Designation: E2721 – 10

StandardTest Method for Evaluation of Effectiveness of Decontamination Procedures for Surfaces When Challenged with Droplets Containing Human Pathogenic Viruses[1]

This standard is issued under the fixed designation E2721; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

INTRODUCTION

Many communicable diseases can often spread through droplets containing infectious agents. Such "contagious droplets" may expose susceptible individuals directly or contaminate environmental surfaces in the immediate vicinity and render them as fomites for further spread of the disease. The characteristics of the droplets (particle size and composition) will influence the viability of the microorganisms when exposed to environmental stresses but also shield them from physical and chemical decontaminants. The wide variations in the types and levels of such protective/shielding ingredients can impact on the effectiveness of surface decontaminants. This test method is designed to simulate surface deposition of contagious droplets from human respiratory secretions. It is primarily focused on influenza viruses but other respiratory viruses or surrogates could be used. Protocols for assessing the microbicidal activity of disinfectants are also described.

1. Scope

1.1 This test method is designed to evaluate decontamination methods (physical, chemical, self-decontaminating materials) when used on surfaces contaminated with virus-containing droplets.

1.2 This test method defines the conditions for simulating respiratory droplets produced by humans and depositing the droplets onto surfaces.

1.3 The method is specific to influenza viruses but could be adapted for work with other types of respiratory viruses or surrogates (Appendix X5).

1.4 This test method is suitable for working with a wide variety of environmental surfaces.

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

1.6 This test method 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

E2720 Test Method for Evaluation of Effectiveness of Decontamination Procedures for Air-Permeable Materials when Challenged with Biological Aerosols Containing Human Pathogenic Viruses

[1] This test method 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.

2.2 *EPA Standards:*

EPA 600/4-84/013 (N16) USEPA Manual of Methods for Virology[3]

2.3 *WHO Standards:*

WHO Manual on Animal Influenza Diagnosis and Surveillance[4]

3. Terminology

3.1 *Definitions:*

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

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

3.1.3 *contact transmission, n*—infections caused by direct skin-to-skin contact or indirect contact with objects contaminated with pathogens.

3.1.4 *contagious respiratory droplet, n*—respiratory secretions containing infectious microorganisms that form large droplets (≥5 µm) and settle out of the air over short distances.

3.1.5 *droplet transmission, n*—direct transfer of pathogen-containing droplets to conjuncitval or mucous membranes.

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

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

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

4. Summary of Test Method

4.1 The test method describes the steps required to deposit droplets onto surfaces and quantitatively assess decontamination efficiency.

4.1.1 Using an aerosol device capable of meeting the data quality objectives set for in this test method, influenza virus or surrogates are aerosolized to form droplets that are subsequently applied to surfaces.

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—Nonviable 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 test 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 test method defines procedures for validation of the droplet generator, preparation of the test specimen, application of the challenge virus, enumeration of viable viruses, assessing data quality, and calculation of decontamination efficiency.

5.3 This test method provides defined procedures for creating droplets that approximate those produced by human respiratory secretions, with particular emphasis on droplet size distribution and aerosolization media.

5.4 Safety concerns associated with aerosolizing microbial agents are not addressed as part of this test method. 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 method. Users are encouraged to consult the manual *Biosafety in Microbiological and Biomedical Laboratories*[5] published by the U.S. Centers for Disease Control and Prevention (CDC).

5.5 This test method differs from Test Methods E1052 and E2197 in the presentation of virus to the surface. The aforementioned test methods use liquid inoculum to contaminate carrier surfaces, whereas this method presents the virus in droplets that are representative of human respiratory secretions

5.6 This method differs from Test Method E2720, because (*1*) larger droplets are being formed, (*2*) the droplets will not be completely dried prior to application to surfaces, (*3*) the droplets can be applied to any surfaces, not just those that are air permeable, and (*4*) unique equipment is required to create droplets.

6. Apparatus

6.1 *Droplet Apparatus*—The apparatus used to load microorganisms onto a substrate is composed of several commercially available components and can be readily constructed.[6,7,8] 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 contains the description of a prototypical

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

[4] 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.

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

[6] Vo, E., Rengasamy, S., Shaffer, R., "Development of a Test System to Evaluate Decontamination Procedures for Viral Droplets on Respirators." *Applied and Environmental Microbiology*, Vol 75, No. 23, 2009, pp. 7303–7309.

[7] Woo, M. H., Hsu, Y. M., Wu, C. Y., Heimbuch, B. K., Wander, J. D., "A Device for a Consistent and Controlled Delivery of Aerosolized Droplets Containing Viral Agents Onto Surfaces." *Journal of Aerosol Science*, Vol 41, 2010, pp. 941-952.

[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.