Designation: E3273 – 21

Standard Practice to Assess Microbial Decontamination of Indoor Air using an Aerobiology Chamber[1]

This standard is issued under the fixed designation E3273; 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

Indoor air normally contains a variety of microbes released from animate and inanimate sources (**1**).[2] Exposure to airborne microbes can occur either directly by inhalation or by contact with surfaces and objects where airborne particles have settled (**2**). Such exposure can lead to infections or allergic reactions, and microbial decontamination of indoor air can reduce the risk of exposure to potentially harmful microbes. While many devices for indoor air decontamination are either already on the market or under development, the continuing absence of suitable test protocols makes their claims difficult to assess. This practice gives the design, construction and operation of an economical and versatile aerobiology chamber to test technologies for a temporary reduction in the number of viable microbes in indoor air.

1. Scope

1.1 This practice is to assess technologies for microbial decontamination of indoor air using a sealed, room-sized chamber (~24 m^3) as recommended by the U.S. Environmental Protection Agency (**3**). The test microbe is aerosolized inside the chamber where a fan uniformly mixes the aerosols and keeps them airborne. Samples of the air are collected and assayed, firstly to determine the rates of physical and biological decay of the test microbe, and then to assess the air decontaminating activity of the technology under test as $\log_{10}$ or percentage reductions in viability per m^3 (**1**). The air temperature and relative humidity (RH) in the chamber are measured and recorded during each test.

1.2 The chamber can be used to assess microbial survival in indoor air as well as to test the ability of physical (for example, ultraviolet light) and chemical agents (for example, vaporized hydrogen peroxide) to inactivate representative pathogens or their surrogates in indoor air.

1.3 This practice does not cover testing of microbial contamination introduced into the chamber as a dry powder.

1.4 This practice does not cover work with human pathogenic viruses, which require additional safety and technical considerations.

1.5 *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.*

1.6 *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]

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

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

3. Terminology

3.1 For definitions of general terms used in this practice, refer to Terminologies D1129 and E2756.

[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] The boldface numbers in parentheses refer to a list of references at the end of this standard.

[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.

3.2 *Definitions:*

3.2.1 *nebulizer*—a device used to create an aerosol from a liquid.

3.2.2 *soil load, n*—a solution of one or more organic, or inorganic substances, or both, added to the suspension of the test organism to simulate the presence of body secretions, excretions, or other extraneous substances.

3.2.3 *test organism, n*—an applied inoculum of an organism that has characteristics which allow it to be readily identified.

3.2.3.1 *Discussion*—The test organism is used to simulate a transient topical microbial contaminant. It may also be referred to as a marker organism, bacterial simulant, or bacterial contaminant.

3.2.4 *test substance, n*—a formulation that incorporates antimicrobial ingredients.

3.3 *Definitions of Terms Specific to This Standard:*

3.3.1 *test device, n*—a piece of equipment meant for microbial decontamination of air using a physical or chemical process.

4. Summary of Practice

4.1 The test device is placed in the leak-free aerobiology chamber and operated either remotely or via the access gloves built into the chamber (Fig. 1); a port on one side of the chamber can be used to attach aerosol cans to release test chemicals.

4.2 Aerosols of the test microorganism are released into the chamber using a nebulizer; a fan inside it uniformly distributes the aerosols and keeps them airborne. An air sample is collected using a slit-to-agar (STA) sampler to determine the initial level of microbial contamination.

4.3 The test device is switched on or the test chemical introduced into the chamber and allowed to operate for the desired length of time. During this period, additional air samples are collected to determine the levels of viable airborne microorganisms over the test period.

