



**US Environmental Protection Agency
Office of Pesticide Programs**

**Office of Pesticide Programs
Microbiology Laboratory
Environmental Science Center, Ft. Meade, MD**

**Standard Operating Procedure for
Screening of Polished Stainless Steel Penicylinders,
Porcelain Penicylinders, and Glass Slide Carriers
Used in Disinfectant Efficacy Testing**

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Title	Screening of Polished Stainless Steel Penicylinders, Porcelain Penicylinders, and Glass Slide Carriers Used in Disinfectant Efficacy Testing
Scope	This protocol describes the procedures for the preparation of carriers for the AOAC Use-Dilution Methods, the AOAC Tuberculocidal Activity of Disinfectants Test, the AOAC Germicidal Spray Products Test Method, the Disinfectant Towelette Test, and the AOAC Sporocidal Activity of Disinfectants Test. See section 15.
Application	The carriers discussed in this SOP are used in methods designed to evaluate the performance of disinfectants and sterilants against the prescribed microbes.

	Approval	Date
SOP Developer:	_____	_____
	Print Name: _____	
SOP Reviewer	_____	_____
	Print Name: _____	
Quality Assurance Unit	_____	_____
	Print Name: _____	
Branch Chief	_____	_____
	Print Name: _____	

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1. Definitions	Abbreviations/definitions are provided in the text.
2. Health and Safety	Follow procedures specified in SOP MB-01, Laboratory Biosafety. The Study Director and/or lead analyst should consult the Material Safety Data Sheet for specific hazards associated with products.
3. Personnel Qualifications and Training	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	Refer to SOP EQ-01 (pH meters), EQ-02 (thermometers), EQ-03 (weigh balance), EQ-04 (spectrophotometer) and EQ-05 (timers) for details on method and frequency of calibration.
5. Sample Handling and Storage	<ol style="list-style-type: none"> 1. Refer to SOP MB-22, Disinfectant Sample Preparation, and SOP COC-01, Chain of Custody Procedures. 2. BTC 835 should be stored at room temperature in a cabinet designed to contain flammable materials. 3. BTC 835 is diluted prior to use. Testing should be conducted within three hours of dilution. Diluted product should be stored at room temperature.
6. Quality Control	For quality control purposes, the required information is documented on the appropriate form(s) (see section 14).
7. Interferences	<ol style="list-style-type: none"> 1. Failing a physical screen may involve varying levels of physical damage or imperfection. Thus, examples of carriers passing and failing physical screens are studied during the training process.
8. Non-conforming Data	<ol style="list-style-type: none"> 1. Manage non-conforming data as specified in the study protocol; procedures will be consistent with SOP ADM-07, Non-Conformance Reports. 2. Polished stainless steel, porcelain, and glass slide carriers that fail physical screening will not be used in testing. 3. Polished stainless steel carriers that fail the biological screening will not be used for testing, unless carriers are physically re-screened, re-cleaned and pass biological screening. 4. All polished stainless steel carriers used for product efficacy testing that subsequently give a positive (with growth) result must be cleaned and biologically rescreened in the same manner before reusing.
9. Data	<ol style="list-style-type: none"> 1. Data will be archived consistent with SOP ADM-03, Records and

Management	Archives.
10. Cautions	1. All carriers used for disinfectant testing must be screened in advance according to procedures outlined in this protocol.
11. Special Apparatus and Materials	<ol style="list-style-type: none"> 1. <i>Disinfectant for biological screening of steel penicylinders</i>: BTC 835: 50% n-Alkyl (50% C₁₄, 40% C₁₂, 10% C₁₆) Dimethyl Benzyl Ammonium Chloride (Stepan Co., Northfield, IL 60093). 2. <i>Carriers, polished stainless steel penicylinders for the AOAC Use-dilution Method</i>. Polished stainless steel cylinders, 8 ± 1 mm outer diameter, 6 ± 1 mm inner diameter, 10 ± 1 mm length; type 304 stainless steel, SS 18-8 (S & L Aerospace Metals, Maspeth, NY or Fisher Scientific catalog number 07-907-5Q as of July 2012). 3. <i>Carriers, porcelain penicylinders for the AOAC Tuberculocidal Activity of Disinfectants Test and the AOAC Sporocidal Activity Test</i>. Porcelain, 8 ± 1 mm outer diameter, 6 ± 1 mm inner diameter, 10 ± 1 mm long (CeramTec Ceramic; Cat. No. LP15819 0645). 4. <i>Carriers, glass slide carriers for the AOAC Germicidal Spray Products as Disinfectants Test Method</i>. 25 mm × 25 mm (or comparable size) borosilicate glass cover slips with number 4 thickness. 5. <i>Carriers, glass slide carriers for the Disinfectant Towelette Test</i>. 25 mm × 75 mm (or comparable size) borosilicate glass cover slips with number 4 thickness (Bellco Glass, Inc., item number: 1916-SO134) or Fisherfinest® Premium Frosted Microscope Slides (Fisher Scientific, catalog number 12-544-2). 6. <i>Phenolphthalein</i>. For detecting the presence of NaOH in the rinse water during cleaning of polished stainless steel penicylinders. Phenolphthalein 1% (w/v) solution in alcohol. 7. <i>Sterile deionized water</i>. For preparing 500 ppm solution of BTC 835. 8. <i>Sodium hydroxide (NaOH) 1N solution</i>. For cleaning polished stainless steel penicylinders. 9. <i>Triton X-100</i>. For washing porcelain penicylinders for the AOAC Sporocidal Activity Test.
12. Procedure and Analysis	

