



Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure¹

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1. Scope

1.1 This guide covers an example of a method that measures the changes in a population of aerobic microorganisms within a specified sampling time when antimicrobial test materials are present. Several options for organism selection and growth, inoculum preparation, sampling times and temperatures are provided. When the technique is performed as a specific test method, it is critical that the above mentioned variables have been standardized. Antimicrobial activity of specific materials, as measured by this technique, may vary significantly depending on variables selected. It is important to understand the limitations of *in vitro* tests, especially comparisons of results from tests performed with different parameters. As an example, test results of microorganisms requiring growth supplements or special incubation conditions may not be directly comparable to organisms evaluated without those stated conditions.

1.2 Knowledge of microbiological techniques is required for this procedure.

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 and health practices and determine the applicability of regulatory requirements prior to use.*

2. Referenced Documents

2.1 ASTM Standards:²

D1193 Specification for Reagent Water

¹ This guide 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.

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E2783 Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure

3. Terminology

3.1 Definitions:

3.1.1 *inoculum suspension, n*—the initial suspension of test organism used to inoculate the test material. This may also be known as the organism inoculum (see 8.3).

3.1.2 *microbial population, n*—the microbial count (cfu/mL) in the final volume of test material (see 9.4). This may also be known as the “numbers control.” The measurement may be taken at time zero which may be termed “Initial Population.” Alternatively, the measurement may be taken at each exposure time or the longest exposure time used during testing to simulate the test procedure which may be termed “Final Population.”

3.1.3 *neutralization, n*—the process for inactivating or quenching the activity of a test material. This may be achieved through physical means (for example, filtration, dilution) and/or the addition of chemical agents, called neutralizers.

3.1.4 *neutralizer, n*—a chemical agent used to inactivate, neutralize, or quench the microbicidal properties of an antimicrobial agent.

3.1.5 *total test volume, n*—the volume of test material plus the volume of inoculum suspension.

4. Summary of a Basic Test Method

4.1 The test material or a dilution of the test material is brought into contact with a known population of microorganisms for a specified period of time at a specified temperature. An appropriate and specified neutralization technique is applied to quench the antimicrobial activity of the test material at specified sampling intervals (for example, 30 s, 60 s, or any range covering several minutes or hours), and the surviving microorganisms are enumerated. The percent and/or log₁₀ reduction is calculated by comparison with the microbial population.