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Standard Practice for Evaluation of Effectiveness of Decontamination Procedures for Air-Permeable Materials when Challenged with Biological Aerosols Containing Human Pathogenic Viruses[1]

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INTRODUCTION

Many communicable diseases are often spread through the aerosol route of exposure. The droplet nuclei formed in these aerosols may infect susceptible individuals directly or contaminate environmental surfaces and render them fomites for further spread of disease. The characteristics of the droplet nuclei (particle size and composition) will influence the viability of microorganisms when exposed to environmental stresses but may also shield them from physical and chemical decontaminants. The wide variations in the types and levels of such protective/shielding ingredients can have impact on the effectiveness of surface decontaminants. This practice is designed to simulate the deposition of droplet nuclei from human respiratory secretions onto and into air-permeable membranes. It is primarily focused on influenza viruses but other respiratory viruses or surrogate viruses could be used. Protocols for assessing the microbicidal activity of disinfectants are also described.

1. Scope

1.1 This practice is designed to evaluate decontamination methods (physical, chemical, self-decontaminating materials) when used on air-permeable materials contaminated with virus-containing droplet nuclei.

1.2 This practice defines the conditions for simulating respiratory droplet nuclei produced by humans.

1.3 The practice is specific to influenza viruses, but could be adapted for work with other types of respiratory viruses or surrogates.

1.4 This practice is suitable only for air-permeable materials.

1.5 This practice does not address the performance of decontaminants against microbes expelled via blood splatter, vomit, or fecal contamination.

1.6 This practice should be performed only by those trained in bioaerosols, microbiology, or virology, or combinations thereof.

1.7 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.8 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E1052 Test Method to Assess the Activity of Microbicides against Viruses in Suspension

E2197 Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals

[1] This practice is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

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2.2 *IEST Standards:*

IEST-RP-CC003.3 Garment System Considerations for Clean Rooms and Other Controlled Environments[3]

2.3 *Department of Defense Standards:*

CA06PRO411 Method for Evaluating Air Purification Technologies for Collective Protections Using Viable Microbial Aerosols[4]

2.4 *EPA Standards:*

EPA 600/4-84/013 (N16) USEPA Manual of Methods for Virology[5]

2.5 *WHO Standards:*

WHO Manual on Animal Influenza Diagnosis and Surveillance[6]

3. Terminology

3.1 *Definitions:*

3.1.1 *aerosol, n*—a suspension of solid or liquid particles in a gas medium.

3.1.2 *air-permeable material, n*—no standard definition is available; for the purpose of this practice, air-permeable material is described as any membrane that has a pressure drop ≤ twice that of high efficiency particulate air (HEPA) media in the same test environment.

3.1.3 *biological aerosol, n*—aerosol comprising particles of biological origin or activity which may affect living things through infectivity, allergenicity, toxicity, or pharmacological and other processes.

3.1.4 *influenza, n*—an infectious disease of birds and mammals caused by RNA viruses of the family *Orthomyxoviridae*.

3.1.5 *protective factor, n*—soluble or insoluble material co-deposited with microorganisms that directly protects the microorganism from environmental stresses or decontaminants.

3.1.6 *respiratory droplet nuclei, n*—evaporatively condensed, pathogen-containing particles of respiratory secretions expelled into the air by coughing, sneezing, or talking, which can remain airborne for long periods of time.

3.1.7 *self-sanitizing material, n*—a substrate containing an antimicrobial agent that collectively acts as a germicide.

4. Summary of Practice

4.1 The practice describes the steps required to deposit droplet nuclei onto air-permeable membranes and quantitatively assess decontamination efficiency.

4.1.1 Using an aerosol device capable of meeting the data quality objectives set for in this practice, influenza virus or surrogates are aerosolized to form droplet nuclei that are subsequently applied to air-permeable materials.

4.1.2 The virus-contaminated carriers are subjected to disinfection protocols and incubated for the specified time and conditions. Control samples are incubated under identical conditions, but are not exposed to the disinfection protocols.

NOTE 1—Carriers with incorporated microbicides do not receive any additional disinfection treatment. An untreated control is needed to assess antimicrobial efficacy.

4.1.3 Virus particles are eluted from the test and control carriers and viability is assessed by 50 % tissue culture infectious dose assay ($\log_{10}TCID_{50}$).

NOTE 2—Non-viable quantification techniques for viral enumeration such as polymerase chain reaction (PCR) or hemagglutination cannot be used.

