



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

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

**Standard Operating Procedure for
Tuberculocidal Activity of Disinfectants:
II. Confirmative in vitro
Test for Determining Tuberculocidal Activity**

SOP Number: MB-07-04

Date Revised: 09-29-08

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

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for
Tuberculocidal Activity of Disinfectants:
II. Confirmative *in vitro* Test for Determining Tuberculocidal Activity

SOP Number: MB-07-04

Date Revised: 09-29-08

Initiated By: _____ Date: ___/___/___

Print Name: _____

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1.0 SCOPE AND APPLICATION:

1.1 This SOP describes the methodology used to determine tuberculocidal activity of disinfectants against *Mycobacterium bovis* (BCG) on hard surfaces (AOAC Method 965.12 Part II).

2.0 DEFINITIONS:

2.1 AOAC = AOAC INTERNATIONAL

2.2 MPB = Modified Proskauer Beck medium

2.3 M7H9 = Middlebrook 7H9

2.4 M7H11 = Middlebrook 7H11

2.5 Carrier Set = One carrier “set” is defined as the primary MPB tube containing the carrier and the two additional subculture media tubes (e.g., M7H9 broth, Kirchners medium, TB broth) that were inoculated from the carrier’s corresponding neutralizer tube. There are 10 carrier sets per lot of product sample tested.

3.0 HEALTH AND SAFETY:

3.1 All manipulations of the test organism are required to be performed in accordance with biosafety practices stipulated in the SOP MB-01, Lab Biosafety. All *M. bovis* (BCG) manipulations are performed in a biosafety level 3 isolation laboratory (i.e., room B202 or room B207).

3.2 Disinfectants may contain a number of different active ingredients, such as quaternary ammonium compounds, halogens, phenolics, aldehydes, peroxides, and heavy metals. Latex gloves and other personal protective clothing or devices are worn during the handling of these items. A chemical fume hood or other containment equipment is employed when performing tasks with products.

4.0 CAUTIONS:

4.1 To ensure the stability of the test disinfectant solution, perform testing within 3 hours of preparation.

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

4.3 Use appropriate aseptic techniques for all test procedures involving the manipulation of test organisms and associated test components.

5.0 INTERFERENCES:

5.1 Touching the interior sides of the medication tube should be avoided while the carrier is being lowered into the disinfectant and the hook is being removed. Contact with the interior sides of the medication tube may cause adhesion of test microbe cells which are not in contact with the disinfectant. This may result in re-inoculation of the carrier with the test microbe as it is being removed from the medication tube. Re-inoculation of the carrier with the test microbe can lead to false positive results.

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 Culture media.

7.1.1 *Modified Proskauer-Beck medium.* Dissolve 2.5 g KH_2PO_4 , 5.0 g asparagine, 0.6 g $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, 2.5 g magnesium citrate, 20.0 mL glycerol, 0.0046 g FeCl_3 , and 0.001 g $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$ in 1 L H_2O . Adjust to pH 7.2-7.4 with 1 N NaOH. Filter through paper, place 20 mL portions in separate 25 × 150 mm tubes, and steam sterilize 20 min at 121°C. Use this broth for propagating test cultures and for recovery of test organism from treated carriers.

7.1.2 *Middlebrook 7H9 agar* (dehydrated M7H9 medium + agar). Dissolve 4.7 g in 900 mL H_2O containing 2 mL glycerol and 15.0 g agar. Heat to boiling to dissolve completely. Steam sterilize 15 min at 121°C. Cool sterile medium to 45°C, add 100 mL Middlebrook ADC Enrichment under aseptic conditions, and mix thoroughly. Distribute in 20 mL portions in sterile 25 × 150 mm screw-capped tubes and slant or dispense into sterile Petri plates. Use slants to maintain stock culture and plates for inoculum enumeration.

7.1.3 *Middlebrook 7H11 agar* (dehydrated M7H11 medium). Dissolve 21.0

g dehydrated M7H11 agar medium in 895 mL H₂O containing 5 mL glycerol. Heat to boiling to dissolve completely. Steam sterilize 15 min at 121°C. Cool sterile medium to 50-55°C, add 100 mL OADC enrichment under aseptic conditions, and mix thoroughly. Alternatively, M7H11 agar plates may be purchased (e.g., Remel catalog #R01605). Note: M7H11 agar may only be used for plating and enumeration.

