



**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 Carriers Used in
Disinfectant Efficacy Testing**

SOP Number: MB-03-04

Date Revised: 08-14-08

EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

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Initiated By: _____ Date: ___/___/___

Print Name: _____

Technical Review: _____ Date: ___/___/___

Print Name: _____

Technical Staff

QA Review: _____ Date: ___/___/___

Print Name: _____

QA Officer

Approved By: _____ Date: ___/___/___

Print Name: _____

Branch Chief

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1.0 SCOPE AND APPLICATION:

1.1 This protocol describes the procedures for the physical and biological screening of carriers for the AOAC Use-Dilution Methods (see 15.1, 15.2 and 15.3), the physical screening and cleaning of carriers for the AOAC Confirmative *In vitro* Test for Determining Tuberculocidal Activity (see 15.4), the physical screening and cleaning of carriers for the AOAC Germicidal Spray Products Test Method (see 15.5), the physical screening and cleaning of carriers for the Disinfectant Towelette Test Against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (MB-09) and the physical screening and cleaning of carriers for the AOAC Sporocidal Activity of Disinfectants Test (see 15.6).

2.0 DEFINITIONS:

2.1 AOAC = AOAC International

2.2 OD = outside diameter

2.3 ID = inside diameter

2.4 DI water = De-ionized water

3.0 HEALTH AND SAFETY:

3.1 Personal protective equipment should be used to handle BTC 835.

3.2 All manipulations of the test organism are required to be performed in accordance with biosafety practices stipulated in SOP MB-01, Lab Biosafety.

4.0 CAUTIONS:

4.1 All carriers used for disinfectant testing must be screened in advance according to procedures outlined in this protocol.

4.2 Strict adherence to the protocol is necessary for the validity of the test results.

5.0 INTERFERENCES:

5.1 Examples of carriers passing and failing physical screens are studied during the training process. The physical screening task is assigned only to personnel who display consistent decision-making for passing and failing carriers.

6.0 PERSONNEL QUALIFICATIONS:

- 6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

- 7.1 BTC 835: 50% n-Alkyl (50% C₁₄, 40% C₁₂, 10% C₁₆) Dimethyl Benzyl Ammonium Chloride (Stepan Co., Northfield, IL 60093)
- 7.2 Polished stainless steel carriers cylinders, 8∇1 mm OD, 6∇1 mm ID, length 10 ∇1mm; type 304 stainless steel, SS 18-8 (S&L Aerospace Metals, Maspeth, NY, 11378, or Fisher Catalog No. 7-907-5).
- 7.3 Porcelain [carriers] cylinders, 8∇1 mm OD, 6∇1 mm ID, length 10∇1 mm (Fisher Catalog No. 07-907).
- 7.4 Glass Slide Carriers, Bellco 25 H 75 mm (or comparable size) borosilicate glass cover slips with number 4 thickness (Bellco Glass, Inc., item number: 1916-SO134)
- 7.5 Glass Slide Carriers, Bellco 25 H 25 mm (or comparable size) borosilicate glass cover slips with number 4 thickness (Bellco Glass, Inc., item number: 1916-SO134)
- 7.6 Phenolphthalein 1% (w/v) solution in alcohol.
- 7.7 Sterile deionized water.
- 7.8 Sodium hydroxide (NaOH) 1N solution.

8.0 INSTRUMENT OR METHOD CALIBRATION: Not applicable

9.0 SAMPLE HANDLING AND STORAGE:

- 9.1 BTC 835 should be stored at room temperature in a cabinet designed to contain flammable materials.
- 9.2 BTC 835 is diluted. Dilution should be performed within three hours of testing and diluted product should be stored at room temperature.

10.0 PROCEDURE AND ANALYSIS:

- 10.1 For the polished stainless steel carriers used in the AOAC Use-Dilution Test Method (see

15.1), proceed as follows:

- 10.1.1 Physical Screening: Visually screen polished stainless steel carriers. Discard carriers that are visibly damaged (dull, chipped, dented, or gouged). Carriers that do not pass the screening process are not used in testing. Explore the option of returning discarded carriers to the manufacturer for repolishing. Record screening results in the Physical Screening of Carriers Record Form (see 16.1).
- 10.1.2 Cleaning: Soak the 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. Rinse twice more with DI water.
- 10.1.2.1 Place the cleaned carriers into 25 mm x 150 mm test tubes, 20 per tube.
- 10.1.2.2 Cover the carriers with reagent grade water (i.e., deionized water from Barnstead B-Pure unit in B206) and cap.
- 10.1.2.3 Fill out a media/reagent preparation sheet and assign a preparation number (see SOP QC-15, Media Prep and Sterilization Run Numbers). Autoclave at 121EC for 20 min; cool and store at room temperature for 3 months. After 3 months, reclean and sterilize prior to use.
- 10.1.3 Biological Screening: Before the polished stainless steel carriers can be used for use-dilution testing, each individual carrier must be screened biologically. This is accomplished by performing an AOAC Use-Dilution test (see SOP MB-05, Use Dilution Method) on each carrier using a 48-54 hour old culture of *Staphylococcus aureus*.
- 10.1.3.1 BTC 835 is diluted to 500 ppm with sterile deionized water and used as the test disinfectant.
- 10.1.3.2 All assays occur at 20±1EC, without organic soil, and with a ten minute exposure period to BTC-835.
- 10.1.3.3 Positive tubes (with growth) are confirmed by Gram stain.

