervals of each microbiological sample. It is acceptable to calculate all concentrations if desired. Subtract off the background concentration of the same analyte. Tabulate the results.

6.4 Average noise results either mathematically or L_{ave} from your direct read instrument. See Appendix C for additional information.

7 Reporting Results

7.1 Minimum required information

7.1.1 Name and location of the test laboratory

7.1.2 Date of the test

7.1.3 Laboratory test operators' names

7.1.4 In-room air cleaner (IRAC) manufacturer's name if known. The company submitting the IRAC for testing, how the IRAC was obtained, who requested the test

7.1.5 Description of the test IRAC to be tested, including the following:

7.1.5.1 Brand and model number (or description of the prototype)

7.1.5.2 Physical description of the IRAC will be provided by the manufacturer and verified by the laboratory, including a specification sheet that includes at least the dimensions and the components including technologies used for inactivation or removal (including technology-specific details such as peak UV wavelength). Other details of the IRAC should be included to describe it. These may include, if applicable, filters, catalyst type, heating coils, fans in cfm, type of air purifier, power supply, and so forth.

7.1.5.3 State whether or not IRAC components comply with the manufacturer's specifications.

7.1.5.4 If the IRAC has options, those used in the test must be specified. These may include output settings, fan speed, filters, or shielding.

7.1.5.5 Airflow rate, inactivation medium generation rate, as determined by the manufacturer.

7.1.5.6 Photos or drawings of the IRAC as positioned or located in the test rig example in the ceiling, on the floor, middle of the chamber, and against the wall.

7.1.5.7 Number of IRACs in the test chamber

7.1.5.8 Location of IRAC(s) in the test chamber

7.1.5.9 EPA establishment number and/or FDA registration number of the manufacturer or the device, if applicable

7.1.6 Operation information as stated by the manufacturer (such as recommended installation location). List procedures that were performed to run test that came from manual or follow the manufacturer's use guidelines.

7.1.7 Operating conditions for reporting purposes during the test: airflow rate of IRAC in m^3/s (cfm), the temperature in degrees Celsius (degrees Fahrenheit), relative humidity (RH), voltage input of air cleaner (V_{rms}), current draw (amps), static pressure relative to space external to the chamber.

7.1.8 Chamber/Equipment description (as required in Section 4)

- 7.1.8.1 Actual chamber dimensions, volume in m3 (ft3), type of test chamber BSL 1-3.
- 7.1.8.2 Mixing fans (airflow, make/model, rotation speed, location)
- 7.1.8.3 Chamber airflow
- 7.1.8.4 Temperature/RH control system
- 7.1.8.5 Air cleanup method
- 7.1.8.6 Nebulizers used, including estimated particle size distribution and location
- 7.1.8.7 Sampler/ing description and location
- 7.1.8.8 Bioassay/enumeration methods used
- 7.1.8.9 LOD/LOQ of test methods for each analyte.
- 7.1.8.10 Materials of construction of the interior walls, floor, and ceiling

7.1.9 Test data

- 7.1.9.1 Diagram of sampling points, IRAC locations, mixing fan location(s), and temperature/RH control device.
- 7.1.9.2 Test air temperature and RH (average and range)
- 7.1.9.3 For the baseline, natural decay test (No-IRAC) and IRAC test
  - 7.1.9.3.1 Organisms and suspension used
  - 7.1.9.3.2 Summary of generation and collection methods
  - 7.1.9.3.3 Summary of laboratory and/or sample analytical methods (specific to the organisms used)
  - 7.1.9.3.4 Length (time) of each test run
  - 7.1.9.3.5 Organism test data expressed in CFU/m^3 or PFU/m^3 (CFU/ft^3 or PFU/ft^3), both tables and graphs, with respect to time
  - 7.1.9.3.6 Show calculations for % removal/inactivation.
  - 7.1.9.3.7 Tabulate Net % Removal/Inactivation for each organism tested at all acceptable time points. Also, tabulate % Removal/Inactivation for both IRAC OFF and IRAC ON tests.
  - 7.1.9.3.8 Clean air delivery rate (CADR) for all four organisms
  - 7.1.9.3.9 All analyte testing results in appropriate Imperial & SI units, including tabulated concentrations. Justification is required if any test is not conducted.
- 7.1.9.4 IRAC

7.1.9.4.1 Input volts, input amperage, and nominal wattage

7.1.9.4.2 Airflow rate and air changes per hour (ACH)

7.1.10 Required disclaimer to be on the cover or page 1 of the report: The data presented in this report represent conditions existing in this test chamber on the day of this study. Caution must be exercised in drawing conclusions from the data contained in this report as to the efficacy of the IRAC at other times or under different circumstances. Reports are valid for five years. 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 an example.