4.1.4 The virucidal activity of the decontamination procedure is determined from the log difference in viability between treated and control carriers.

5. Significance and Use

5.1 The efficacy of disinfection technologies can be evaluated on finished products, as well as on developmental items.

5.2 This practice defines procedures for validation of the aerosol generator, preparation of the test specimen, application of the challenge virus, enumeration of viable viruses, assessing data quality, and calculation of decontamination efficacy.

5.3 This practice provides defined procedures for creating droplet nuclei that approximate those produced by human respiratory secretions with particular emphasis on particle size distribution and aerosolization media.

5.4 Safety concerns associated with aerosolizing microbial agents are not addressed as part of this practice. Individual users should consult with their local safety authority, and a detailed biological aerosol safety plan and risk assessment should be conducted prior to using this practice. Users are encouraged to consult the manual *Biosafety in Microbiological and Biomedical Laboratories*[7] published by the U.S. Centers for Disease Control and Prevention (CDC).

5.5 This practice differs from Test Methods E1052 and E2197 in the presentation of the virus to surface. The aforementioned test methods use liquid inoculum to contaminate carrier surfaces, whereas this practice presents the virus in the absence of water as droplet nuclei.

5.6 This practice differs from Test Method E2721 because (*1*) smaller particles are being formed, (*2*) the droplets will be dried, thus forming droplet nuclei, prior to application to

[3] Available from Institute of Environmental Sciences and Technology (IEST), Arlington Place One, 2340 S. Arlington Heights Rd., Suite 100, Arlington Heights, IL 60005-4516, http://www.iest.org.

[4] Foarde, K., Heimbuch, B. K., Maxwell, A., VanOsdell, D., "Method for Evaluating Air Purification Technologies for Collective Protection Using Viable Microbial Aerosols," Test Operating Procedure (TOP) Under the Army Test and Evaluation Command (ATEC), Edgewood Chemical and Biological Center, Edgewood, Md., 2010 in press.

[5] Available from United States Environmental Protection Agency (EPA), Ariel Rios Bldg., 1200 Pennsylvania Ave., NW, Washington, DC 20460, http://www.epa.gov.

[6] Webster, R., Cox, N., Stohr, K. WHO Manual on Animal Influenza Diagnosis and Surveillance. World Health Organization, Department of Communicable Disease Surveillance and Response. WHO/CDS/CDR/2002.5 Rev. 1.

[7] CDC-NIH, *Biosafety in Microbiological and Biomedical Laboratories*, 5th Edition, U.S. Department of Health and Human Services, Washington, D.C., 2009.

air-permeable materials, and (*3*) unique equipment is required to create the droplet nuclei.

6. Apparatus

6.1 *Biological Aerosol Generators*—The apparatus to load microorganisms onto a substrate is composed of several commercially available components and can be readily constructed (see IEST-RP-CC003.3).[4,8,9] The overall design of the apparatus can take various forms and can be fashioned in different dimensions while meeting the validation requirements and data quality objectives listed below. Appendix X1 and Appendix X2 contain the description of a prototypical device that can be used to load droplet nuclei onto surfaces. However, it is the responsibility of the user of this standard to validate the performance of the device prior to use.

6.1.1 Validation requirements and baseline testing.

6.1.1.1 *Environmental Conditions*—Generator must be capable of delivering air with a relative humidity of 70 ± 10 %.

6.1.1.2 *Leak Test*—The device must maintain a positive pressure of ~ 50 cm of water for at least 10 min.

6.1.1.3 *Flow Rate Consistency*—All ports containing specimen holders must maintain a constant flow with a coefficient of variation (CV) ≤ 10 % over the duration of the sampling period.

6.1.1.4 Loading uniformity across the diameter of the test specimen is required to ensure the even distribution of the droplet nuclei over the surface of the carrier. A standard deviation of ± 0.5 $\log_{10}TCID_{50}$ is desired.

6.1.1.5 *Sample-to-Sample Variation*—The variability of virus loading for multiple samples loaded for a single test must have a standard deviation of ± 0.5 $\log_{10}TCID_{50}$.

6.1.1.6 *Droplet Nuclei Characteristics*—The droplet nuclei generated for this practice will have a count median diameter (CMD) of ~0.8 µm. The virus will be aerosolized in a saliva substitute (Table 1) that will add the appropriate "protective factors." This practice would be suitable for simulating other fluids of interest; however, if a different fluid is used, the formulation and recipe listing the protective factors and particle size must be reported.

