Designation: E2783 − 11 (Reapproved 2016)

Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure[1]

This standard is issued under the fixed designation E2783; 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 This test method measures the changes of a population of aerobic and anaerobic microorganisms within a specific sampling time when tested against antimicrobial test materials *in vitro*. The organisms used are standardized as to growth requirements and inoculum preparation and must grow under the conditions of the test. The primary purpose of this test method is to provide a set of standardized conditions and test organisms to facilitate comparative assessments of antimicrobial materials miscible in aqueous systems.

1.2 This test method allows the option of using a test sample size of 10 mL or 100 mL.

1.3 Knowledge of microbiological techniques is required for this procedure.

1.4 Aseptic technique should be practiced at all times.

1.5 In this test method, SI units are used for all applications, except for distance in which case inches are used and SI units follow in parentheses.

1.6 *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:*[2]

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

3. Terminology

3.1 *Definitions:*

3.1.1 *antimicrobial, n*—describes an agent that kills or inactivates microorganisms or suppresses their growth or reproduction.

3.1.2 *drug, n*—articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals. Drugs are intended to affect the structure or any function of the body of man or other animals.

3.1.3 *initial microbial population, n*—bacterial count (CFU/mL) in the final volume of test material. Also known as initial bacterial population, numbers control or control.

3.1.4 *inoculum, n*—the viable microorganisms used to contaminate a sample, device or surface, often expressed as to number and type.

3.1.5 *microbiocide, n*—a physical or chemical agent that kills microorganisms.

3.1.6 *neutralization, n*—the process for inactivating or quenching the activity of a microbiocide. Often achieved through chemical or physical means (for example, filtration or dilution).

3.1.7 *room temperature, n*—temperature in the range of 20 to 30°C (68 to 85°F).

3.1.8 *test material, n*—a formulation which incorporates antimicrobial ingredient(s). Also known as test formulation.

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

4. Summary of Test Method

4.1 A dilution/aliquot of the test material is brought into contact with a known population of test organisms for specified periods of time, at a specified temperature. The activity of the test material is quenched at specified sampling intervals (example 15, 30, and 60 s, or any range covering several minutes or hours) with an appropriate neutralizing technique. The test material is neutralized at the sampling time and the surviving microorganisms enumerated. The percent and $\log_{10}$ reduction, from an initial microbial population is calculated.

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[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] 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.

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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 *Adjustable or Fixed Volume Pipet*—Capable of dispensing 0.1 mL and 1.0 mL.

6.2 *Anaerobic Jar or Incubator*—Any incubator or apparatus in an incubator that creates an environment having a level of oxygen that does not support the growth of oxygen-requiring microorganisms. Required only for organisms that need an anaerobic environment to grow.

6.3 *Balance*—Any suitable laboratory balance with a minimum readability of 0.01 g.

6.4 *Beakers and Magnetic Stir Bars*—For 100 mL sample size. A 250 mL beaker containing a 51 by 8 ± 2 mm magnetic stir bar. The beaker and stir bar should be sterile.

6.5 *Colony Counter*—Any of several types may be used.

6.6 *Incubator*—Any incubator capable of maintaining a suitable temperature ±2ºC may be used.

6.7 *Laboratory Centrifuge*—Any centrifuge capable of producing 3200 r/min (1520 RCF).

6.8 *Magnetic Stirring Plate*—Any rotor powered magnetic stirrer.

6.9 *Positive Displacement Pipet*—1.0 mL capacity. Required for viscous test materials.

6.10 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterility.

6.11 *Sterile Container*—Any container of sufficient size to dilute test material into.

6.12 *Test Tubes with Cap*—Sterile. Alternate sample container to (7.5) for 10 mL sample size. Size of test tube should allow the thorough mixing of samples.

6.13 *Timer (Stop Clock)*—One that displays minutes and seconds.

6.14 *Vortex Mixer*—Any suitable vortex mixer capable of mixing test material and diluents.

6.15 *Waterbath*—Any waterbath capable of maintaining suitable temperature.

