





SOP Number	MB-03-17
Title	Screening of Polished Stainless Steel Penicylinders, Porcelain Penicylinders, and Glass Slide Carriers Used in Disinfectant Efficacy Testing
Revisions Made	<ul style="list-style-type: none">• Minor editorial changes for clarification purposes.• Added part numbers to carriers in Section 11.• Increased the amount of forms

SOP Number	MB-03-09
Title	Screening of Polished Stainless Steel Penicylinders, Porcelain Penicylinders, and Glass Slide Carriers Used in Disinfectant Efficacy Testing
Scope	Describes the procedures for the preparation of carriers used in the AOAC Use-Dilution Methods, AOAC Tuberculocidal Activity of Disinfectants Test, AOAC Germicidal Spray Products as Disinfectants Test, Disinfectant Towelette Test, and AOAC Sporidical 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.

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1. Definitions	<ol style="list-style-type: none"> 1. Additional abbreviations/definitions are provided in the text. 2. BTC 835: An alkyl dimethyl benzyl ammonium chloride with alkyl chain distribution 50% C₁₄, 40%, C₁₂, 10%, C₁₆, used for biologically screening stainless steel penicylinders.
2. Health and Safety	Follow procedures specified in SOP MB-01, Laboratory Biosafety. Consult the 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 SOPs EQ-01 (pH meters), EQ-02 (thermometers), EQ-03 (weigh balance), EQ-04 (spectrophotometer), EQ-05 (timers), and QC-19 (pipettes).
5. Sample Handling and Storage	<ol style="list-style-type: none"> 1. Refer to SOP MB-22, Preparation and Sampling Procedures for Antimicrobial Test Substances, and SOP COC-01, Chain of Custody Procedures, as necessary. 2. Store BTC 835 at room temperature in a cabinet designed to contain flammable materials. 3. Dilute BTC 835 prior to use. Conduct testing within three hours of dilution. Store diluted product at room temperature.
6. Quality Control	For quality control purposes, document the required information on the appropriate form(s) (see section 14).
7. Interferences	Failing a physical screen may involve varying levels of physical damage to or imperfection on a carrier. 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 are consistent with SOP ADM-07, Non-Conformance Reports. 2. Do not use polished stainless steel, porcelain, or glass slide carriers that fail physical screening. 3. Do not use polished stainless steel carriers that fail the biological screening 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 result (i.e., with growth) must be cleaned and biologically rescreened in the same manner before reusing.
9. Data Management	Archive data consistent with SOP ADM-03, Records and Archives.

10. Cautions	Screen all carriers used for disinfectant testing in advance according to procedures outlined in this SOP.
11. Special Apparatus and Materials	<ol style="list-style-type: none"> 1. <i>Disinfectant for biological screening of steel penicylinders</i>: 50% n-Alkyl (50% C₁₄, 40% C₁₂, 10% C₁₆) Dimethyl Benzyl Ammonium Chloride (e.g., BTC 835, Stepan Co.). 2. <i>Carriers, polished stainless steel penicylinders for the AOAC Use-dilution Methods</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, or Fisher Scientific Cat. No. 07-907-5Q as of January 2023). 3. <i>Carriers, porcelain penicylinders for the AOAC Tuberculocidal Activity of Disinfectants Test and the AOAC Sporidical Activity Test</i>. Porcelain, 8±1 mm outer diameter, 6±1 mm inner diameter, 10±1 mm long (CeramTec North America LLC; Cat. No. 1010368, formerly #LP15819 0645). 4. <i>Carriers, glass slide carriers for the AOAC Germicidal Spray Products as Disinfectants Test</i>. 25×25 mm (or comparable size) borosilicate glass cover slips with number 4 thickness (Chemglass Life Sciences, Cat. No. EPA-1112-151CLSR). 5. <i>Carriers, glass slide carriers for the Disinfectant Towelette Test</i>. 25×75 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, Cat. No. 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 and rinsing carriers. 8. <i>Sodium hydroxide (NaOH) 1N solution</i>. For cleaning polished stainless steel penicylinders. 9. <i>Triton X-100</i> (e.g., 1% (v/v) solution). For washing used porcelain penicylinders for the AOAC Sporidical Activity Test.
12. Procedure and Analysis	
12.1 Polished Stainless Steel Penicylinders	<ol style="list-style-type: none"> a. <u>Physical Screening</u>: Visually screen polished stainless steel carriers. Discard carriers that fail physical screening due to visible damage (dull, chipped, dented, or gouged). Place carriers that pass physical