<p>12.1 Polished Stainless Steel Penicylinders (AOAC Use Dilution Methods)</p>	<ul style="list-style-type: none">a. <u>Physical Screening</u>: Visually screen polished stainless steel carriers. Dispose of carriers that fail physical screening due to visible damage (dull, chipped, dented, or gouged). Place carriers that pass physical screening in a container and label with date and “Physically Screened”.b. <u>Cleaning</u>: Soak the physically screened carriers overnight (approx. 12 hr) in 1N NaOH and rinse several (3-4) times with tap water. Collect a portion of the last rinse water and add 2-3 drops of 1% phenolphthalein. If any NaOH remains, the phenolphthalein turns pink, indicating the need for additional rinsing. Continue to rinse the carriers until the addition of phenolphthalein to the collected portion of the rinse water does not produce a color change (to pink). Rinse twice more with DI water. Allow carriers to air dry and store in a closed container marked with date and “Cleaned Carriers/Not Biologically Screened”.c. <u>Biological Screening</u>:<ul style="list-style-type: none">i. Place the cleaned carriers into 25 mm x 150 mm test tubes, 20 per tube.ii. Cover the carriers with reagent grade water (i.e., deionized water from Barnstead B-Pure unit in B206) and cap.iii. Autoclave at 121°C for 20 min; cool and store at room temperature.iv. Perform AOAC Use-Dilution testing (see SOP MB-05, Use Dilution Method) on each carrier using the following parameters: <i>Staphylococcus aureus</i>, 500 ppm solution of BTC 835 prepared using sterile deionized water, 20±1°C, no organic soil, ten minute exposure period, and letheen broth as the neutralizer. Use primary subculture tubes only. Use test forms from SOP MB-05, Use Dilution Method, as necessary (e.g., Processing Sheet, Carrier Counts Form, etc.).v. Conduct control counts (e.g., six total carriers per test day) as per section 12.3 of SOP MB-05, Use Dilution Method.vi. Record screening results in the Use-Dilution Test Results Sheet for Screening Carriers (see section 14).
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	<ul style="list-style-type: none">vii. If growth is observed in the subculture tube, the carrier fails. Autoclave, wash and repeat biological screening of failed carriers.viii. Collect all carriers that pass biological screening. Autoclave and re-wash carriers as per section 12.1.b. After air drying, assign a master media/reagent preparation number (see SOP QC-15, Media Prep and Sterilization Run Numbers) to the set. These passing carriers represent a master pool of official carriers to be used in efficacy evaluations. <p>d. Preparation of Penicylinders for Efficacy Testing.</p> <ul style="list-style-type: none">i. Prior to conducting an AOAC Use-Dilution test, remove the required number of cleaned carriers from the master pool of biologically screened carriers (i.e., current master prep; see section 12.1.c.viii).ii. Place the cleaned carriers into 25 mm x 150 mm test tubes, 20 per tube.iii. Cover the carriers with reagent grade water (i.e., deionized water from Barnstead B-Pure unit in B206) and cap.iv. Autoclave at 121°C for 20 min; cool and store at room temperature.v. Fill out a media/reagent preparation sheet and assign a preparation number (see SOP QC-15, Media Prep and Sterilization Run Numbers) to the autoclaved carriers.vi. Following efficacy testing, identify/separate (e.g., place all positive carriers in one container and mark with tape) carriers giving a positive result (i.e., growth). Autoclave all carriers. Re-clean (see section 12.1.b) the following: carriers for which test results were negative, carriers used for enumeration, and unused carriers still in 25 × 150 mm test tubes. Allow carriers to air dry and return to the master pool of biologically screened carriers.vii. Carriers giving a positive result (i.e., growth) in efficacy
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	<p>testing must be cleaned and biologically rescreened prior to subsequent reuse in testing. Do not return these carriers to the master pool.</p>
<p>12.2 Porcelain Penicylinders (AOAC Tuberculocidal Activity of Disinfectants Test)</p>	<p>a. <u>Physical Screening</u>: Porcelain carriers are examined individually for scratches, nicks, spurs, and discolorations. Carriers that do not pass the screening process are not used in testing. Record screening results in the Physical Screening of Carriers Record Form (see section 14).</p> <p>b. <u>Cleaning</u>: Rinse unused carriers gently in water three times to remove loose material and drain.</p> <p>i. Place clean porcelain carriers in multiples of 10 or 20 in capped Erlenmeyer flasks or 20 mm × 150 mm tubes.</p> <p>ii. Fill out a media/reagent preparation sheet to assign a preparation number to the carriers. Sterilize 20 minutes at 121°C or for 2 hours at 180°C in air oven; cool and store at room temperature. Handle porcelain carriers with care. Minimize carrier movement and avoid excessive contact between carriers that might result in damage.</p> <p>iii. All porcelain carriers used in TB product testing are discarded; carriers are single use.</p>
<p>12.3 Porcelain Penicylinders (AOAC Sporocidal Activity Test Method)</p>	<p>a. <u>Physical Screening</u>: Prior to use, examine porcelain carriers individually and discard those with scratches, nicks, spurs, or discolorations. Record screening results in the Physical Screening of Carriers Record Form (see section 14).</p> <p>b. <u>Cleaning</u>: Rinse unused carriers gently in water three times to remove loose material and drain. Place rinsed carriers into Petri dishes matted with 2 layers of filter paper in groups of 15 carriers per Petri dish or place carriers into 25 × 150 mm tubes (10 carriers per tube).</p> <p>i. Sterilize 20 minutes at 121°C or for 2 hours at 180°C in air oven; cool and store at room temperature. Note: Handle porcelain carriers with care when placing in Petri dishes. Minimize carrier movement and avoid excessive contact between carriers that might result in chips and cracks.</p> <p>ii. Wash carriers with Triton X-100 and rinse with water four</p>