7. Reagents and Materials

7.1 *Bacteriological Loops*—Any type of disposable sterile or sterilizable bacteriological loop is suitable.

7.2 *Bacteriological Pipets*—Sterile. 1.1, 2.0, 5.0, 10.0 mL capacity.

NOTE 1—Pre-sterilized/disposable bacteriological pipets are available from most local laboratory supply houses.

7.3 *Broth Growth Medium*—Sterile soybean-casein digest broth (tryptic soy broth) or other broth media appropriate to support growth of the test organisms.

7.4 *Dilution Fluid or Diluent*—Sterile Butterfield's buffered phosphate diluent[3] or other suitable diluent with appropriately validated neutralizers. Perform Test Method E1054 to determine what diluent or neutralizers are required. Volume is 9.0 mL after sterilization.

7.5 *Flip Top Centrifuge Tubes*—Sterile. For 10 mL sample size. Minimum of 50 mL capacity.

NOTE 2—Pre-sterilized/ disposable flip-top centrifuge tubes are available from most laboratory supply houses.

7.6 *Petri Dishes*—100 by 15 mm. Required to plate samples and control.

NOTE 3—Pre-sterilized/disposable plastic petri dishes are available from most local laboratory supply houses.

7.7 *Physiological Saline*—Sterile. Used to prepare inoculum.

7.8 *Pipet Tips*—Sterile. 0.1 and 1.0 mL capacity.

7.9 *Positive Displacement Pipet Tips*—Sterile. 1.0 mL capacity.

7.10 *Solid Growth and Plating Medium*—Sterile soybean-casein digest agar (tryptic soy agar) or other solid media appropriate to support growth of the test organism(s) with appropriately validated neutralizers. Perform Test Method E1054 to determine what neutralizers are required. Should be tempered to between 40 to 50°C if using a pour plate method.

7.11 *Water*—Sterile deionized or distilled water.

8. Hazards

8.1 All test organisms listed for use in this method fall under the Biosafety level 1 or 2 categories and should be handled in accordance with CDC and NIH guidelines.[4]

9. Calibration and Standardization

9.1 Ensure that all equipment used in this test method has been calibrated and standardized as required for that piece of equipment.

10. Test Organisms

10.1 The following list of organisms may be used in this procedure. This list is not all inclusive and other organisms may be used. Organisms selected may be representative of the microbial flora encountered under the conditions of use, or may represent standardized strains. The organism should be capable of providing reproducible results under the test conditions.

10.2 The organisms listed shall be maintained as specified by ATCC or other validated methods.

10.2.1 *Acinetobacter* species.
10.2.2 *Candida albicans* ATCC 10231.
10.2.3 *Enterobacter* species.
10.2.4 *Enterococcus faecalis* ATCC 29212.
10.2.5 *Enterococcus faecium.*

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[3] Horowitz, W., (Ed.), *Official Methods of Analysis of the AOAC*, 17th Ed., Sec. 6.3.03 A.(f), Chapter 6, 2000, p.10. Official Methods of Analysis of AOAC International, Gaithersburg, MD.

[4] Richmond, J. Y. and McKinney, R. W. (eds.), 1999, *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed., Washington DC, U.S. Government Printing Office.

10.2.6 *Escherichia coli* ATCC 8739, 11229, or 25922.
10.2.7 *Klebsiella pneumoniae* ATCC 10031 or 51504.
10.2.8 *Micrococcus luteus* ATCC 7468.
10.2.9 *Pseudomonas aeruginosa* ATCC 9027, 15442, or 27853.
10.2.10 *Proteus mirabilis* ATCC 4675 or 7002.
10.2.11 *Salmonella enterica* ATCC 10708.
10.2.12 *Serratia marcescens* ATCC 14756.
10.2.13 *Shigella species.*
10.2.14 *Staphylococcus aureus* ATCC 6538, 29213, 33591, or 33592.
10.2.15 *Staphylococcus epidermidis* ATCC 12228.
10.2.16 *Staphylococcus haemolyticus.*
10.2.17 *Staphylococcus hominis.*
10.2.18 *Staphylococcus saprophyticus.*
10.2.19 *Streptococcus pyogenes.*
10.2.20 *Streptococcus pneumoniae.*

11. Preparation of Organism

11.1 All organisms used in the test method shall be prepared using a validated method. The same method shall be used to prepare all organisms for the test.