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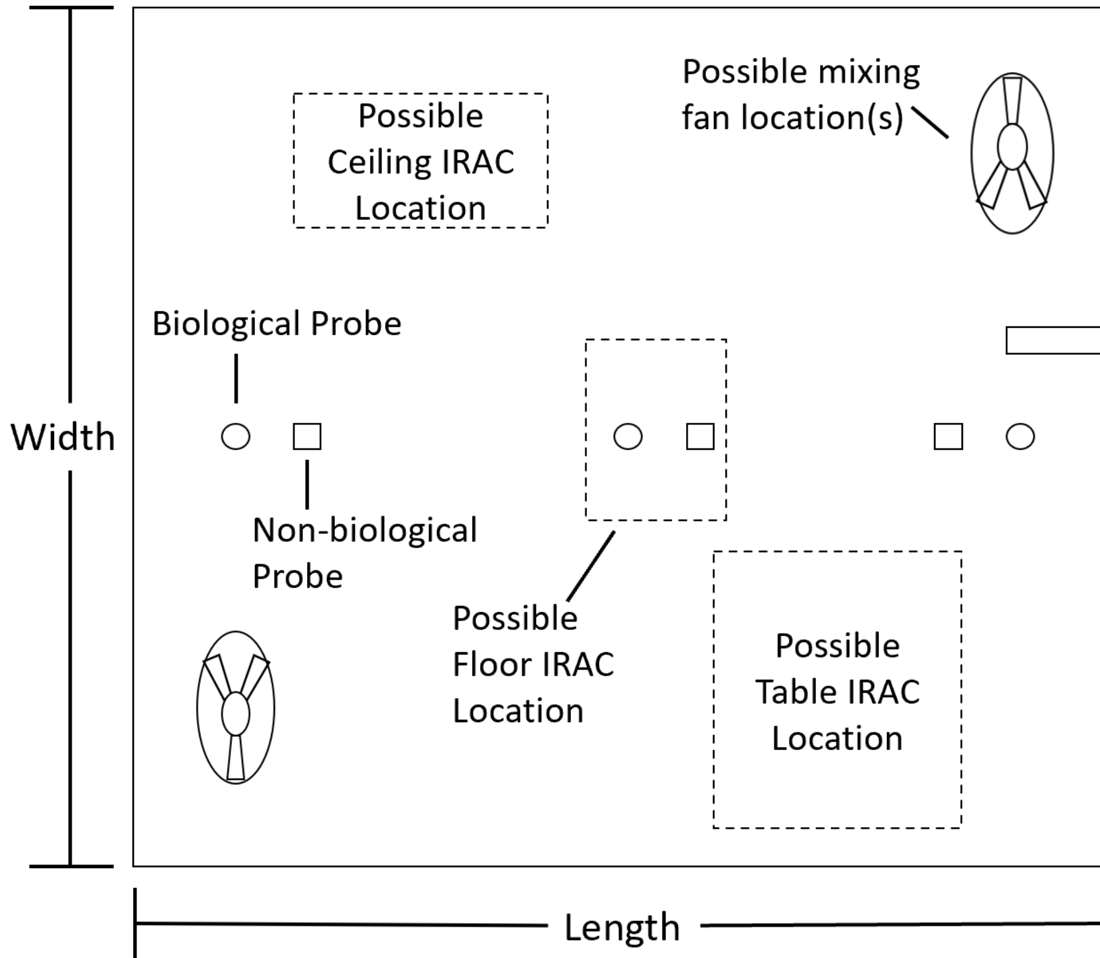
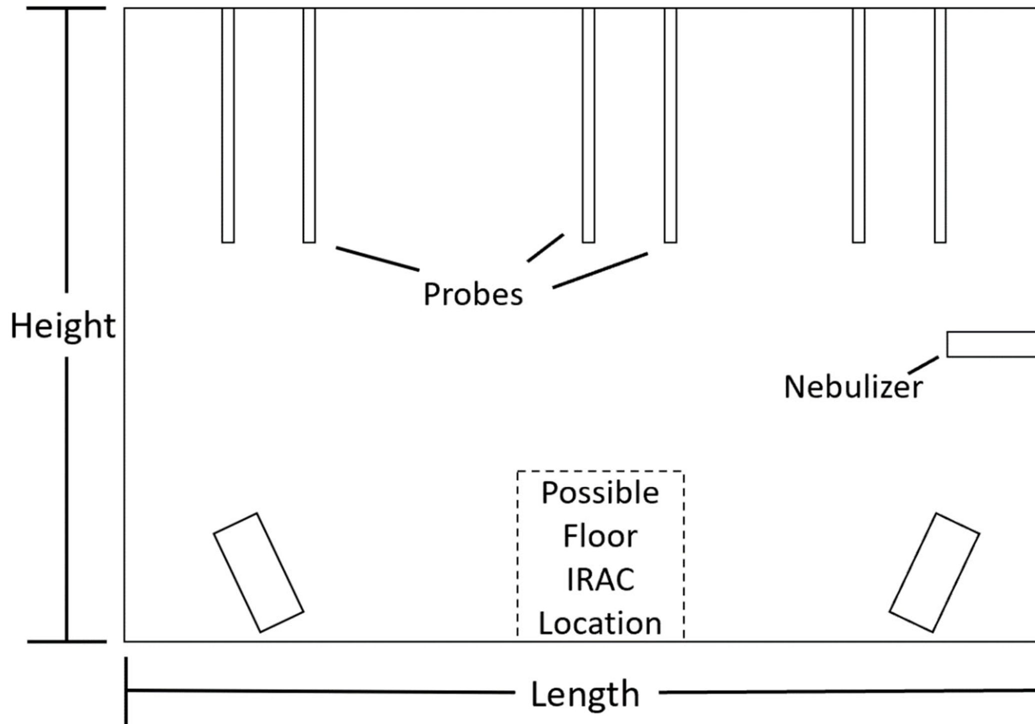
Figure: A