7.1.4 *Middlebrook 7H9 broth* (dehydrated M7H9 medium). Dissolve 4.7 g in 900 mL H₂O containing 2 mL glycerol and 1.0 g Bacto agar. Heat to boiling to dissolve completely. Steam sterilize 15 min at 121°C. Cool sterile medium to 45°C, add 100 mL Middlebrook ADC Enrichment under aseptic conditions and mix thoroughly. Distribute in 20 mL portions in sterile 25 × 150 mm tubes. Use for recovery of test organism from treated carriers.

7.1.5 *Kirchners medium*. Dissolve 5 g asparagine, 2.5 g sodium citrate, 0.6 g magnesium sulfate, 2.5 g monopotassium phosphate, and 1.5 g dipotassium phosphate, in 900 mL H₂O containing 20 mL glycerol and 1.0 g Bacto agar. Heat to boiling to dissolve completely. Steam sterilize 15 min at 121°C. Cool sterile medium to 45°C, add 100 mL Middlebrook ADC Enrichment under aseptic conditions, and mix thoroughly. Distribute in 20 mL portions in sterile 25 × 150 mm tubes. Use for recovery of test organism from treated carriers.

7.1.6 *TB broth base*. Dissolve 2.0 g yeast extract, 2.0 g proteose peptone No. 3, 2.0 g casitone, 1.0 g potassium phosphate monobasic, 2.5 g sodium phosphate dibasic, 1.5 g sodium citrate, and 0.6 g magnesium sulfate (heptahydrate) in 900 mL H₂O containing 50 mL glycerol and 1.0 g Bacto-agar. Heat to boiling to dissolve completely. Steam sterilize 15 min at 121°C. Cool sterile medium to 45°C, add 100 mL Dubos Medium Serum under aseptic conditions, and mix thoroughly. Distribute in 20 mL portions in sterile 25 × 150 mm tubes. Use for recovery of test organism from treated carriers.

7.2 *Test organism. Mycobacterium bovis* (BCG) (Organon Teknika Corp., Durham, NC, USA, or equivalent). For stock culture, streak inoculate M7H9 slants. Incubate 15-20 days at 36 ± 1°C. Following incubation, maintain at 2-5°C for 4-6 weeks.

7.3 Reagents.

- 7.3.1 *Sterile water.* Prepare stock supply of H₂O (e.g., in 1 L flasks with closures), steam sterilize a minimum of 20 min at 121°C, and use to prepare dilutions of the test substance. Use reagent-grade water. Reagent-grade water should be free of substances that interfere with analytical methods. Any method of preparation of reagent-grade water is acceptable provided that the requisite quality can be met. Reverse osmosis, distillation, and deionization in various combinations all can produce reagent-grade water when used in the proper arrangement. See Standard Methods for the Examination of Water and Wastewater for details on reagent-grade water.
- 7.3.2 *Neutralizer.* Normal horse serum or other chemical to inactivate the germicide.
- 7.3.3 *0.1% polysorbate 80 in saline.* Add 0.1 mL polysorbate 80 to 100 mL sterile 0.85% aqueous saline (sodium chloride) solution, filter sterilize. Used in test culture preparation.
- 7.3.4 *Octylphenoxypolyethoxyethanol nonionic surfactant* (e.g. Triton X-100).
- 7.4 Apparatus.
- 7.4.1 *Pipets and glassware.* Volumetric pipets and volumetric flasks. Various volumes for disinfectant preparation.
- 7.4.2 *Test tubes.* For disinfectant, autoclavable 25 × 150 mm or 25 × 100 mm (Bellco Glass Inc., or equivalent); reusable or disposable 20 × 150 mm (for cultures/subcultures). Cap with closures before sterilizing.
- 7.4.3 *Tissue grinder.* Thomas Scientific No. 3431E20, size B, or equivalent. Sterilize all glassware 2 h in hot air oven at 180°C or steam sterilize for a minimum of 20 min at 121°C with drying cycle.
- 7.4.4 *Water bath.* Constant temperature, relatively deep water bath capable of maintaining 20 ± 1°C.
- 7.4.5 *Racks or other tube holding device.* Any convenient style.
- 7.4.6 *Inoculating loop (transfer loop).* 95% platinum, 3.5% rhodium alloy, 18 or 19 gauge, 4 mm loop with 75 mm shank (Baxter Scientific Products, or equivalent) or 100 mm disposable loops.