11.2 A minimum starting inoculum level of 1.0×10^8 CFU/mL should be used for the test.

11.3 Other starting inoculum levels may be used depending on the organisms growth potential.

11.4 All organisms shall be no more than five passages removed from the original source.

11.5 The inoculum shall be used within 4 h of preparation.

11.6 If a validated method is not available, the following instructions shall be used to prepare the inoculum.

11.7 *Inoculum Preparation Directly from Agar:*
11.7.1 The stock culture, frozen or lypholized should be at least one 24 h broth growth media (7.3) transfer from the original source.
11.7.2 Inoculate a sufficient number of solid growth media slants or plates (7.10).
11.7.3 Incubate at appropriate temperature and environment for the organism.
11.7.4 Wash each slant by adding 5 to 10 mL of physiological saline (7.7) to each slant.
11.7.5 Using bacteriological loop (7.1), gently scrape or rub surface of agar to remove growth.
11.7.6 Using a sterile pipet, collect the washings in a 50 mL centrifuge tube (7.5). Repeat for all slants, adding washing to the centrifuge tube.
11.7.7 Centrifuge at conditions appropriate to sediment the culture completely.
11.7.8 Decant or pipet supernatant and re-suspend organism pellet in 20 mL physiological saline (7.7).
11.7.9 Centrifuge at conditions appropriate to sediment the culture completely.
11.7.10 Decant or pipet supernatant and re-suspend organism in an amount of physiological saline (7.7) sufficient to achieve a minimum final suspension of 1.0×10^8 CFU/mL. Use McFarland Barium Sulfate Standard #2, turbidimetry, optical density, or other technique that correlates to an aerobic plate count.

11.8 *Inoculum Prepared Directly from Broth:*
11.8.1 The stock culture, frozen, or lypholized should be at least one 24 h liquid growth media (7.3) transfer from the original source.
11.8.2 Incubate at appropriate temperature and environment for the organism.
11.8.3 Centrifuge at conditions appropriate to sediment the culture completely.
11.8.4 Decant or pipet supernatant and re-suspend organism pellet in 20 mL physiological saline (7.7).
11.8.5 Centrifuge at conditions appropriate to sediment the culture completely.
11.8.6 Decant or pipet supernatant and re-suspend organism in an amount of physiological saline (7.7) sufficient to achieve a minimum final suspension of 1.0×10^8 CFU/mL. Use McFarland Barium Sulfate Standard #2, turbidimetry, optical density, or other technique that correlates to an aerobic plate count.

NOTE 4—Antimicrobials sensitive to organic material (for example alcohol and iodine) may have reduced activity by even the slightest organic load and therefore only use thoroughly washed inoculum suspensions, whether initially grown in broth or from solid media.

12. Test Conditions

12.1 Test should be performed at room temperature.

12.2 Test materials and diluents should be at room temperature.

12.3 Test materials may require a lower or higher temperature than room temperature (for example, solids that require warming to and be held at 45°C, or test material may only be stable at a certain temperature). If this is a requirement, all steps of the test should be run at that temperature.

12.4 This test method allows the use of either a 10 or 100 mL sample size. The test shall be run using the same sample size for all test materials.

13. Test (Contact) Times

13.1 For selection of contact times, a minimum time period should be selected based on the ability to reproduce the test sampling in this short time frame (for example 15, 30 and 60 s).

13.2 Time points should reflect the intended use of the test material.

13.3 Times may be chosen to construct a kinetic kill model.

14. Test Material Concentration

14.1 Select the test concentrations of the test material. The concentrations selected may reflect the anticipated concentration of the test material during use. Each concentration is tested in triplicate.