Front View
Probes
Height
Nebulizer
Possible
Floor
IRAC
Location
Length

Normative Appendix A: **Test Organisms (Select one from each organism type)**

This test method requires one organism from each category to be tested and reported as a single ASHRAE 185.3 test. This is required because different types of organisms have different levels of resistance to different killing mechanisms. Four organisms of different types should give a realistic expectation of how a device works and allow reasonable comparisons across devices. If organisms other than those listed in the table below are used, it is preferred that the lab complete the 185.3 test plus the test for any additional organisms. Should other organisms be tested with the same procedures, the test report shall be labeled "modified" and the changes indicated clearly on the cover, summary results, and first page if there is no cover.

Acceptable Test Organisms for a 185.3 Test

	Organism(s)	**ATCC Cat. #**	**Biosafety Level**	**Comment(s)**
Gram positive Bacteria	*Staphylococcus aureus*	ATCC 6538	2	Organisms found on BEI, but not exact strain
	Staphylococcus epidermidis	ATCC 12228	1	
	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 not exact strain
	Klebsiella pneumoniae	ATCC 4352	2	
	Pseudomonas aeruginosa	ATCC 9027	2	
	Serratia marcescens	N/A		Only available on ATCC
Bacteriophages	Phi X174	ATCC 13706-B1	1	Only available on ATCC
	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	Only available on ATCC
	Penicillium rubens	ATCC 11709	1	
	Stachybotrys chartarum	N/A	1	
	Aspergillus brasiliensis	ATCC 16404	1	

We believe all pathogens specified in the standard should require a ATCC or BEI number and ideally Certificate of Authenticity (COA).

The rationale for selecting different organisms is supported by the two tables below showing relative resistance for organisms from Favero and Bond, 2001 and, below that, from McDonnell and Russell, 1999.

Enforcement Policy for Sterilizers, Disinfectant Devices, and Air Purifiers During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency Guidance for Industry and Food and Drug Administration Staff March 2020

- **Most Resistant**

- **Least Resistant**

- **Bacterial Spores**
- **Mycobacteria**
- **Nonlipid or Small Viruses**
- **Fungi**
- **Vegetative Bacteria**
- **Lipid or Medium-Size Viruses**

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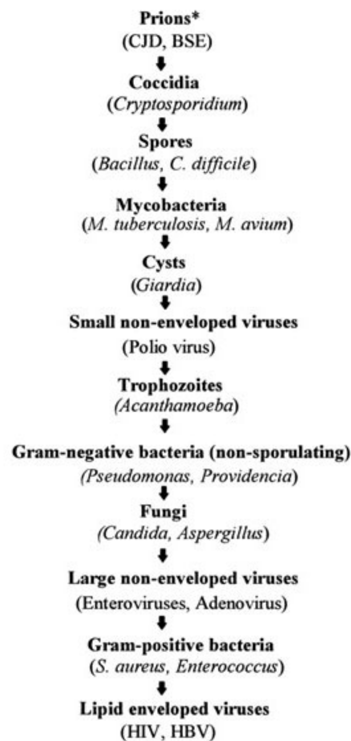
Descending order of resistance to antiseptics and disinfectants.