4.4 The plates of the recovery medium from the STA are incubated for CFU development.

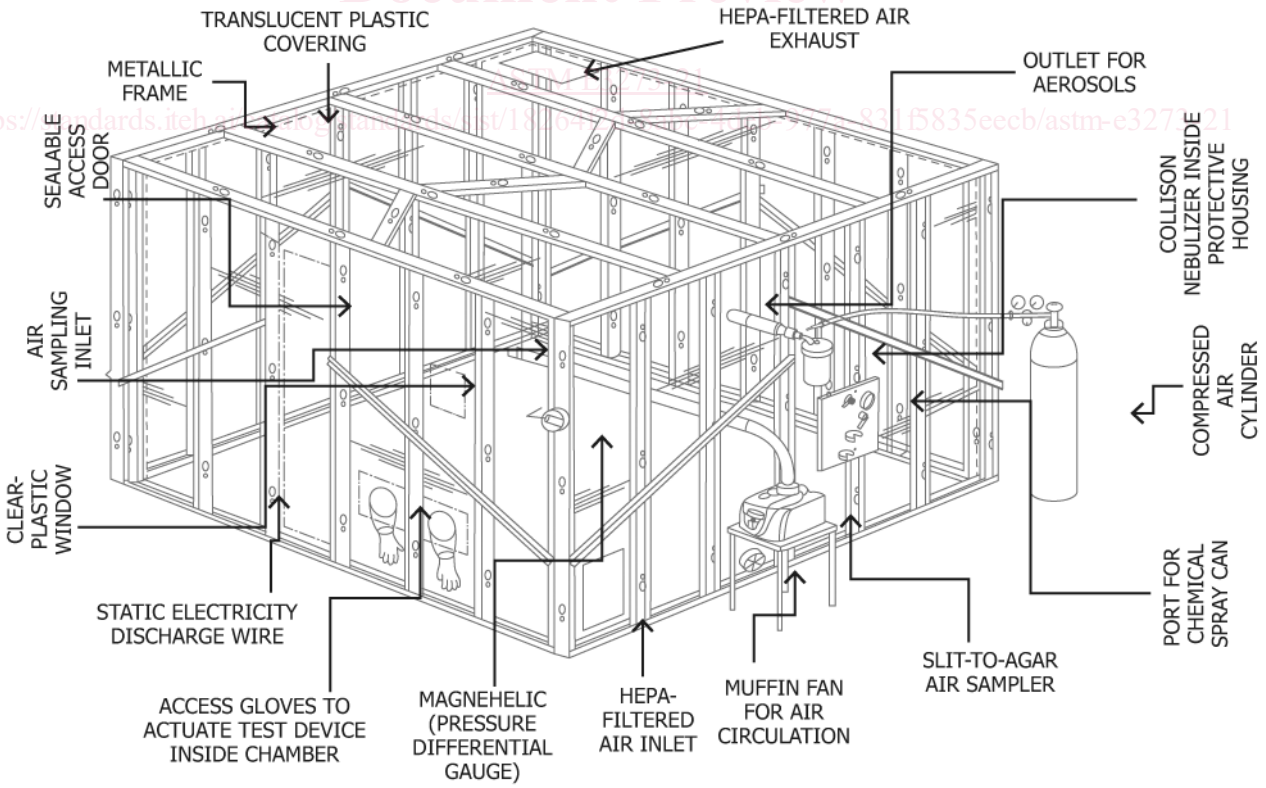
4.5 The CFU are counted to determine $\log_{10}$ or percent reductions in the viability of the test microorganism/m^3.

4.6 Between tests, the air in the chamber is flushed with fresh air to purge out any microbial and/or chemical residues in it.

5. Significance and Use

5.1 This practice is to help in the development of protocols to assess the survival, removal and/or inactivation of human pathogens or their surrogates in indoor air. It accommodates the testing of technologies based on physical (for example, UV light) and chemical agents (for example, vaporized hydrogen peroxide) or simple microbial removal by air filtration or a combination thereof.

5.2 While this practice is designed primarily for work with aerobic, mesophilic vegetative bacteria, it can be readily adapted to handle other classes of microbial pathogens or their surrogates.



(10.5 ft × 11.83 ft × 6.92 ft -860.0 ft^3 or 24.0 m^3)

FIG. 1 Aerosol Test Chamber with Essential Components

5.3 The pieces of equipment given here are as examples only. Other similar devices may be used as appropriate.

6. General Equipment and Labware

6.1 *Air Displacement Pipettes,* 100 µL to 1000 µL with disposable tips.

6.2 *Analytical Balance,* to weigh chemicals and to standardize inoculum delivery volumes by pipettes.

6.3 *Centrifuge,* for sedimentation and washing of cells of the test microorganism(s).

6.4 *Colony Counter,* for example, Quebec Colony Counter.

6.5 *Filter Sterilization System for Media and Reagents,* a membrane or cartridge filtration system (0.22 µm pore diameter) is required to sterilize heat-sensitive solutions.

6.6 *Glassware,* 1 L flasks with a side arm and appropriate tubing to capture the filtrates from 47 mm diameter membrane filters; 250 mL Erlenmeyer flasks for culture media.

6.7 *Hot Air Oven,* an oven at 60 °C to dry clean and sterile glassware.

6.8 *Incubators,* an aerobic incubator with an adjustable temperature range of 30 °C-56 °C.

6.9 *Biological safety cabinet,* Class II (Type A).

NOTE 1—See reference (**4**) for details on the proper maintenance and use of such cabinets.

6.10 *Magnetic Stir Plate and Stir Bars,* large enough for a 5 L beaker or Erlenmeyer flask for preparing culture media or other solutions.