- 7.4.7 *Wire hook.* For carrier transfer. Make 3-5 mm bend (approximately 60°) at end of suitable platinum or platinum alloy wire, No. 23 B&S gauge, in appropriate holder (Johnson Matthey Inc., or equivalent).
- 7.4.8 *Carriers.* “Penicylinders,” porcelain, 8 ± 1 mm od, 6 ± 1 mm id, 10 ± 1 mm long (CeramTec Ceramic; Cat. No. LE15819). Sterilize 2 h in 180°C air oven. Wash used Penicylinders with Triton X-100 and rinse with H₂O 4 times.
- 7.4.9 *Petri dishes.* Matted with 2 layers of S&S No. 597 or Whatman No. 2, 9 cm filter paper.
- 7.4.10 *Timer.* Any certified timer that can display time in seconds.
- 7.4.11 *Spectrophotometer.* To measure specified wavelengths between 400 nm and 700 nm (e.g., Beckman DU Series 500).

8.0 INSTRUMENT OR METHOD CALIBRATION:

- 8.1 Refer to the laboratory equipment calibration and maintenance SOPs (SOP EQ series) for details on method and frequency of calibration.

9.0 SAMPLE HANDLING AND STORAGE:

- 9.1 Disinfectants are stored according to the manufacturer’s recommendations if stipulated, or at room temperature. Those disinfectants requiring activation or dilution prior to use are activated or diluted within three hours of testing unless test parameter specify otherwise.
- 9.2 Follow chain-of custody guidelines during testing as stipulated in SOP COC-01, Chain of Custody.

10.0 PROCEDURE AND ANALYSIS:

10.1 Carrier Preparation:

- 10.1.1 Prior to use, examine porcelain carriers individually and discard those with scratches, chips, or cracks.
- 10.1.2 Rinse unused carriers gently in water 3 times to remove loose material and drain.

10.1.3 Place clean carriers in multiples of 10 or 20 in capped Erlenmeyer flask or 20 × 150 mm tubes.

10.1.4 Sterilize 20 min at 121°C. Cool and store at room temperature.

Note: Handle porcelain carriers with care. Minimize carrier movement and avoid excessive contact between carriers that might result in damage.

10.1.5 Wash carriers with octylphenoxypolyethoxyethanol nonionic surfactant (e.g., Triton X-100) and rinse with water 4 times for reuse.

10.2 Test Culture Preparation.

10.2.1 Initiate test culture by inoculating several 20 mL tubes (25 × 150 mm) of MPB from a M7H9 stock agar slant by transferring one 4 mm id loopful or equivalent inoculum from the stock culture onto the surface of the broth. Typically, 2-4 M7H9 stock agar slants are used to inoculate 10-20 tubes of MPB. Record all transfers on the Organism Culture Tracking Form (culture notation = -SL).

10.2.2 Incubate the tubes 21–25 days undisturbed at 36 ± 1°C preferably in a slanted position to increase surface area.

Note: The test cultures must be carefully managed. Over-inoculation of MPB may lead to reduced viability due to excessive growth after 21–25 days, and the resulting carrier counts may be negatively impacted. Inoculation of MPB with a smaller amount of inoculum (i.e., a partial loopful) may lead to higher quality cultures.

10.2.3 These tubes may be used as test culture or to conduct another set of transfers. If conducting a second series of transfers, use the 10-20, 21-25 day old cultures grown in MPB and inoculate an additional 10-20 25 × 150 mm tubes, each containing 20 mL of MPB. Incubate in a slanted position without disturbing for 21-25 days at 36 ± 1°C. Record all transfers on the Organism Culture Tracking Form (culture notation = -LL).

10.2.4 Depending on the amount of growth from each 21-25 day old culture, 10-20 of the cultures may be required to generate enough standardized inoculum (approx. 75 mL) for a “typical” test day. A typical test day

will require 24 seeded carriers, 12 carriers per dish. These carriers are used for the following:

Test of 1 product sample: 24 total carriers; 12 for testing, 3 for carrier counts, 9 are extra carriers.

10.2.5 One the day of the test: Using a transfer loop, transfer culture to a heat-sterilized glass tissue grinder, add 1.0 mL 0.1% polysorbate 80 in saline solution, grind to break up large clumps or aggregates of the test organism.