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Normative Appendix B: QA/QC

All instruments shall have Annual or biannual calibration using a NIST-traceable methodology. The specifics should be done per the IAW manufacturer's instructions or per the Lab SOP when the manufacturer does not have specifications.

The following tests shall be performed as indicated in Table B-1.

1. Leak test –
    a. There are several ways to do this.
    b. One simple way to conduct this test is to:
        i. Chose 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).
        ii. Close up the chamber as you would during the test.
        iii. Run the cleanup system as you would before a test.
        iv. Measure background levels in the space surrounding the chamber.
        v. Measure chamber backgrounds.
        vi. Generate contaminant (even a small spritz of liquid VOC can suffice).
        vii. Run mixing fans as you would during the test.
        viii. Wait until the value stabilizes (is not fluctuating indicating lack of mixing or contaminant still evaporating). If too high for your normal test needs, you can run a cleanup system to reduce the level. Then make sure the value stabilized.
        ix. Monitor for an hour.
        x. Calculate k value or equivalent clean air going in.
        xi. If high, re-gasket, seal openings, make sure the cleanup system is sealed off, etc.
        xii. Try again.
        xiii. Learn what level is usual for your chamber for ease in noticing that a leak has occurred.
        xiv. If an air mixing test is done after this one and results in changes to the chamber, repeat this test.
2. Air mixing
    a. Initially mix air in the room to ensure generated bioaerosols are uniformly distributed before natural decay test according to ASTM well-mixed room definition. The duration of initial mixing after bioaerosol generation is stopped shall be five minutes after generation is stopped. Some laboratories may require more than five minutes to reach well-mixed conditions, and they should follow their validated SOP to ensure uniform bioaerosol distribution. Whatever procedure is done in 5.2.5 shall be performed in 5.3.3.
    b. A different air-mixing protocol may be necessary for the actual test to ensure that the airflow patterns/currents do not affect the air sampling results or the performance of the IRAC. Continue to mix air in room to ensure generated bioaerosols remain uniformly distributed during the natural decay test. The mixing criteria from 4.1.9 (initial and decay test) may be different or the same.

3. Temp/RH maintenance

a. Set up chamber as you would for a no device test, run fans as usual, monitor T/RH for maximum length of expected tests. Determine if T/RH go out of spec. Tabulate the time, T and RH for your QA records.

4. Baseline microbes check

Table B-1 Required QA/QC Tests			
Test	Requirement	Every 2 yr*	
Chamber Leak Test	Kvalue <0.04	X	
Air Mixing Test	CV<15%	X	
T/RH Test	Within limits	X	
*or after any substantial change			

Informative Appendix C: IRAC Safety and Health Considerations

C.1 Occupied vs non occupied spaces

The health and safety considerations for IRAC devices intended for non-occupied spaces varies considerably from those intended for occupied spaces, especially if there are provisions for a ventilation period following operation and prior to potential occupant exposure. For instance, hydrogen peroxide generating devices are not meant to operate during occupancy and typically require an engineered ventilation period after each operational cycle because of the documented potential health effects of hydrogen peroxide. For IRAC devices intended for operation in occupied spaces, it is important to perform preliminary or triage quantitative evaluations to ensure hazardous analytes are either below recognized health hazard exposure limits or have engineering controls implemented to prevent occupant exposure above health hazard exposure limits.

C.2 All hazards exposure risk assessment (ozone, UV, etc.)

The potentially anticipated analytes listed in C.3 have some history of risk assessment and exposure level applied to them to offer guidance in at least triaging IRACs for this test's purposes. Evaluation of these analytes is not meant for in-depth analysis but for early detection of potentially hazardous analytes that occupants may be exposed to upon operation of the IRAC device. For any additional analytes that do not have any regulatory or guideline exposure limits, risk assessment is typically carried out in two stages, including qualitative and quantitative analyses according to the NIOSH Decision Logic at https://www.cdc.gov/niosh/docs/87-108/default.html, and it is recommended that guidance from a Certified Industrial Hygienist or Occupational Toxicologist is sought out.

C.3 Potential Hazardous Analytes (This is not an exhaustive list.)

Table C1.