14.2 If test material is to be diluted, dilute using sterile water (7.11). Dilution using other materials, such as sterile saline or sterile buffer may be appropriate if test material is

typically diluted that way under conditions of use. Dilute all test material needed at once. Use sterile container (6.11). Mix thoroughly. Test material can be mixed using a sterile magnetic stir bar and a magnetic stir plate.

15. Inoculum (Start) Count

15.1 To be done at the start and end of the test phase. The start and end counts must be within ±0.5 $\log_{10}$ for test to be valid.

15.2 Prepare serial ten-fold dilutions of the inoculum prepared in Step 11 using 9.0 mL dilution blanks (7.4).

15.3 Enumerate, in duplicate, using standard plating techniques. Ensure that the proper dilutions are plated in order to obtain plate(s) that are within a countable range.

15.4 Incubate at the appropriate temperature, time, and environment for the test organism.

15.5 Count colonies using standard plate count rules and record raw data as CFU/plate. Average duplicate plates and multiply by the dilution factor to calculate CFU/mL.

NOTE 5—Inoculum suspension should be thoroughly mixed prior to each instance of use.

16. 100 mL Sample Size Test Procedure

16.1 The control and all replicates of the test material should be prepared and weighed out prior to start of the test.

16.2 *Control (Numbers Control):*

16.2.1 Prepare a water control blank by adding 100 mL water (7.11) to a beaker (6.4) and place on a magnetic stirring plate (6.8). Speed of stirring should ensure that water and inoculum will completely mix in the time before the first sample is taken.

16.2.2 At time 0, add 1.0 mL of the inoculum prepared in Step 11 to the water control blank prepared in 16.2.1.

16.2.3 Start the timer (6.13) within ±1 s of adding the inoculum suspension in 16.2.2.

16.2.4 At the longest time point to be tested, remove a 1.0 mL aliquot from the beaker and immediately transfer to a 9.0 mL dilution blank (7.4).

16.2.5 Prepare serial ten-fold dilutions of the control from 16.2.4 using 9.0 mL dilution blanks (7.4).

16.2.6 Enumerate, in duplicate, using standard plating techniques. Ensure that the proper dilutions are plated in order to obtain plates(s) that are within a countable range.

16.2.7 The plating of the control should be completed within 30 min of completing 16.2.4.

16.2.8 Incubate at the appropriate temperature, time, and environment for the test organism.

16.2.9 Count colonies using standard plate count rules and record raw data as CFU/plate. Average duplicate plates and multiply by the dilution factor to calculate CFU/mL.

16.3 *Test Material:*

16.3.1 Add 100 mL of the test material to a beaker (6.4) and place on a magnetic stirring plate (6.8). Speed of stirring should ensure that test material and inoculum will completely mix in the time before first sample is taken.

16.3.2 At time 0, add 1.0 mL of inoculum prepared in Step 11 to the test material prepared in 16.3.1.

16.3.3 Start the timer (6.13) within ±1 s of adding the inoculum suspension in 16.3.2.

16.3.4 At the first time point, remove a 1.0 mL aliquot from the beaker and immediately transfer to a 9.0 mL dilution blank (7.4).

16.3.5 Repeat 16.3.4 for each time point.

16.3.6 Prepare serial ten-fold dilutions of each time point.

16.3.7 Enumerate, in duplicate, using standard plating techniques. Ensure that the proper dilutions are plated in order to obtain plates(s) that are within a countable range.

16.3.8 The plating of the samples should be completed within 30 min of completing 16.3.4.

16.3.9 Incubate at the appropriate temperature, time, and environment for the test organism.

16.3.10 Count colonies using standard plate count rules and record raw data as CFU/plate. Average duplicate plates and multiply by the dilution factor to calculate CFU/mL.

16.3.11 Each sample, time point, and organism shall be run in triplicate.

17. 10 mL Sample Size Test Procedure

17.1 The control and all replicates of the test material should be prepared and weighed out prior to start of the test.

17.2 *Control (Numbers Control):*

17.2.1 Prepare a water control blank by adding 10 mL water (7.11) to a centrifuge tube (7.5).

17.2.2 Start to vortex mix the test material. Continuous mixing of the control throughout the test time is crucial to evenly disperse the inoculum.