<p>(AOAC Use Dilution Methods)</p>	<p>screening in a container and label with date and “Physically Screened.”</p> <p>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 rinsate 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 rinsate 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.”</p> <p>c. <u>Biological Screening of Stainless Steel Penicylinders</u>:</p> <ol style="list-style-type: none">i. Place the cleaned carriers into 25×150 mm test tubes, 20 per tube.ii. Cover the carriers with reagent grade water (i.e., deionized water) and cap.iii. Steam sterilize 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. See section 14 for forms.v. Conduct control counts (e.g., six total carriers per test day) as per SOP MB-05, Use Dilution Method.vi. Record screening results in the Use-Dilution Test Results Sheet for Screening Carriers (see section 14).vii. If growth is observed in the subculture tube, the carrier fails. Autoclave, physically screen, wash, and repeat biological screening of failed carriers.viii. Collect all carriers that pass biological screening. Autoclave and re-wash the passing carriers as per section 12.1.b. After air drying, assign a master media/reagent preparation number (see SOP MB-10, Media and Reagents) to the set. These passing carriers represent a master pool of official carriers to
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	<p>be used in efficacy evaluations conducted as per SOP MB-05, Use Dilution Method.</p> <p>d. Preparation of Stainless Steel Penicylinders for Efficacy Testing.</p> <ol style="list-style-type: none"> i. Prior to conducting the AOAC Use-Dilution test method, 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 25×150 mm test tubes, 20 per tube. iii. Cover the carriers with reagent grade water (i.e., deionized water) and cap. iv. Steam sterilize 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 MB-10, Media and Reagents) to the sterilized carriers. vi. Following efficacy testing, identify/separate carriers giving a positive result (i.e., growth); for example, place all positive carriers in one container and mark with tape. Steam sterilize 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 testing must be cleaned and biologically rescreened prior to subsequent reuse in testing. Do not return these carriers to the master pool.
<p>12.2 Porcelain Penicylinders (AOAC Tuberculocidal Activity of Disinfectants Test)</p>	<ol style="list-style-type: none"> a. <u>Physical Screening</u>: Examine porcelain carriers individually for scratches, nicks, spurs, and discolorations. Do not use carriers that do not pass the screening process. Record screening results in the Physical Screening of Carriers Record Form (see section 14). b. <u>Cleaning</u>: Rinse unused carriers gently in DI water three times to remove loose material and drain. <ol style="list-style-type: none"> i. Place clean porcelain carriers in multiples of 10 or 20 in capped Erlenmeyer flasks or 20×150 mm tubes.

	<ul style="list-style-type: none"> ii. Fill out a media/reagent preparation sheet to assign a preparation number to the carriers. Steam sterilize 20 minutes at 121°C or for 2 hours at 180°C in hot 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. iii. Discard all porcelain carriers used in TB product testing; carriers are single use.
<p>12.3 Porcelain Penicylinders (AOAC Sporicidal Activity Test Method)</p>	<ul style="list-style-type: none"> 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). b. <u>Cleaning</u>: Rinse unused carriers gently in DI water three times to remove loose material and drain. Place rinsed carriers into 25×150 mm tubes with closures (10 carriers per tube). <ul style="list-style-type: none"> i. Steam sterilize 20 minutes at 121°C or for 2 hours at 180°C in hot air oven; cool and store at room temperature. Minimize carrier movement and avoid excessive contact between carriers that might result in chips and cracks. c. <u>Reuse</u> <ul style="list-style-type: none"> i. Carriers for this test may be re-used after cleaning and sterilization. ii. Wash carriers with Triton X-100 (e.g., a 1% (v/v) solution) and rinse with DI water four times for reuse.
<p>12.4 Glass Slide Carriers (AOAC Germicidal Spray Products as Disinfectants Test and Disinfectant Towelette Test)</p>	<ul style="list-style-type: none"> 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). 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 rinse three additional times with DI water. <ul style="list-style-type: none"> i. Drain and allow carriers to dry before use. 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.