Analytes of Concern	Abbreviation	Considerations	Additional References
Ozone	O_3	Potential product of some technologies	CARB Guidelines at https://ww2.arb.ca.gov/resources/ozone-and-health WHO WHO global air quality guidelines: particulate matter (PM2.5 and PM10), ozone, nitrogen dioxide, sulfur dioxide and carbon monoxide EPA Risk Assessment and Modeling - Ozone Risk Analyses | US EPA NIOSH CDC - NIOSH Pocket Guide to Chemical Hazards - Ozone

Negative and Positive Ions	(-) and (+)	Products of ionization technologies	None for regulatory limits or recommendations
Volatile Organic Compounds	VOC	Product of room/chamber materials. Any increase above background should be investigated	EPA Guidelines at https://www.epa.gov/indoor-air-quality-iaq/volatile-organic-compounds-impact-indoor-air-quality
Peroxide	H_2O_2	Potential product of some technologies	OSHA limits at https://www.osha.gov/chemicaldata/630
Particulate Matter	PM	Measure PM 1.0, 2.5 and 10.0	The OSHA limits are very high so a baseline-relative measure may be the best approach. https://www.cdc.gov/niosh/pel88/dusts.html
Carbon Dioxide	CO_2	Product of occupants	OSHA and other limits at https://www.osha.gov/chemicaldata/183
Ultraviolet Radiation	UV-C or UVGI	Direct and reflective measurements are needed	The latest ACGIH guideline is at https://www.acgih.org/ultraviolet-radiation/
Formaldehyde	CH_2O	Off-gassing	https://www.osha.gov/chemicaldata/377 https://www.ul.com/services/carb-testing-formaldehyde-emissions-composite-wood-products

C.4 Noise Exposure

The three primary methods for evaluating room noise are:

1. The survey method that employs the A-weighted sound level;
2. The engineering method that employs noise criteria (NC) curves; and
3. The method for evaluating low-frequency fluctuating noise using room noise criteria (RNC) curves.

A helpful reference for evaluating noise exposure in rooms is ANSI/ASA S12.2-2019 Criteria for Evaluating Room Noise.

NIOSH Sound level meter App: https://www.cdc.gov/niosh/topics/noise/app.html might be a helpful screening tool for use in evaluating room noise.

Normative Appendix D: Mounting and installation of IRAC

D.1 Be capable of mounting and installing the most common test IRACs, wall-mounted IRACs, tabletop IRACs, ceiling IRACs, and floor IRACs. This includes air-tight ports for power lines and remote controls. IRACs shall be installed according to the manufacturers recommendation or to approximate the intended application mounting. The following installation locations are recommended, but not required:

D.1.1 IRACs that rest on the floor: Located on the IRAC side at the centerline in the lengthwise direction, and ¼ of the width.

D.1.2 IRACs that are suspended from ceilings: The IRAC shall be suspended to a height indicated by the manufacturer or 12 inches below the ceiling if the recommended height is greater than the chamber height. For one IRAC, the IRAC shall be located on the IRAC side, on the centerline in the lengthwise direction, and ¼ of the width. For multiple IRACs, the spacing shall be defined by the manufacturer. The IRAC may be suspended from the chamber by cables, or it may rest on a ladder, scaffolds, etc., so long as the airflow pattern of the IRAC is not modified by the suspension system.

D.1.3 IRACs that are mounted to the surface of ceilings: The IRACs shall be mounted to a solid surface with a minimum size of 1.2 m by 1.2 m (4 ft by 4 ft), constructed of a suitable material to not impact the testing. The solid surface shall be oriented horizontally and located at a height indicated by the manufacturer or in close proximity to the ceiling of the chamber if the recommended height is greater than the chamber height. For one IRAC, the IRAC shall be located on the IRAC side at the centerline in the lengthwise direction, and ¼ of the width. For multiple IRACs, the spacing shall be defined by the manufacturer. The IRAC/mounting surface may be suspended from the chamber by cables, or it may rest on a ladder, scaffolds, etc., so long as the airflow pattern of the IRAC is not modified by the suspension system.

D.1.4 IRACs that are recessed into ceilings: The IRAC shall be mounted within a sealed box per manufacturer guidance to prevent airflow between the chamber and the interior of the box while satisfying any ambient requirements of the IRAC. The lower horizontal surface shall be a minimum of 1.2 m by 1.2 m (4 ft by 4 ft) (representing a ceiling), constructed of a suitable material to not impact the testing. The lower horizontal surface shall be located at a height indicated by the manufacturer or in close proximity to the ceiling of the chamber if the recommended height is greater than the chamber height. For one IRAC, the IRAC shall be located on the IRAC side at the centerline in the lengthwise direction, and ¼ of the width. For multiple IRACs, the spacing shall be defined by the manufacturer. The IRAC/mounting box may be suspended from the chamber by cables, or it may rest on a ladder, scaffolds, etc., so long as the airflow pattern of the IRAC is not modified by the suspension system.