6.11 *Markers,* for permanent marking of labware.

6.12 Disposable or reusable holders for 47 mm diameter membrane filters (0.22 µm or 0.45 µm pore diameter).

6.13 *pH Meter,* to measure pH of buffers, reagents and test formulations.

6.14 *Positive Displacement Pipette,* with pipette tips fitted with "plungers" that can accurately dispense 10 µL.

6.15 *Refrigerator,* a refrigerator at 5 °C ± 3 °C for storage of media, culture plates and reagents.

6.16 *Serological Pipettes,* sterile reusable or single-use pipettes of 10.0 mL, 5.0 mL, and 1.0 mL capacity.

6.17 *Spectrophotometer,* for measuring turbidity of microbial suspensions.

6.18 *Nitrile Disposable Gloves.*

6.19 *Sterile Disposable Plastic Petri Dishes,* 100 mm by 15 mm.

6.20 *Sterile Polypropylene Centrifuge,* Tubes with Caps, 50 mL.

6.21 *Sterilizer,* any steam sterilizer suitable for processing culture media, reagents, and labware is acceptable.

6.22 *Timer,* any stopwatch able to read in minutes and seconds.

6.23 *Vacuum Source,* a vacuum pump, access to an in-house vacuum line or a water faucet vacuum apparatus required to pull the samples through the membrane filters.

6.24 *Vials (Glass),* wide-mouth, 20 mL, for use as dilution vials.

6.25 *Vortex Mixer,* to vortex mix microbial suspensions.

6.26 *Freezer,* at -20 °C ± 2°C for storage of media, reagents and additives.

6.27 *Deep freezer,* -70 °C ± 2 °C or lower to store stocks of test microbes.

6.28 *Centrifuge,* with a minimum speed of 3000 × g to sediment bacteria for concentration or washing, or both.

6.29 *Glass beads,* borosilicate with 5 µm in diameter.

7. General Solutions and Reagents[4]

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (**5**).

7.2 *Phosphate Buffered Stock Solution*—To prepare a stock solution of phosphate buffer, dissolve 34.0 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 mL of water. Adjust pH to 7.2 ± 0.2 with 0.1 N NaOH or 0.1 N HCl and bring to 1000 mL with deionized water.

7.3 *Phosphate Buffered Saline (PBS),* to be used as a diluent and wash for all microorganisms; to prepare PBS, add 1.25 mL of the stock solution and 8.75 g of NaCl to a volumetric flask, fill with deionized water to the 1000 mL mark, and mix; adjust pH to 7.2 ± 0.2, if necessary. Sterilize by filtration or autoclaving.

7.4 *Test formulation,* prepared at its use-dilution and bring it to the test temperature.

7.5 *Growth, Recovery Media, and Media Supplements,* the required types of materials (see below) can be purchased from a variety of sources specializing in laboratory supplies.

7.6 *Distilled Deionized Water (DDW),* or equivalent high-quality water, for making reagent solutions and media. (See Terminology D1129 and Specification D1193.)

7.7 *Plates of Recovery Media,* ready-made plates can be purchased or prepared in-house according to manufacturer's instructions. Sterility and growth-promotion checks must be performed on all batches of culture media.

7.8 *Tryptone, Bovine Serum Albumin (BSA), and Bovine Mucin,* the three ingredients for the soil load (see Section 9) can be purchased from a variety of chemical suppliers.

7.9 *Antifoam,* a concentrate is added to the microbial suspension to be nebulized to reduce frothing. It can be autoclave-sterilized.

[4] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar 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.

8. Soil Load (E2197)

8.1 In case a soil load is to be added to the test microbial suspension, prepare its stock solutions as follows in PBS (pH 7.2):

8.2 Add 0.5 g of Tryptone to 10 mL of PBS.

8.3 Add 0.5 g of BSA to 10 mL of PBS.

8.4 Add 0.04 g of bovine mucin to 10 mL of PBS.

8.5 Prepare the solutions separately and sterilize by passage through a 0.22 µm pore diameter membrane filter, aliquot, and store at either 5 °C ± 3 °C or –20 °C ± 2 °C.

8.6 To prepare the fluid for each nebulization into the aerobiology chamber, add to the nebulizer reservoir 0.75 mL BSA, 1.05 mL yeast extract, 3.0 mL mucin, 50 µL of the test bacterial suspension and 10 µL of Antifoam A to 10.14 mL of Dulbecco's PBS.