10.2.6 Dilute the homogenized culture with 9 mL MPB and transfer the suspension from the tissue grinder to a sterile test tube.

10.2.7 Allow the suspension to settle for 10-15 min.

10.2.8 Remove the upper portion of each culture, leaving behind any debris or clumps, and transfer to a sterile flask; pool cultures in the flask and swirl to mix.

10.2.9 Dilute the pooled culture with MPB to achieve $20.0 \pm 1\%$ T at 650 nm.

Note: Unless using a 1.5 mL semimicrocuvette with appropriate cap to measure the transmittance, wear half face respirators with HEPA filter cartridges during this process.

10.2.10 If an organic soil load is specified in the test parameters for the product test, measure the culture and add the appropriate amount of soil to the standardized culture. Swirl to mix.

10.2.11 Use standardized culture to inoculate porcelain cylinders.

10.3 Carrier Inoculation.

10.3.1 Transfer 10 sterile carriers, using flamed wire hook, into approximately 15-20 mL standardized test culture in 25×150 mm test tube.

10.3.1.1 The test culture must completely cover the carriers. If a carrier is not covered, gently shake the tube, or reposition the carrier within the tube with a sterile wire hook.

- 10.3.1.2 Be sure to inoculate a sufficient number of carriers for the test.
- 10.3.2 After 15 ± 1 min contact period at room temperature, remove cylinders, using flamed wire hook; shake carrier vigorously against side of the tube to remove excess culture, and place on end in vertical position in sterile Petri dish matted with 2 layers of Whatman No. 2 (or equivalent) filter paper, making sure that carriers do not touch to prevent improper drying. Place no more than 12 carriers in a Petri dish.
 - 10.3.2.1 Carriers that touch or fall over cannot be used for testing and must be removed and re-cleaned.
- 10.3.3 Once all of the carriers have been transferred, cover and place in incubator at $36 \pm 1^\circ\text{C}$, and let dry 30 ± 2 min. Record the time on the Time Recording Sheet for Carrier Inoculation (see 16.2).
- 10.3.4 Inoculated carriers should be used for testing as soon as possible on the day of preparation.
- 10.4 Disinfectant Sample Preparation.
 - 10.4.1 Equilibrate water bath and allow it to come to $20 \pm 1^\circ\text{C}$ or the temperature specified ($\pm 1^\circ\text{C}$) by the manufacturer. Prepare the disinfectant dilutions within 3 h of performing the assay unless test parameters specify otherwise. Ready-to-use products are tested as received; no dilution is required.
 - 10.4.2 Prior to opening the container of a liquid product, gently shake the container and thoroughly clean the area around the cap and spout with 70% ethanol. Allow the surface to dry. Remove the cap. Do not touch the inside surface of the cap. If present, carefully remove the seal attached to the lip of the spout with sterile instruments (i.e., razor blade, forceps).
 - 10.4.3 Pour an appropriate aliquot of the sample into a sterile beaker. Do not place a pipette or any other instrument inside the product container. Place cap on the product container and secure tightly. From the beaker, dispense ready-to-use products directly into sterile medication tubes or initiate dilutions for concentrated products.

- 10.4.4 Aseptically prepare disinfectant samples as directed by the test parameters. Prepare all dilutions with sterile standardized volumetric glassware. For diluted products, use ≥ 1.0 mL or 1.0 g of sample disinfectant to prepare the use-dilution to be tested. Use v/v dilutions for liquid products and w/v dilutions for solids. Round to 2 decimal places toward a stronger product.
- 10.4.5 Dispense 10 mL aliquots of the diluted disinfectant or ready-to-use product into 25×100 mm (or 25×150 mm) test tubes, one tube per carrier. Place tubes in the equilibrated water bath for approximately 10 minutes to allow test solution to come to specified temperature. Record the temperature of the water bath and recirculating chiller before and after testing on the Test Information Sheet (see 16.4).
- 10.4.6 Record disinfectant preparation on the Media/Reagent Preparation Sheet (see SOP QC-15, Media Prep and Sterilization Run Numbers).
- 10.5 Test Procedure:
- 10.5.1 After the required drying time, carriers are sequentially transferred from Petri dish to test tubes containing disinfectant at appropriate intervals. Use a certified timer to monitor the transfers. Modify intervals to accommodate exposure times other than 10 min. Record timed transfer activities on the Time Recording Sheet for Carrier Transfers (see 16.3).
- 10.5.2 One carrier is added per tube. The carrier must be deposited in the tube within ± 5 s of the prescribed drop time. Using alternating hooks, sterilize the hook and allow it to cool after each carrier transfer. When lowering the carrier into the disinfectant tubes, neither the carrier nor the wire hook should touch the interior sides of the tube.
- Note: Proper execution of transfer step is one of the most critical technique-sensitive areas of the method. False positives may result from the inadvertent transfer of live organism to sides of the tube followed by contact with the treated carrier during removal.
- 10.5.3 After the carriers have been deposited into the disinfectant, and the exposure time is complete, the carriers are then transferred in a sequentially timed fashion into the 10 mL neutralizer (e.g., horse serum) in 20×150 mm tubes with a sterile hook. Drain excess disinfectant from the carrier prior to transfer.

