



Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents¹

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1. Scope

1.1 These methods are used to determine the effectiveness of procedures and agents for inactivating (neutralizing, quenching) the microbiocidal properties of antimicrobial agents and to ensure that no components of the neutralizing procedures and agents, themselves, exert an inhibitory effect on microorganisms targeted for recovery.

NOTE 1—Knowledge of microbiological and statistical techniques is required for these procedures. These methods are not applicable to testing with viruses (see Test Method E 1482).

1.2 *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:

- E 645 Test Method for Effectiveness of Microbicides Used in Cooling Systems²
- E 1115 Test Method for Evaluation of Surgical Hand Scrub Formulations²
- E 1482 Test Method for Neutralization of Virucidal Agents in Virucidal Effectiveness Evaluations²

3. Terminology

3.1 Definitions of Terms Specific to This Standard:

3.1.1 *antimicrobial agent*—a test formulation, chemical compound, or product designed to prevent the growth of microbes either by inhibiting growth or destroying the microbe.

3.1.2 *antimicrobial effectiveness evaluation*—a determination of microbiocidal properties of an antimicrobial agent by methods, such as Test Methods E 645 and E 1115.

3.1.3 *CFU/mL*—colony-forming units of a microorganism per millilitre of fluid.

3.1.4 *neutralizer*—a procedure or chemical agent used to inactivate, neutralize, or quench the microbiocidal properties of

an antimicrobial agent.

3.1.5 *neutralizer effectiveness*—a neutralizer's ability to inactivate, neutralize, or quench the microbiocidal properties of an antimicrobial agent.

3.1.6 *neutralizer toxicity*—any inhibitory effects a neutralizer may have on the survival of a microbial population.

3.1.7 *test material control*—an evaluation of the activity of an test material in reducing a known population of microorganisms.

3.1.8 *test organism viability*—the population or viability of a challenge microorganism used in a neutralization assay.

4. Summary of Test Methods

NOTE 2—The neutralization test method selected must be identical to the method used in the antimicrobial effectiveness evaluation.

4.1 *Neutralization Assay with Recovery on Solid Medium*—Neutralization assay for antimicrobial effectiveness tests that recover and quantify microorganism populations on solid (agar) media. This method is appropriate for antimicrobial agents that can be chemically inactivated or diluted to sub-inhibitory levels.

4.2 *Neutralization Assay with Recovery in Liquid Medium*—Neutralization assay for antimicrobial effectiveness tests that recover surviving microorganism populations in liquid media for a growth/no growth determination. This method is appropriate for antimicrobial agents that can be 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 microorganism populations by using membrane filtration. This method is appropriate for antimicrobial agents that cannot be chemically inactivated or diluted to sub-inhibitory levels. This method should not be used when difficulties are incurred during the filtration process.

5. Significance and Use

5.1 The effectiveness of antimicrobial agents such as disinfectants, sanitizers and antiseptics are measured by their ability to kill microorganisms at or for a specified contact time. Accurate determination of antimicrobial effectiveness therefore requires efficient and effective 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

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² *Annual Book of ASTM Standards*, Vol 11.05.