9. Preparation of Inocula

9.1 Appendix X1 lists the microorganisms most often used and the media for their cultivation and recovery from indoor air. The number of CFU/mL of each freshly prepared and homogenized microbial test suspension may be estimated spectrophotometrically, based on a standard curve at a specific wavelength, but should be confirmed by titration using membrane filtration or alternative methods.

9.2 The concentration of the viable test microorganisms in the experimentally contaminated air should be high enough to meet the required tested technology's performance criterion. This should be confirmed by determining the numbers of viable microorganisms in the chamber's air before its decontamination.

10. Personal Protective Equipment

NOTE 2—PPE; all items listed are available from suppliers of laboratory safety gear.

10.1 Facemask,

10.2 Hairnet,

10.3 Booties (external to shoe protector and scrub bottoms),

10.4 Lab gown (ties in back),

10.5 Safety glasses, and

10.6 Laboratory gloves

11. Specialized Pieces of Equipment

11.1 *Specialized pieces of equipment.* As a wide variety of specialized pieces of equipment is commercially available, the following list gives only examples of those.

11.1.1 An STA programmable sampler: For event-related collection of bacterial aerosols (for example, Pinpoint Scientific.[5])

11.1.2 *Six-jet Collison nebulizer*—To generate microbial aerosols in the respirable range of 0.5 µm-5.0 µm (for example CH Tech[6]); cylinder of extra-dry compressed air with pressure regulator and a back flow preventer.

11.1.3 *Volatile-gas detector with a gas leak probe*—To check for any air leaks from the chamber.

11.1.4 *Air temperature and RH meter*—To monitor and record the air temperature and RH in the aerosol chamber via a wireless data logger.

11.1.5 *Magnehelic*—To detect any pressure differential between the inside and outside of the chamber (Fig. 1).

11.1.6 A muffin fan for an even distribution the aerosols inside the chamber, and keeping them airborne during testing.

12. Microbial Species for Testing

12.1 The selection of the microbes for the testing should consider *(a)* biosafety, *(b)* ability to grow to high enough titers for experimentation, *(c)* ease of handling and detection, *(d)* relative stability in air and during aerosol generation/capture, and *(e)* relevance to the site of technology use. Table X1.1 lists suitable bacterial species representing airborne pathogens.

13. Maintenance, Passage and Storage of Test Bacteria

13.1 Obtain standard strains of the bacteria to be used in the testing from a reputable source such as the American Type Culture Collection (ATCC).

13.2 Use the following procedures to initiate and maintain in-house stocks of the cultures.

13.3 *Culture Initiation:*

13.3.1 Wipe the outside of the ampule/vial of the freeze-dried culture with a towelette prewetted with 70 % (v⁄v) ethanol and open it inside a laminar flow hood.

13.3.2 Resuspend the freeze-dried material in 1.0 mL of sterile TSB.

13.3.3 Using a pipette with a sterile pipette tip place 0.1 mL of the rehydrated suspension into each one of two 10.0 mL tubes containing 5.0 mL of sterile TSB. Mix well by shaking.

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[5] The sole source of supply of the apparatus known to the committee at this time is Pinpoint Scientific, 1st Floor (SMF), North Road, Bridgend Industrial Estate, Bridgend, UK, CF31 3TP; https://www.pinpointscientific.com/Particle Measuring Systems, Boulder, CO; http://www.pmeasuring.com/home. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

[6] The sole source of supply of the apparatus known to the committee at this time is for example, CH Tech., Westwood, NJ 07675; www.inhalation.org. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

13.3.4 Streak a loopful of the suspension onto two 100 mm diameter TSA plates (predried to remove any accumulated water on the surface of the agar) to obtain isolated colonies, and incubate the plates at 36 °C ±1 °C for 18 h ± 2 h.

13.4 *Cryopreservation of cultures*—Prepare a broth culture of the desired bacterial species by inoculating with a flamed loop a colony from the TSA plate into 9.0 mL of TSB and incubate the tube at 36 °C ± 1 °C for 18 h ± 2 h (temperature and time for optimal growth). Add to this broth culture 1.0 mL of autoclave-sterilized glycerol, shake well and reincubate for 2 h before mixing well and aliquoting into labelled (with indelible ink) cryovials each displaying the source, scientific name, passage number, lot number and date of storage of the test bacterium. Store the vials at -70 °C or below for no longer than 12 months.

13.5 *Test Culture Preparation:*

13.5.1 Thaw frozen test culture quickly by holding the vial under running warm water from a tap or by immersing it in a waterbath at 45 °C ± 1 °C.