TABLE 1 Artificial Saliva[8]

Reagent	Amount
$MgCl_2 \cdot 7\ H_2O$	0.04 g
$CaCl_2 \cdot H_2O$	0.13 g
$NaHCO_3$	0.42 g
0.2 M KH_2PO_4	7.70 mL
0.2 M K_2HPO_4	12.3 mL
NH_4Cl	0.11 g
KSCN	0.19 g
$(NH_2)_2CO$	0.12 g
NaCl	0.88 g
KCl	1.04 g
Mucin	3.00 g
Distilled water	1000 mL
*p*H	7

6.2 *Other Equipment*—The list is specific for influenza virus. Other equipment may be needed if a different virus is used.

6.2.1 *Autoclave,* capable of maintaining 121 to 123°C and [15 to 17 lbs per in.^{2}–gauge (psig)].

6.2.2 *CO_2 Incubator,* capable of maintaining 35 to 37°C and 5 ± 0.5 % CO_2.

6.2.3 *Vortex Mixer.*

6.2.4 *Analytical Balance,* capable of weighing 0.001 g.

6.2.5 *Refrigerator,* capable of maintaining 2 to 8°C.

6.2.6 *Stopwatch or Electronic Timer.*

6.2.7 *Pipettor,* with a precision of 0.001 mL.

7. Reagents and Materials

7.1 *Reagents*—The list is specific for influenza use. Other reagents may be needed if a different virus is used.

7.1.1 *Influenza virus (H1N1; A/PR/8/34)—cell culture adapted*, ATCC VR-1469.

7.1.1.1 The WHO Manual on Animal Influenza Diagnosis and Surveillance contains specific procedures for preparing influenza virus and titering samples. Other viruses may be used, but conditions for propagation and enumeration are not provided.

7.1.2 *Madin–Darby Canine Kidney (MDCK) Cell Line,* ATCC CCL-34.

7.1.3 *Artificial Saliva,* see Table 1.

7.1.4 *Eagle's Minimum Essential Medium With Earle's Balanced Salts (EMEM).*

7.1.5 *Heat-Inactivated Fetal Bovine Serum (45 min at 56°C).*

7.1.6 *Penicillin/Streptomycin,* 10 000 units penicillin and 10 mg streptomycin per mL.

7.1.7 *L-Glutamine,* 200 m*M* in 0.85 % NaCl.

7.1.8 *Crystal Violet.*

7.1.9 *Glutaraldehyde.*

7.1.10 *TPCK–Trypsin.*

7.1.11 *Phosphate Buffered Saline (PBS).*

7.1.12 *Bovine Serum Albumin.*

7.1.13 *Trypsin–EDTA Solution,* 0.05 % trypsin, 0.53 m*M* EDTA in Hank's balanced salts solution without sodium bicarbonate, calcium, and magnesium.

7.1.14 *Distilled Water and Purified Water.*

7.1.15 *Ethanol,* laboratory grade.

7.1.16 *Household Bleach.*

7.2 *Materials*—The list is specific for influenza use. Other reagents may be needed if a different virus is used.

7.2.1 *Tissue Culture Treated Flasks*—T-25, T-75, T-175, 24-well plate.

7.2.2 *Pipettes,* 1, 5, 10, and 25 mL.

7.2.3 *Test Tube Rack.*

7.2.4 *Micropipettes,* capable of delivering 0.001 mL accurately and consistently.

7.2.5 *1.7-mL Sterile Microcentrifuge Tubes.*

7.2.6 *15-mL Sterile Centrifuge Tubes.*

[8] Heimbuch B. K., Wallace, W. H., Kinney, K., Lumley, A. E., Wu, C-Y, Woo, M-H, Wander, J. D., "A Pandemic Influenza Preparedness Study: Use of Energetic Methods to Decontaminate Filtering Facepiece Respirators Contaminated with H1N1 Aerosols and Droplets," *American Journal of Infection Control*, 2010, DOI 10.1016/j.ajic.2010.07.004.

[9] Fisher E, Rengasamy S, Viscusi DJ, Vo E, Shaffer R., Development of a test system to apply virus-containing particles to filtering facepiece respirators for the evaluation of decontamination procedures, *Appl Environ Microbiol*, Vol 75, No. 6, 2009, pp. 1500–1507.