Designation: E3179 – 18

Standard Test Method for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation against Influenza Virus on Fabric Carriers with Simulated Soil[1]

This standard is issued under the fixed designation E3179; 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.

1. Scope

1.1 This test method defines test conditions to evaluate ultraviolet germicidal irradiation (UVGI) light devices (mercury vapor bulbs, light-emitting diodes, or xenon arc lamps) that are designed to kill/inactivate influenza virus deposited on inanimate carriers.

1.2 This test method defines the terminology and methodology associated with the ultraviolet (UV) spectrum and evaluating UVGI dose.

1.3 This test method defines the testing considerations that can reduce UVGI surface kill effectiveness (that is, soiling).

1.4 Protocols for adjusting the UVGI dose to impact the reductions in levels of viable influenza virus are provided (Annex A1).

1.5 This test method does not address shadowing.

1.6 The test method should only be used by those trained in microbiology and in accordance with the guidance provided by Biosafety in Microbiological and Biomedical Laboratories.[2]

1.7 This test method is specific to influenza viruses

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

1.9 **Warning—**Mercury has been designated by many regulatory agencies as a hazardous substance that can cause serious medical issues. Mercury, or its vapor, has been demonstrated to be hazardous to health and corrosive to materials. Use caution when handling mercury and mercury-containing products. See the applicable product Safety Data Sheet (SDS) for additional information. The potential exists that selling mercury or mercury-containing products, or both, is prohibited by local or national law. Users must determine legality of sales in their location.

1.10 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.11 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1053 Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces

E1316 Terminology for Nondestructive Examinations

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

E2721 Practice for Evaluation of Effectiveness of Decontamination Procedures for Surfaces When Challenged with Droplets Containing Human Pathogenic Viruses

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

E3135 Practice for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil

G130 Test Method for Calibration of Narrow- and Broad-Band Ultraviolet Radiometers Using a Spectroradiometer

[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] *Biosafety in Microbiological and Biomedical Laboratories* (5th edition), 2009, HHS Publication No. (CDC) 21-1112.

[3] 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 *ISO Standards:*[4]

ISO 9370 – Plastics -- Instrumental Determination of Radiant Exposure in Weathering Tests -- General Guidance and Basic Test Method. 21348 – Definitions of Solar Irradiance Spectral Categories

ISO 21348 –Space environment (natural and artificial) — Process for determining solar irradiances

3. Terminology

3.1 For Definitions of Terms used in this Method, refer to Terminologies in E3135, E1316, E2756, and ISO 21348:

3.2 *Definitions:*

3.2.1 *carrier, n*—a surrogate surface or matrix that facilitates the interaction of test microorganisms and treatment(s).

3.2.2 *irradiance (E), n*—a radiometric term for the radiant flux that is incident upon a surface ($W \cdot m^{-2}$).

3.2.3 *joule (J), n*—a unit of work or energy in the SI system of units.

3.2.3.1 *Discussion*—One Joule is one watt-second.

3.2.4 *light-emitting diode (LED), n*—a solid-state electronic device or transistor which emits light.

3.2.4.1 *Discussion*—An LED is a p-n junction diode, which emits light when activated. When a suitable voltage is applied to the leads, electrons are able to recombine with electron holes within the device, releasing energy in the form of photons.

3.2.5 *mercury vapor lamp, n*—a gas discharge lamp that uses electric arc through vaporized mercury to produce light.

3.2.6 *radiometer, n*—a device for measuring the radiant power that has an output proportional to the intensity of the input power.

3.2.7 *shadowing, v*—creating a dark area or shape by blocking light rays.

3.2.8 *soiling agent, n*—substance applied either along with or on top of the test microorganism that can reduce the effectiveness of the antimicrobial technology.

3.2.9 *ultraviolet germicidal irradiation (UVGI), n*—a method that uses short-wavelength ultraviolet (UV-C) light to kill or inactivate microorganisms primarily by forming pyrimidine dimers, leaving them unable to perform vital cellular functions.

3.2.10 *ultraviolet (UV) light, n*—radiation having wavelengths shorter than wavelengths of visible light (~400 nm) and longer than those of X-rays (~100 nm).

3.2.11 *UV-A, n*—radiation within the ultraviolet spectrum that extends from approximately 315 to 400 nm in wavelength.

3.2.12 *UV-B, n*—radiation within the ultraviolet spectrum that extends from approximately 280 to 315 nm in wavelength.

3.2.13 *UV-C, n*—radiation within the ultraviolet spectrum which extends from approximately 100 to 280 nm in wavelength.

3.2.14 *xenon arc lamp, n*—a specialized type of gas discharge lamp, an electric light that produces light by passing electricity through ionized xenon gas at high pressure.

4. Summary of Test Method

4.1 This test method describes the steps required to deposit influenza virus onto fabric carriers.

4.2 This test method defines the process for adding soiling agents on top of influenza virus, which can reduce the effectiveness of UV antimicrobial activity.

4.3 This test method defines a protocol for quantifying the dose a UVGI device delivers to a surface.

4.4 This test method defines the process for exposure of influenza virus to UVGI.

4.5 This test method defines protocols for extraction of viable influenza virus from carriers followed by viable enumeration.

5. Significance and Use

5.1 This test method determines the effectiveness of UVGI devices for reducing viable microorganisms deposited on carriers.

5.2 This test method evaluates the effect soiling agents have on UVGI antimicrobial effectiveness.

5.3 This test method determines the delivered UVGI dose.

6. Hazards

6.1 UV light becomes increasingly hazardous as the wavelength decreases, shifting from longer wavelengths (UV-A, UV-B) to shorter wavelengths (UV-C). UV-A and UV-B are part of the normal solar spectrum found in our atmosphere and are responsible for UV-related aging, sunburns, and mutagenic effects. UV-C radiation is filtered out by the Earth's atmosphere and is not part of the received solar spectrum. UV-C is highly mutagenic and is harmful to all life forms.