13.5.2 Inoculate 10 mL of TSB with 100 µL the thawed culture and incubate at 36 °C ± 1 ºC for 18 h ± 2 h.

13.5.3 To prepare a "Refrigerated Stock Culture", inoculate 10 mL of TSB with 100 µL of the culture and incubate at 36 °C ± 1 ºC for 18 h ± 2 h. Place at 4 °C ± 2 °C for no longer than seven (7) days.

13.5.4 Inoculate 100 µL of the "Refrigerated Stock Culture" into 10 mL of TSB and incubate at 36 °C ± 1 °C for 18 h ± 2 h.

13.5.5 Using a vortex mixer, resuspend the culture for 3 s - 4 s. When working with *P. aeruginosa* cultures, remove any pellicle prior to resuspension.

13.5.6 The culture should be adjusted to deliver approximately 1.6×10^4 CFU/m^3- 1.0×10^5 CFU/m^3 such that recovery from the parallel untreated control is 4.2 – 5 $\log_{10}$ CFU/m^3.

13.5.7 To prepare the fluid for each nebulization into the aerobiology chamber, add to the nebulizer reservoir 0.75 mL BSA, 1.05 mL yeast extract, 3.0 mL mucin, 50 µL of the test bacterial suspension and 10 µL of Antifoam A to 10.14 mL of Dulbecco's PBS.

13.5.8 Assay the fluid to be nebulized for CFU before and after nebulization by making five 10-fold dilutions (for example, add 100 µL to 900 µL of PBS). Plate appropriate dilutions in duplicate by pour/spread plating or filtration.

13.5.9 Incubate plates for 18 h ± 2 h at 36 °C ± 1 °C. Record the CFU and observe them for any extraneous microbial contamination. The test data would be invalid in case any contamination is detected.

14. Aerobiology Chamber Design

14.1 Details on the basic materials required for the construction of the chamber are given in Appendix X2. The equipment and materials listed are examples only and may be substituted with equivalent items from other sources.

14.2 Fig. 1 depicts a typical aerobiology chamber designed to conform to the guideline from the U.S. EPA (**3**).

14.2.1 It has a volume of 24.00 m^3. Polyethylene sheeting (0.006 in./0.1524 mm thick)

14.2.2 It is affixed to a steel-framed structure with polyvinyl chloride (PVC) adhesive tape to represent the walls, ceiling and floor to maintain an airtight seal.

14.2.3 Sealable ports, window and door provide access to the inside of the chamber for maintenance and to place and remove any monitoring devices to be used.

14.2.4 The plastic sheeting can be easily and safely removed, decontaminated as biohazardous waste by autoclaving and discarded when no longer required.

14.2.5 The sheeting is grounded with a copper wire to dissipate any static electricity that may accumulate. Similarly, the copper wire used to suspend the PVC air sampling pipe acts as grounding wire.

14.2.5.1 In accordance with the current EPA guidelines (**3**), the chamber does not permit any air exchanges; furniture or fixtures may be placed in it as needed.

14.3 The chamber's internal environment can be monitored throughout an experiment with a wireless relative humidity (RH)/air temperature sensor/data logger system and recorded on an externally placed computer for subsequent download and analysis.

14.4 To assess the airborne survival of the test bacteria or to determine the activity of an air decontamination technology, the air in the chamber can be sampled at the rate of 1 ft^3 (28.3 L)/min using an externally-placed slit-to-agar air (STA) sampler with a built-in vacuum pump.[7]

14.4.1 This programmable device can be set to operate for a minimum air sampling time of two (2) min to as long as five hours, and the actual length of sample collection time will be determined by the anticipated load of viable bacteria in the air of the chamber.

14.5 Between experiments, the air inside the chamber is flushed with fresh air and the exiting air discharged directly through a HEPA-filtered exhaust for a minimum of one hour.

14.6 A magnehelic is affixed to the outside of the chamber to visually indicate on a continuing basis any pressure differential between its internal and external atmospheres.

14.6.1 Any change in pressure differential would indicate a breach in the integrity of the chamber resulting in the immediate termination of the test.

14.7 The period of exposure (contact time) of the test airborne microbe to the technology under assessment may vary from minutes to hours.

15. Operation of the Aerosol Chamber

15.1 Leak test the chamber after each change in the plastic sheeting using a mixed gas with 10 % (v/v) hydrogen to fill the chamber and then pass the probe of a volatile-gas detector over all the seams in the plastic sheeting to search for and correct any leaks with the sealing tape.

[7] The sole source of supply of the apparatus known to the committee at this time is, Pinpoint Scientific, 1st Floor (SMF), North Road, Bridgend Industrial Estate, Bridgend, UK, CF31 3TP; https://www.pinpointscientific.com/). If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.