If Gram-positive cocci are observed, the carrier fails and will not be used for testing. Positive carriers will be re-screened prior to re-circulation for official use.

- 10.1.3.4 If an organism other than Gram positive cocci is identified, the carrier is cleaned following the procedure in 10.1.2, and biological screening is performed again.
 - 10.1.3.5 Fill out a media/reagent preparation sheet and assign a preparation number to the Apass@ carriers (see SOP QC-15, Media Prep and Sterilization Run Numbers).
 - 10.1.3.6 Record screening results in the Use-Dilution Test Results Sheet for Screening Carriers (see 16.3).
- 10.2 For the porcelain carriers used in the AOAC Confirmative *In vitro* Tuberculocidal Test Method (see SOP MB-07, Confirmatory Tuberculocidal Method), proceed as follows:
- 10.2.1 Physical Screening: 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 16.1).
 - 10.2.2 Cleaning: Rinse unused carriers gently in water three times to remove loose material and drain.
 - 10.2.2.1 Place clean porcelain carriers in multiples of 10 or 20 in capped Erlenmeyer flasks or 20 mm x 150 mm tubes.
 - 10.2.2.2 Fill out a media/reagent preparation sheet to assign a preparation number to the carriers (see SOP QC-15, Media Prep and Sterilization Run Numbers). 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.
 - 10.2.2.3 All porcelain carriers used in product testing are discarded.
 - 10.2.2.4 Wash carriers with octylphenoxypolyethoxyethanol nonionic surfactant (e.g. Triton X-100) and rinse with water four times for reuse.

- 10.3 For the glass slide carriers used in the AOAC Germicidal Spray Products Test Method (see SOP MB-06, Testing Spray Disinfectants), proceed as follows:
- 10.3.1 Physical Screening: Visually screen glass slide carriers 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 16.1).
 - 10.3.2 Cleaning: 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.
 - 10.3.2.1 Drain and allow carriers to dry before use
 - 10.3.2.2 Place one glass slide carrier into a 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 (see SOP QC-15, Media Prep and Sterilization Run Numbers).
 - 10.3.2.3 Autoclave for 45 minutes at 121EC with a 30 minute dry cycle; cool; store at room temperature.
 - 10.3.3 All glass slide carriers used in testing are discarded.
- 10.4 For the glass slide carriers used in the Disinfectant Towelette Test (see SOP MB-09, Disinfectant Towelette Test Against *Staphylococcus aureus* and *Pseudomonas aeruginosa*), proceed as follows:
- 10.4.1 Physical Screening Visually screen glass slide carriers (25 H 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 16.1).
 - 10.4.2 Cleaning: Prior to carrier preparation for testing, rinse the carriers once with deionized water, rinse three times with 95% ethyl alcohol, and finally rinse three times with DI water.
 - 10.4.2.1 Drain and allow carriers to dry before use. Record screening results in the Physical Screening of Carriers Record form of SOP MB-03, Screening Carriers Used in Disinfectant Efficacy Testing.

- 10.4.2.2 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 (see SOP QC-15, Media and Reagent Preparation: Assigning Prep and Sterilization Run Numbers).
- 10.4.2.3 Autoclave for 45 minutes at 121EC with a 30 minute dry cycle; cool; store at room temperature.
- 10.4.2.4 The 25 H 75 mm glass slide carriers may be reused. Slides giving a positive result in a test must not be reused.

10.5 For the porcelain carriers used in the AOAC Sporicidal Activity Test (see SOP MB-15, AOAC Sporicidal Activity of Disinfectants-*Bacillus* species), proceed as follows:

- 10.5.1 Physical Screening Cleaning: 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 16.1).
- 10.5.2 Cleaning: 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).
 - 10.5.2.1 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.
 - 10.5.2.2 Wash carriers with Triton X-100 and rinse with water 4 times for - reuse.