- 10.5.4 Shake tube containing carrier in neutralizer thoroughly and immediately transfer the carrier to the tube containing 20 mL MPB. Sterilize hook after each carrier transfer. Contact of the carrier to the interior of the tube during transfer should be avoided as much as possible.
- 10.5.5 Once all carriers have been transferred, sequentially transfer 2 mL aliquots from each neutralizer tube into 2 additional subculture media, M7H9 broth, Kirchners medium, or TB broth, as specified. This portion of the assay is not timed, but the aliquots should be sequentially transferred to the subculture media within 30 ± 5 min. Repeat this with each of the 10 carriers.
- 10.5.6 Refer to Attachment A (Testing Footnotes and Explanations) for a list of footnotes which may be used to record certain observations which occurred during testing.
- 10.5.7 Determine the carrier counts (bacterial carrier load) on three carriers selected at random. Enumeration will be performed as stipulated in SOP MB-04: Enumeration of Bacterial Inocula on Carriers (Carrier Counts).
- 10.5.8 Positive controls. On the day of testing, place a dried inoculated carrier into a tube of MPB and a tube of each subculture media. Incubate tubes as in the test. Growth in the tubes validates the test system viability.
- 10.5.9 Negative controls. On the day of testing, expose 1 sterile carrier to a tube with 10 mL disinfectant for the appropriate contact time (i.e. the contact time used in the efficacy test). After the contact time, drain the excess disinfectant from the carrier and transfer it into a tube with 10 mL neutralizer using a sterile hook. Shake the tube containing the neutralizer thoroughly and immediately transfer the carrier to a tube containing 20 mL MPB. Transfer 2 mL aliquots from the neutralizer tube into 1 tube of each of the additional subculture media. Incubate tubes as in the test. Growth observed in any of the tubes invalidates the test. Record any observations of interactions between the disinfectant and the subculture media on the Test Results Sheet (see 16.5).
- 10.5.10 Neutralizer controls. Incubate 1 tube of each subculture medium with

2 mL sterile neutralizer for quality control purposes. Shake each subculture tube thoroughly; incubate 60 days at $36 \pm 1^\circ\text{C}$. Growth observed in any of the tubes invalidates the test. Record any observations of interactions between the neutralizer and the subculture media on the Test Results Sheet (see 16.5).

10.6 Recording results.

10.6.1 Report results as + (growth) or 0 (no growth).

10.6.2 Record results at 60 days. If the 60th day of incubation falls on a weekend or holiday, record the results on the first workday following the 60th day of incubation. Recording of results beyond the 60th day should be notated in the Comments section on the Test Results Sheet (see 16.5).

10.6.3 If no growth or occasional (insufficient for confirmation) growth occurs within a tube, incubate an additional 30 days and record the results. Growth should be checked by using standard confirmation procedures (e.g., acid fast staining and growth on selective media) to ensure that no contamination is present.

10.6.4 Record results at 90 days. If the 90th day of incubation falls on a weekend or holiday, record the results on the first workday following the 90th day of incubation. Recording of results beyond the 90th day should be notated in the Comments section on the Test Results Sheet (see 16.5).

10.7 Confirmation Procedures for Product Testing:

10.7.1 To confirm the results of product testing, representative positive subculture tubes are selected for further investigation.