NOTE 1—This method is not designed to evaluate safety concerns surrounding UV exposure.

6.2 Some UVGI bulbs produce ozone, which is harmful to all life forms. Consult the manufacturer of the UVGI bulbs or the device, or both, to determine if ozone is produced. If so, you must follow Occupational Safety and Health Administration (OSHA) regulations to ensure worker safety.

NOTE 2—This test method is not designed to evaluate safety concerns surrounding ozone production by UVGI devices

6.3 Safety measures are required to ensure workers are not exposed to UV light during testing, especially UV-B and UV-C. Safety glasses with appropriate UV protection and appropriate lab attire shall be used at all times when working with UV devices

6.4 Signage shall be posted on the laboratory when UV lights are in use to prevent accidental exposure to coworkers.

[4] Available from International Organization for Standardization (ISO), ISO Central Secretariat, BIBC II, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, http://www.iso.org.

7. Reagents and Materials[5]

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

NOTE 3—The WHO Manual on Animal Influenza Diagnosis and Surveillance[6] contains specific procedures for preparing and assaying influenza viruses and titering samples. Other influenza strains and viruses may be used, with conditions for propagation and enumeration provided.

7.2 *Sodium Hypochlorite,* ~0.5%.

7.3 *Artificial Sebum Soiling Agent.*[7,8]

Reagent	Amount
Palmitic Acid	10%
Stearic Acid	5%
Coconut Oil	15%
Paraffin Wax	10%
Synthetic Spermacetti	15%
Olive Oil	20%
Squalene	5%
Cholesterol	5%
Oleic Acid	10%
Linoleic Acid	5%

7.4 *Artificial Saliva (Mucin) Soiling Agent (See Practice E2720 and Practice E2721.):*

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

7.5 *50-mL Polypropylene Centrifuge Tubes*—sterile, with caps.

7.6 *1.7-mL Sterile Polypropylene Microcentrifuge Tubes.*

7.7 *15-mL Sterile Polypropylene Centrifuge Tubes.*

7.8 *Cell Culture Treated Flasks*—T-25, T-75, T-175,

7.9 *Cell Culture Cluster Plates*—24-well plate.

7.10 *Cell Spreaders*—disposable, plastic triangular 60 mm, sterile.

7.11 *Petri Dishes*—100×15 mm, sterile; *glass* or *plastic*.

7.12 *Carriers*—2.5 cm in diameter, and composed of any fabric material.

8. Equipment

8.1 *UV Exposure Device*—UVGI light source that is a standalone bulb or contained within an exposure system.

8.2 *Radiometer*—calibrated to measure 254-nm irradiation in accordance with Test Method G130 or ISO 9370.

8.3 *Spectrophotometer*—calibrated to measure wavelengths ranging from at least 200 to 315 nm. Typically, a cosine corrector is used that will allow light to be collected from 180° field of view.

8.4 *Vortex Mixer.*

8.5 *Thermometer or Thermocouple*—accuracy of ± 0.5 °C and range of 10 to 100 °C.

8.6 *Hygrometer*—accuracy of ± 5 % RH and a range of 1 to 99 % RH.

8.7 *Analytical Balance*—capable of weighing 0.001 g and a range of up to 500 g.

8.8 *Refrigerator*—capable of maintaining 2 to 8 °C.

8.9 *Electronic Timer.*

8.10 *Autoclave (steam)*—capable of maintaining 121 °C to 123 °C and 103 kPa to 117 kPa.

8.11 *Pipettor*—capable of a volume up to 1 mL and a precision of 0.001 mL.

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

8.13 *Biological Safety Cabinet*—Class II, A2

9. UV Dose Determination

9.1 Turn on the UVGI light source and allow it to stabilize as indicated by the manufacturer.

9.2 Measure and record air temperature and humidity in the UV Exposure Device (If the UVGI light source is unshielded, measure and record air temperature and humidity in the room).

NOTE 4—Some UVGI light sources will generate heat during operation. The delivered dose of UV light can be reduced due to elevated temperature. It is important to quantify changes in air temperature over the course of the exposure and measure the effect it has on UVGI dose.

NOTE 5—The delivered dose of UVGI light can be reduced due to elevated relative humidity. It is important to quantify humidity over the course of the exposure and measure the effect it has on UVGI dose.

9.3 Measure output from mercury vapor or LED UVGI light using a calibrated radiometer. If using a xenon arc lamp, refer to 9.4.

NOTE 6—Radiometers are normally set to measure a band of wavelengths surrounding the peak intensity wavelength, which will vary among manufacturers.

9.3.1 The peak intensity for UV-C is 254 nm, and this wavelength shall be used as the reference point for UV output from the UVGI light source and for measurement with the radiometer.

9.3.2 Measure the irradiance at the distance the carrier will be positioned from the UVGI light source.

[5] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For Suggestions on the testing of reagents not listed by the American Chemical Society, see *Annual Standards for Laboratory Chemicals,* BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary,* U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[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] Lawrence C, DA Harnish, M S-Powers, D Mills, BK Heimbuch. Assessment of half-mask elastomeric respirator and powered air-purifying respirator reprocessing for an influenza pandemic.*American Journal of Infection Control*. 2017:45(12); 1324-1330

[8] Mills D., DA Harnish, C Lawrence, M S-Powers, BK Heimbuch. Ultraviolet Germicidal Irradiation of Influenza-Contaminated N95 Filtering Facepiece Respirators, *American Journal of Infection Control*, 2018 Jul;46(7):e49-e55