17.2.3 At time 0, add 0.1 mL of the inoculum prepared in Step 11 to the water control blank prepared in 17.2.1.

17.2.4 Start the timer (6.13) within ±1 s of adding the inoculum suspension in 17.2.3.

17.2.5 At the longest time point to be tested, remove a 1.0 mL aliquot from the centrifuge tube and immediately transfer to a 9.0 mL dilution blank (7.4).

17.2.6 Prepare serial ten-fold dilutions of the control from 17.2.5 using 9.0 mL dilution blanks (7.4).

17.2.7 Enumerate, in duplicate, using standard plating techniques. Ensure that the proper dilutions are plated in order to obtain plates(s) that are within a countable range.

17.2.8 The plating of the control should be completed within 30 min of completing 17.2.5.

17.2.9 Incubate at the appropriate temperature, time, and environment for the test organism.

17.2.10 Count colonies using standard plate count rules and record raw data as CFU/plate. Average duplicate plates and multiply by the dilution factor to calculate CFU/mL.

17.3 *Test Material:*

17.3.1 Add 10 mL of the test material to a centrifuge tube (7.5).

17.3.2 Start to vortex mix the test material. Continuous mixing of the test material throughout the test time is crucial to evenly disperse the inoculum.

17.3.3 At time 0, add 0.1 mL of inoculum prepared in Step 11 to the test material prepared in 17.3.1.

17.3.4 Start the timer (6.13) within ±1 s of adding the inoculum suspension in 17.3.3.

17.3.5 At the first time point, remove a 1.0 mL aliquot from the centrifuge tube and immediately transfer to a 9.0 mL dilution blank (7.4).

17.3.6 Repeat 17.3.5 for each time point.

17.3.7 Prepare serial ten-fold dilutions of each time point.

17.3.8 Enumerate, in duplicate, using standard plating techniques. Ensure that the proper dilutions are plated in order to obtain plates(s) that are within a countable range.

17.3.9 The plating of the samples should be completed within 30 min of completing 17.3.5.

17.3.10 Incubate at the appropriate temperature, time, and environment for the test organism.

17.3.11 Count colonies using standard plate count rules and record raw data as CFU/plate. Average duplicate plates and multiply by the dilution factor to calculate CFU/mL.

17.3.12 Each sample, time point, and organism shall be run in triplicate.

18. Calculation and Interpretation of Results

18.1 If no countable plates are found (all dilutions Too Numerous to Count or outside of the countable range) the test is invalid and must be repeated in order to achieve countable plates.

18.2 If plates from lowest plated dilution do not contain growth:

18.2.1 The results are valid if the results from Test Method E1054 showed that the test material was adequately neutralized at that dilution. If the neutralization study showed that the product was neutralized at a higher dilution, then that dilution should be used in the calculation of results.

18.3 Obtain the CFU/mL of both the start and end inoculum count (15) and convert each to Log_{10}. The start and end counts shall not vary more than ± 0.5 log_{10} for test to be valid.

18.4 *Calculation of Percent (%) Reduction:*

18.4.1 Obtain CFU/mL of the control (16.2.9 or 17.2.10).

18.4.2 Obtain CFU/mL of each test material replicate for a time point (16.3.10 or 17.3.11). Average all three replicates.

18.4.3 Percent Reduction (% reduction) = ((CFU/mL Control – CFU/mL test material) / CFU/mL Control) × 100

18.5 *Calculation of Log_{10} Reduction:*

18.5.1 Obtain CFU/mL of the control (16.2.9 or 17.2.10).

18.5.2 Convert to log_{10}.

18.5.3 Obtain CFU/mL of each test material replicate for a time point (16.3.10 or 17.3.11). Convert each replicate to log_{10}. Average all three replicates.

18.5.4 Log_{10} reduction (LR) = Log_{10} of control – Log_{10} of test material

18.6 If multiple contact times are run for a test material, each contact time will have a percent and Log_{10} reduction for each test organism.