10.7.2 The maximum number of tubes that is confirmed per product sample tested is 10.

10.7.3 At least one positive subculture tube for each carrier set with growth is confirmed.

10.7.4 If more than one subculture tube for a carrier set is positive, only growth in one subculture tube is confirmed.

- 10.7.5 If the MPB in the set is positive, it is the representative subculture tube used for confirmation. If MPB is not positive, then the order of selecting the representative subculture tubes for confirmation is: M7H9, Kirchners, and TB.
- 10.7.6 If growth is observed in only one carrier set, then all subculture tubes showing growth for that carrier are subject to confirmation.
- 10.8 Identification (presumptive) of *M. bovis* (BCG):
 - 10.8.1 The tests used to verify the identity of *M. bovis* (BCG) are acid fast staining and typical growth on selective media (e.g., M7H9 or M7H11).
 - 10.8.2 Growth for acid fast staining is taken from the selected positive tubes (based on the hierarchy of media in section 10.7.5) on the day that results are read. Acid fast rods are typical for *M. bovis* (BCG). The acid fast staining results should be read promptly prior to assigning a (+) or (0) to the results.
 - 10.8.3 If acid fast rods are observed from the selected tubes then a (+) is assigned to the results. If no cells are observed for the acid fast stain then a (0) is applied to the results.
 - 10.8.4 In addition, growth from these positive tubes is streaked over the surface of an M7H9 or M7H11 agar plate and incubated for 21-25 days at 36±1°C.
 - 10.8.5 Following the 21-25 day incubation period, the colony morphology of the organism on M7H9 or M7H11 agar should be evaluated. *M. bovis* (BCG) typically appears as colorless to buff-colored, raised, rough growth on M7H9 or M7H11 agar (see SOP MB-02, Test Microbes: Initiation, Maintenance and Quality Control).
 - 10.8.6 If a satisfactory smear cannot be obtained directly from the tube, the smear for acid fast staining will be taken from the 21-25 day old M7H9 or M7H11 agar plate that was inoculated with the growth from the tube.
 - 10.8.7 In the event that no cells were observed with acid fast staining initially but typical growth was observed on the M7H9 or M7H11, then the (0) will be corrected to read (+) on the test sheet. An entry error will be

noted in the comments section of the Test Results Sheet (see 16.5).

10.8.8 Record results on the Test Microbe Confirmation Sheet (see 16.6).

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 forms. Completed forms are archived in notebooks kept in secured file cabinets in room D217. Only authorized personnel have access to the secured files. Archived data is subject to OPP's official retention schedule contained 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 form(s) (see 16.0).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Strict adherence to the protocol is necessary for the validity of the test results. Any deviation from the standard protocol must be recorded on the form and an explanation for the deviation given.

15.0 REFERENCES:

15.1 Official Methods of Analysis. 2008. 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, (Method 965.12 Part II: Confirmative in vitro Test for Determining Tuberculocidal Activity).

15.2 Standard Methods for the Examination of Water and Wastewater. 2005. 21st Ed., American Public Health Association, Washington, D.C.

16.0 FORMS AND DATA SHEETS:

16.1 Physical Screening of Carriers Record

16.2 AOAC Confirmative Tuberculocidal Test: Time Recording Sheet for Carrier Inoculation

16.3 AOAC Confirmative Tuberculocidal Test: Time Recording Sheet for Carrier

Transfers

- 16.4 AOAC Confirmative Tuberculocidal Test: Test Information Sheet
- 16.5 AOAC Confirmative Tuberculocidal Test: Results Sheet
- 16.6 AOAC Confirmative Tuberculocidal Test: Test Microbe Confirmation Sheet

Attachment A: Testing Footnotes and Explanations

16.2

AOAC Confirmative Tuberculocidal Test: Time Recording Sheet for Carrier Inoculation
 OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by: _____	
Test Date	
Type of Test	AOAC Confirmative Tuberculocidal Test
Product Reg. No.	
Product Name	
Sample No(s).	

Date/Initials						
Inoculum Settle Time (from clock/timer)		Test Culture %T	Inoculation Time*		Dry Time**	
Start Time	End Time		Start Time	End Time	Start Time	End Time
/	/		/	/	/	/
/	/		/	/	/	/
Comments:						

* Start time = when all carriers have been transferred into the culture; end time = time when last carrier has been removed from culture (from clock/timer).

** Start time = when carriers are placed in the incubator; end time = when carriers are removed from the incubator (from clock).