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

- 12.1 Data will be recorded promptly, legibly, and in indelible ink on the appropriate screening record forms (see 16.0). Completed forms are archived in notebooks kept in secured file

cabinets in D217. Only authorized personnel have access to the secured files. Archived data is subject to OPP=s official retention schedule as outlined in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 For quality control purposes, the required information is documented on the appropriate forms (see 16.0).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Polished stainless steel, porcelain, and glass slide carriers that fail physical screening will not be used in testing.

14.2 Polished stainless steel carriers that fail the biological screening will not be used for testing. If an organism other than a Gram positive coccus is identified as a contaminant resulting in a Apositive@ carrier, then the carrier may be cleaned and rescreened.

14.3 All polished stainless steel carriers used for product testing that subsequently give a positive (with growth) result must be cleaned and biologically rescreened in the same manner before reusing. In addition a new preparation number will be assigned to these carriers.

15.0 REFERENCES:

15.1 Official Methods of Analysis. 2006. 18th Ed. AOAC INTERNATIONAL, Gaithersburg, MD, (AOAC Official Methods, 955.15: Testing Disinfectants Against *Staphylococcus aureus*).

15.2 Official Methods of Analysis. 2006. 18th Ed. AOAC INTERNATIONAL, Gaithersburg, MD, (AOAC Official Methods 964.02: Testing Disinfectants Against *Pseudomonas aeruginosa*).

15.3 Official Methods of Analysis. 2006. 18th Ed. AOAC INTERNATIONAL, Gaithersburg, MD, (AOAC Official Methods 955.14: Testing Disinfectants Against *Salmonella enterica*).

15.4 Official Methods of Analysis. 2008. 18th Ed. AOAC INTERNATIONAL, Gaithersburg, MD, (AOAC Official Methods 965.12: Tuberculocidal Activity of Disinfectants.

15.5 Official Methods of Analysis. 2006. 18th Ed. AOAC INTERNATIONAL, Gaithersburg, MD, (AOAC Official Methods 961.02: Testing Spray Disinfectants Against

Staphylococcus aureus and *Pseudomonas aeruginosa*).

15.6 Official Methods of Analysis. 2006. 18th Ed. AOAC INTERNATIONAL, Gaithersburg, MD, (AOAC Official Method 966.04: Sporicidal Activity of Disinfectants).

16.0 FORMS AND DATA SHEETS:

16.1 Physical Screening of Carriers Record Form

16.2 AOAC Use-Dilution Test Information Sheet for Biological Screening of Carriers

16.3 AOAC Use-Dilution Test Results Sheet for Carrier Screening

16.2

AOAC Use-Dilution Test Information Sheet for Biological Screening of Carriers

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by: _____			
EPA Reg. No.	1839-32	SOP	
Name	BTC-835	Test Date	
Sample No.		Comments/Modifications:	
Lot No.			
Expiration Date			

TEST PARAMETERS/Confirmed by: _____			
Use Dilution	Specified	As Prepared/ Date/Initials	
		↔	↔
Neutralizer	Specified		
Temperature (°C)	Specified	Chiller Unit Display	Test tube Water Bath
		Before: After:	Before: After:
Contact Time	Specified	As Tested	
Other Parameters	Specified		

TEST MICROBE INFORMATION/Confirmed by: _____				
Test Microbe	<i>Staphylococcus aureus</i>	48-54 Hour Culture		
Org. Control No.		Date/Time	Initiated	Harvested
Avg. CFU/Carrier				

REAGENT/MEDIA INFORMATION/Confirmed by: _____			
Reagent/Media	Prep. No.	Reagent/Media	Prep. No.

16.3

AOAC Use-Dilution Test Results Sheet for Carrier Screening
 OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by: _____			
EPA Reg. No.		Test Date	
Name		Organism	
Sample No.			

CARRIER INFORMATION (to be completed by Analyst)		
Carrier Drop Time Interval	Carrier Set	Analyst

TEST RESULTS									
Date Recorded/Intials:									
Primary Subculture/Secondary Subculture (contains carrier)									
1	2	3	4	5	6	7	8	9	10
/	/	/	/	/	/	/	/	/	/
11	12	13	14	15	16	17	18	19	20
/	/	/	/	/	/	/	/	/	/
21	22	23	24	25	26	27	28	29	30
/	/	/	/	/	/	/	/	/	/
31	32	33	34	35	36	37	38	39	40
/	/	/	/	/	/	/	/	/	/
41	42	43	44	45	46	47	48	49	50
/	/	/	/	/	/	/	/	/	/
51	52	53	54	55	56	57	58	59	60
/	/	/	/	/	/	/	/	/	/
Results Summary		Number of Carrier Sets with Growth							
		Number of Carrier Sets without Growth							
Modifications/Comments:									