



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

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1. Scope

1.1 This guide covers examples of a basic method to measure the changes of a population of aerobic microorganisms within a specified sampling time when tested against antimicrobial test materials *in vitro*. Several options for organism selection and growth, inoculum preparation, sampling times and temperatures are provided. When the basic technique is performed as a specific test method it is critical when evaluating the results to ensure that such variables have been standardized. Antimicrobial activity of specific materials, as measured by this technique, may vary significantly on variables selected. It is important to understand the limitations of *in vitro* tests, especially comparisons of results from tests performed under different circumstances. As an example, test results of microorganisms requiring growth supplements, or special incubation conditions, may not be directly comparable to more robust organisms under the conditions of a single procedure.

1.2 Knowledge of microbiological techniques is required for this test.

1.3 The values stated in SI units are to be regarded as standard.

1.4 *This standard may involve hazardous materials, operations and equipment. 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:

D 1193 Specification for Reagent Water

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic or Preserved Products

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 8.2).

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 “initial population” or “numbers control.”

3.1.3 *neutralization, n*—a process which results in the inactivation or quenching of the antimicrobial activity of a test material. This may be achieved through dilution of the test material(s) or with the use of chemical agents, called neutralizers, to reduce or quench the antimicrobial activity.

3.1.4 *neutralizer, n*—a procedure or chemical agent used to inactivate, neutralize, or quench the microbiocidal 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. The activity of the test material is quenched at specified sampling intervals (for example, 30 s, 60 s, or any range covering several minutes or hours) with an appropriate neutralization technique. The test material is neutralized at the sampling time and the surviving microorganisms enumerated. The percent or $\log_{10}$ reduction, or both, from either an initial microbial population, or test blank is calculated.

5. Significance and Use

5.1 This procedure may be used to assess the *in vitro* reduction of a microbial population of test organisms after exposure to a test material.

6. Apparatus

6.1 *Sterile Vials or Test Tubes*, or equivalent.

6.2 *Timer (Stop-clock)*, one that displays minutes and seconds.

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