Designation: E1054 – 22

Standard Practices for Evaluation of Inactivators of Antimicrobial Agents[1]

This standard is issued under the fixed designation E1054; 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 These test procedures are used to determine the effectiveness of methodologies procedures and materials intended for inactivating (neutralizing, quenching) the microbicidal properties of antimicrobial agents; to ensure that no components of the neutralizing procedures and materials, themselves, exert an inhibitory effect on microorganisms targeted for recovery; and to demonstrate that the antimicrobial chemistry tested is microbicidal.

1.2 Knowledge of microbiological and statistical techniques is required for these procedures.

NOTE 1—These methods are not suitable when testing the virucidal activity of microbicides (see Test Method E1482).

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

1.4 *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.*

1.5 *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:*[2]

E645 Practice for Evaluation of Microbicides Used in Cooling Water Systems

E1115 Test Method for Evaluation of Surgical Hand Scrub Formulations

E1482 Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in these practices, refer to Terminology E2756.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *antimicrobial effectiveness evaluation, n*—a determination of microbicidal properties of an antimicrobial agent by methods, such as Practice E645 and Test Method E1115.

3.2.2 *CFU/mL (abbrev.)*—colony-forming units of a microorganism per millilitre of fluid.

3.2.3 *neutralizer effectiveness, adj/n*—ability of a neutralization procedure to inactivate or quench the microbicidal properties of an antimicrobial agent.

3.2.4 *neutralizer toxicity, adj/n*—any inhibitory effects a neutralization procedure may have on the survival of a microbial population.

3.2.5 *test material control, adj/n*—an evaluation of the activity of a test material in reducing a known population of microorganisms.

3.2.6 *test organism viability, adj/n*—the population of a challenge microorganism used in a neutralization assay.

3.2.7 *viability, n*—the ability of a challenge microorganism to form colonies or grow on a nutrient medium.

3.2.7.1 *Discussion*—In the context of these test methods, "viability" is understood to be synonymous with cultivability.

4. Summary of Practices

NOTE 2—The neutralization test procedure selected must be consistent with the methods of testing used in the antimicrobial effectiveness evaluation.

4.1 *Neutralization Assay with Recovery on Semi-solid Medium*—Neutralization assay for antimicrobial effectiveness tests that recover and quantify microbial populations on semi-solid (agar) media. This method is appropriate for antimicrobial agents that are chemically inactivated or diluted to

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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.

sub-inhibitory levels and performed entirely *in vitro* or including an *in vivo* component, such as to verify neutralization of an antimicrobial formulation sampled from the skin of a human volunteer.

4.2 *Neutralization Assay with Recovery in Liquid Medium*—Neutralization assay for antimicrobial effectiveness tests that recover surviving microbial populations in liquid media for a growth/no growth determination. This method is appropriate for antimicrobial agents that are chemically inactivated or diluted to sub-inhibitory levels.

4.3 *Neutralization Assay with Recovery by Membrane Filtration*—Neutralization assay for antimicrobial effectiveness tests that recover and quantify microbial populations by using membrane filtration. This method is appropriate for antimicrobial agents that cannot be chemically inactivated or diluted to sub-inhibitory levels, as well as for those that can be.

5. Significance and Use

5.1 The effectiveness of antimicrobial agents incorporated into disinfectants, sanitizers, and antiseptics is measured by their ability to kill microorganisms within a specified contact time. Hence, accurate determination of antimicrobial effectiveness requires complete and immediate inactivation (neutralization) of the antimicrobial agent. Inefficient or incomplete neutralization will permit killing or inactivation of microorganisms to continue beyond the experimental exposure time, resulting in an overestimation of antimicrobial activity.

5.2 The neutralization methods commonly used in antimicrobial effectiveness evaluations are chemical inactivation, dilution, and filtration. All critical parameters of an antimicrobial effectiveness evaluation—for example, media, equipment, microorganism(s), and temperature of solutions—must be duplicated in the performance of selected neutralization procedure.

5.3 The neutralization evaluation must include at least three replications (five replications in Section 9) so that a statistical analysis of the microbial recovery data can be performed. The number of replicates used in the evaluation depends on the statistical significance required for the expected results, the variability encountered in the data, and the relative effectiveness of the neutralization procedure.

5.4 A limitation of these evaluation procedures is that they use microorganisms that have not been exposed to an antimicrobial agent. Under experimental conditions, cells exposed to neutralization procedures are likely to be damaged to different degrees by the antimicrobial agent. Sublethal injury may be a factor in recovery, and the effect of the neutralization procedure on recovery of injured organisms should be examined. This method is not intended to assess recovery of injured organisms.

NOTE 3—Ideally, all microorganisms used in the antimicrobial effectiveness evaluation should be tested in the neutralization assay. However, representative organisms may be selected for testing, as judged appropriate by the investigator. The investigator is cautioned that failure to identify neutralizer efficacy and toxicity for all microorganisms could result in biased microbial reductions in an antimicrobial effectiveness evaluation. Also, for a study testing multiple antimicrobial formulations, and in which samples will contain multiple species of microorganisms (for example, skin flora) that are exposed to the formulations, a single procedure and/or combination of agents suitable for neutralizing the antimicrobial activities of the multiple formulations must be used for testing.

6. Apparatus

6.1 Standard bacteriological devices and equipment should be used for performance of the neutralization assay.

6.2 *Colony Counter*—Any of several types may be used; for example, Quebec colony counters and similar devices, or automated, computerized plater/counter systems.

6.3 *Incubator*—Any incubator capable of maintaining a temperature appropriate for growth of the test microorganism(s) may be used.

6.4 *Sterilizer*—Any steam sterilizer capable of producing the conditions of sterilization.

6.5 *Timer (stopwatch)*—One that displays hours, minutes, and seconds.

6.6 *Vortex Mixer or equivalent.*

6.7 *Membrane Filter Units*—Any sterilizable unit that permits filtration of microorganisms for enumeration. The membrane filter unit must be chemically compatible with the antimicrobial agent and appropriate to efficient recovery of the test microorganisms.

7. Reagents and Materials

7.1 *Phosphate Buffered Saline Dilution Water*—PBS (see Practice E645).

7.1.1 *Phosphate Buffer Solution, Stock*—Dissolve 34 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.

7.1.2 *Phosphate Buffer Saline Dilution Water*—Add 1.25 mL of stock phosphate buffer solution and 8.75 g of NaCl to a volumetric flask, fill with deionized water to the 1000 mL mark, and mix. Final pH should be adjusted to 7.2 ± 0.2, if necessary. Sterilize by filtration or autoclave.

7.2 Because the types of materials and reagents required for various antimicrobial effectiveness evaluations are so diverse, it is impractical to list them in this method. The specific materials and reagents to be used in the antimicrobial effectiveness evaluation, however, must be used in the neutralization assay to confirm that the antimicrobial agent is being neutralized in a particular evaluation.

7.3 Table 1 provides a list of materials employed by researchers to inactivate the microbicidal properties of various antimicrobial agents. This list is provided as a guide for selecting neutralizers and is not exhaustive. A neutralization assay must be performed to determine a selected neutralizer's effectiveness.

8. Neutralization Assay with Recovery on Semi-solid Medium (Fig. 1)

8.1 At least three replicates of each test condition are required for these procedures. The number of additional replicates necessary to the evaluation is dictated by the statistical significance required for the expected results, the