19. Report

19.1 Report the following information:

19.1.1 Test temperature.

19.1.2 Specific test evaluation times.

19.1.3 Specific test organism strains.

19.1.4 Test material concentration.

19.1.5 Type of media used.

19.1.6 How the organism was prepared.

19.1.7 Neutralization test data.

19.1.8 Test material data—Raw data and calculated data.

19.1.9 Inoculum count—Both the start and end count, raw and calculated data.

19.1.10 Control count—Raw and calculated data.

20. Precision and Bias

20.1 The precision of this test method is based on an interlaboratory study of Test Method E2783, conducted in 2009 and 2010. Four independent laboratories participated in this study. Each lab tested one test material at 15 s against *S. aureus* ATCC 6538, performing the test with both the 10 and 100 mL sample size. The interlaboratory study utilized six replicates of the test material for each test volume in order to gather additional data points. Practice E691 was followed for the design and analysis of the data: the details are given in ASTM Research Report Number E35-1005.[5] In addition to the analysis of the data per Practice E691, equivalency testing was also performed to show that there was no statistical difference between the two sample sizes of 10 and 100 mL.

20.2 Reproducibility limits are listed in Table 1.

20.3 Repeatability limits are listed in Table 2.

20.4 *Equivalency Testing:*

20.4.1 The TOST (two one-sided test) procedure was applied to the log reduction data within each lab. The criteria used for the procedure was that the log reductions would be considered equivalent if they differed by no more than 0.5. In other words, $\theta_1 = -0.5$ and $\theta_2 = 0.5$. The testing was performed at a 95 % confidence level. Summary of the data from each lab is listed in Table 3, Table 4, Table 5, and Table 6.

21. Keywords

21.1 antimicrobial agent; antimicrobial ingredient; antimicrobial product; drug; *in vitro*; log_{10} reduction; percent reduction; time kill

[5] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:E35-1005.

TABLE 1 Reproducibility Limit

	Reproducibility Limit R	Reprodcubility Standard Deviation S_R
10 mL	0.9568	0.3417
100 mL	0.9856	0.3520

TABLE 2 Repeatability Limit

	Repeatability Limit r	Repeatabiltiy Standard Deviation Sr
10 mL	0.3777	0.1349
100 mL	0.2954	0.1055

TABLE 3 Laboratory 1—Equivalency Testing

Lab 1 Amount	Method	Mean	Lower Bound		90% CL Mean			Upper Bound	Assessment
Diff (1-2)	Pooled	0.0448	-0.5	<	0.0225	0.0672	<	0.5	Equivalent
Diff (1-2)	Satterthwaite	0.0448	-0.5	<	0.0225	0.0672	<	0.5	Equivalent

TABLE 4 Laboratory 2—Equivalency Testing

Lab 2 Amount	Method	Mean	Lower Bound		90% CL Mean			Upper Bound	Assessment
Diff (1-2)	Pooled	0.0308	-0.5	<	-0.1851	0.2468	<	0.5	Equivalent
Diff (1-2)	Satterthwaite	0.0308	-0.5	<	-0.1868	0.2484	<	0.5	Equivalent

TABLE 5 Laboratory 3—Equivalency Testing

Lab 3 Amount	Method	Mean	Lower Bound		90% CL Mean			Upper Bound	Assessment
Diff (1-2)	Pooled	0.2807	-0.5	<	0.1633	0.3981	<	0.5	Equivalent
Diff (1-2)	Satterthwaite	0.2807	-0.5	<	0.1614	0.4000	<	0.5	Equivalent

TABLE 6 Laboratory 4—Equivalency Testing

Lab 4 Amount	Method	Mean	Lower Bound		90% CL Mean			Upper Bound	Assessment
Diff (1-2)	Pooled	0.2442	-0.5	<	0.1341	0.3542	<	0.5	Equivalent
Diff (1-2)	Satterthwaite	0.2442	-0.5	<	0.1329	0.3554	<	0.5	Equivalent

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