	<p>times for reuse.</p> <p>iii. Carriers for this test may be re-used after cleaning and autoclaving.</p>
<p>12.4 Glass Slide Carriers (AOAC Germicidal Spray Products as Disinfectants Test and Disinfectant Towelette Test)</p>	<p>a. <u>Physical Screening</u>: Visually screen glass slide carriers (25 × 25 mm and 25 × 75 mm) for scratches, chips or cracks and discard those which are damaged or defective. Record screening results in the Physical Screening of Carriers Record Form (see section 14).</p> <p>b. <u>Cleaning</u>: Prior to carrier preparation for testing, rinse the carriers once with DI water, rinse three times with 95% ethyl alcohol, and finally rinse three times with DI water.</p> <p>i. Drain and allow carriers to dry before use.</p> <p>ii. For slides prepared for use in the AOAC Germicidal Spray Products as Disinfectants Test (25 × 25 mm), place one glass slide carrier into a glass Petri dish with 2 pieces of Whatman No. 2 filter paper. Fill out a media/reagent preparation sheet to assign a preparation number to a set of carriers.</p> <p>iii. For slides prepared for use in the Disinfectant Towelette Test (25 × 75 mm), place one glass slide carrier into a glass Petri dish, directly onto the glass surface of the dish (no filter paper will be added to the Petri dishes). Fill out a media/reagent preparation sheet to assign a preparation number to a set of carriers.</p> <p>iv. Autoclave for 45 minutes at 121°C with a 30 minute dry cycle; cool; store at room temperature.</p> <p>v. Discard all glass slide carriers used in testing. They may not be reused.</p>
<p>13. Data Analysis/ Calculations</p>	<p>1. For control counts for biological screening, calculations will be computed using a Microsoft Excel spreadsheet (see section 14 of SOP MB-05, Use Dilution Method). Both electronic and hard copies of the spreadsheet will be retained. For direct plating, counts up to 300 and their associated dilutions will be included in the calculations.</p> <p>2. Alternatively, Petrifilm may be used for enumeration of bacterial organisms. Follow manufacturer’s instructions for preparation and</p>

	incubation of Petrifilm cards. <i>Note:</i> At a minimum, conduct a culture purity check (isolation streak) using suspension from one dilution tube of one carrier or pooled set.
14. Forms and Data Sheets	<p>Test Sheets. Test sheets are stored separately from the SOP under the following file names:</p> <p>Physical Screening of Carriers Record Form MB-03-06_F1.docx</p> <p>AOAC Use-Dilution Test Information Sheet for Biological Screening of Carriers MB-03-06_F2.docx</p> <p>AOAC Use-Dilution Test Results Sheet for Biological Screening of Carriers MB-03-06_F3.docx</p>
15. References	<ol style="list-style-type: none"> 1. Official Methods of Analysis. Method 955.14 – <i>Salmonella enterica</i>. Posted March 2013. AOAC INTERNATIONAL, Gaithersburg, MD. 2. Official Methods of Analysis. Methods 955.15 – <i>Staphylococcus aureus</i>. Posted September 2013. AOAC INTERNATIONAL, Gaithersburg, MD. 3. Official Methods of Analysis. Method 964.02 – <i>Pseudomonas aeruginosa</i>. Posted September 2013. AOAC INTERNATIONAL, Gaithersburg, MD. 4. Official Methods of Analysis. 2012. 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, (Method 965.12 AOAC Tuberculocidal Activity of Disinfectants Test). 5. Official Methods of Analysis. Method 961.02. Posted March 2013. AOAC INTERNATIONAL, Gaithersburg, MD. 6. Official Methods of Analysis. Method 966.04. Posted March 2013. AOAC INTERNATIONAL, Gaithersburg, MD.