	<ul style="list-style-type: none"> 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. iv. Steam sterilize for 45 minutes at 121°C with a 30-minute dry cycle; cool and store at room temperature. v. Discard all glass slide carriers used in testing; carriers are single use. 																
13. Data Analysis/ Calculations	<ol style="list-style-type: none"> 1. For biological screening, calculate control counts using a Microsoft Excel spreadsheet (see SOP MB-05, Use Dilution Method). Retain both electronic and hard copies of the spreadsheet. For direct plating, counts up to 300 and their associated dilutions will be included in the calculations. 																
14. Forms and Data Sheets	<p>Test Sheets. Test sheets are stored separately from the SOP under the following file names:</p> <table border="0" style="width: 100%;"> <tr> <td style="padding-left: 40px;">Physical Screening of Carriers Record Form</td> <td style="text-align: right;">MB-03-09_F1.docx</td> </tr> <tr> <td style="padding-left: 40px;">AOAC Use-Dilution Test Information Sheet for Biological Screening of Carriers</td> <td style="text-align: right;">MB-03-09_F2.docx</td> </tr> <tr> <td style="padding-left: 40px;">AOAC Use-Dilution Test Results Sheet for Biological Screening of Carriers</td> <td style="text-align: right;">MB-03-09_F3.docx</td> </tr> <tr> <td style="padding-left: 40px;">AOAC Use-Dilution Method Time Recording Sheet for Carrier Transfers</td> <td style="text-align: right;">MB-05_F3.docx</td> </tr> <tr> <td style="padding-left: 40px;">AOAC Use-Dilution Method Carrier Counts Form</td> <td style="text-align: right;">MB-05_F8.docx</td> </tr> <tr> <td style="padding-left: 40px;">AOAC Use-Dilution Method Processing Sheet</td> <td style="text-align: right;">MB-05_F9.docx</td> </tr> <tr> <td style="padding-left: 40px;">Carrier Count Spreadsheet MS Excel spreadsheet: Carrier Count Template_UDT_v4</td> <td style="text-align: right;">MB-05_F10.xlsx</td> </tr> <tr> <td style="padding-left: 40px;">AOAC Use-Dilution Method Carrier Counts Form (Pooled Carriers)</td> <td style="text-align: right;">MB-05_F11.docx</td> </tr> </table>	Physical Screening of Carriers Record Form	MB-03-09_F1.docx	AOAC Use-Dilution Test Information Sheet for Biological Screening of Carriers	MB-03-09_F2.docx	AOAC Use-Dilution Test Results Sheet for Biological Screening of Carriers	MB-03-09_F3.docx	AOAC Use-Dilution Method Time Recording Sheet for Carrier Transfers	MB-05_F3.docx	AOAC Use-Dilution Method Carrier Counts Form	MB-05_F8.docx	AOAC Use-Dilution Method Processing Sheet	MB-05_F9.docx	Carrier Count Spreadsheet MS Excel spreadsheet: Carrier Count Template_UDT_v4	MB-05_F10.xlsx	AOAC Use-Dilution Method Carrier Counts Form (Pooled Carriers)	MB-05_F11.docx
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AOAC Use-Dilution Method Processing Sheet	MB-05_F9.docx																
Carrier Count Spreadsheet MS Excel spreadsheet: Carrier Count Template_UDT_v4	MB-05_F10.xlsx																
AOAC Use-Dilution Method Carrier Counts Form (Pooled Carriers)	MB-05_F11.docx																
15. References	<ol style="list-style-type: none"> 1. Official Methods of Analysis. Method 955.14 – Testing Disinfectants against <i>Salmonella enterica</i>, Use-Dilution Method. Posted March 2013. AOAC INTERNATIONAL, Gaithersburg, MD. 2. Official Methods of Analysis. Methods 955.15 – Testing Disinfectants against <i>Staphylococcus aureus</i>, Use-Dilution Method. Posted September 2013. AOAC INTERNATIONAL, Gaithersburg, MD. 																

	<ol style="list-style-type: none">3. Official Methods of Analysis. Method 964.02 – Testing Disinfectants against <i>Pseudomonas aeruginosa</i>, Use-Dilution Method. Posted September 2013. AOAC INTERNATIONAL, Gaithersburg, MD.4. Official Methods of Analysis. 2012. 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, (Method 965.12 In vitro Test for Determining Tuberculocidal Activity).5. Official Methods of Analysis. Method 961.02 – Germicidal Spray Products as Disinfectants. Posted March 2013. AOAC INTERNATIONAL, Gaithersburg, MD.6. Official Methods of Analysis. Method 966.04 – Sporicidal Activity of Disinfectants. Posted March 2013. AOAC INTERNATIONAL, Gaithersburg, MD.
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