16.3

AOAC Confirmative Tuberculocidal Test: Time Recording Sheet for Carrier Transfers
 OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by: _____	
Test Date	
Test Type	AOAC Confirmative Tuberculocidal Test
Product Reg. No.	
Product Name	
Sample No(s).	

Initials/date	Drop Interval	Carrier Drop Start Time (into the disinfectant)		Carrier Drop End Time (into the neutralizer media/MPB tube)		Transfer of Neutralizer (into additional subculture media)
		Clock	Timer	Clock	Timer	Start Time ¹
Comments: Carriers transferred by: _____. Neutralizer transferred by: _____.						

¹Transfer of neutralizer into secondary subculture taken from the clock.

16.4

AOAC Confirmative Tuberculocidal Test: Test Information Sheet
 OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by: _____			
EPA Reg. No.		SOP	MB-07-04
Name		Test Date	
Sample No.		Comments/Modifications:	
Lot No.			
Expiration Date			

TEST PARAMETERS/Confirmed by: _____			
H ₂ O Hardness (CaCO ₃) ppm	Specified	Titrated(Buret)/Date/Init	HACH/Date/Init
		/ /	/ /
Use Dilution	Specified	As Prepared/Date/Init	
		/ /	
Organic Soil	Specified	As Prepared/Date/Init	
		/ /	
Neutralizer	Specified		
Temperature (°C)	Specified	Chiller Display	Test tube Water Bath
		Before: After:	Before: After:
Contact Time	Specified	As Tested	
Other Parameters	Specified		

TEST MICROBE INFORMATION/Confirmed by: _____			
Org. Control No.		21-25 Day Culture	
% Transmittance		Date	Initiated Harvested
Avg. CFU/Carrier			

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

16.5
 AOAC Confirmative Tuberculocidal Test: Results Sheet
 OPP Microbiology Laboratory

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

TEST RESULTS											
Date Recorded/Initials		60 Day: _____/90Day: _____									
		60 Day Results and 90 Day Results ¹									
Media		Carrier									
		1	2	3	4	5	6	7	8	9	10
MPB	60 day										
	90 day										
M7H9	60 day										
	90 day										
Kirchners ² TB Broth	60 day										
	90 day										
Results Summary		Number of carrier sets with growth									
		Number of carrier sets without growth									

CONTROLS ¹	MPB	M7H9	Kirchners/TB Broth ²	Acceptable?
Positive Controls*	/	/	/	___Yes ___No
Negative Controls*	/	/	/	___Yes ___No
Neutralizer Controls				___Yes ___No
Comments: *60 day results/90 day results				

¹ Record positive (+) or negative (0) results as indicated by the presence or absence of typical mycobacterial growth.
² Circle the medium used in the test.

16.6

AOAC Confirmative Tuberculocidal Test: Test Microbe Confirmation Sheet
 OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by: _____			
EPA Reg. No.		Test Date	
Name		Test Organism	<i>Mycobacterium bovis</i> (BCG)
Sample No.		Comments:	

Source: Tube/Plate ID	Date/ Initials	Stain Results*	Media Information			Results	
			Type	Prep. No.	Inc. Time/ Temp.	Date/Initials	Colony Characteristics

*Record Acid Fast results as AFR = acid fast rods.

Attachment A:

Testing Footnotes and Explanations
OPP Microbiology Laboratory

Footnote	Description
A	Indicates that the inoculated carrier, hook, or forceps hit the interior sides of the medication tube containing disinfectant as the carrier was being dropped.
B	Indicates that the carrier was lost (dropped) during a transfer and was not recovered.
C	Indicates that a tube of a positive carrier set (one showing growth) was later determined to be a contaminant and not the test microbe. In "Comments" refer to the confirmation information for details.
D	Indicates that the primary or secondary subculture tube containing the carrier broke during vortexing. In the "Comments" indicate if carrier was recovered or if the remaining broth was placed in another tube.
E	Indicates that the carrier was exposed to the disinfectant late or early, outside of the ± 5 second drop, spray, or wipe interval. In "Comments" indicate the approximate number of seconds outside (\pm) of the 5 second interval.
F	Indicates that the carrier was placed in the neutralizer late or early, outside of the ± 5 second drop interval. In "Comments" indicate the approximate number of seconds outside (\pm) of the 5 second interval.