US Environmental Protection Agency
Office of Pesticide Programs

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

Standard Operating Procedure for
Use of Petrifilm and PetriScan

SOP Number: EQ-09-02

Date Revised: 08-13-13

SOP Number	EQ-09-02
Title	Use of Petrifilm and PetriScan®
Scope	This SOP describes the use of 3M Petrifilm™ Aerobic Count (AC) Plate and PetriScan® with *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella enterica*.
Application	To enumerate microorganisms used in disinfectant and sporicidal efficacy testing.

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

Date SOP issued:	
Controlled copy number:	
Date SOP withdrawn:	

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1. Definitions	Additional abbreviations/definitions are provided in the text. 1. Petrifilm™ = A sample-ready culture medium which contains "Standard Methods" nutrients, a cold water soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration. 2. CFU = Colony forming units 3. Audit View = A secure database that records and displays user, date, time, and edited data when records are changed. 4. Flag = A code notation assigned by PetriScan® indicating plate edits or changes in other plate characteristics. 5. Plate = Petrifilm plate
2. Health and Safety	1. 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. 2. Counting the Petrifilm using the scanner will be performed on the bench-top.
3. Personnel Qualifications and Training	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	1. The PetriScan® was validated after purchase; these data are archived. 2. Verification is conducted annually, refer to section 6.
5. Sample Handling and Storage	For additional information, refer to 15.5. 1. Store unopened Petrifilm plate pouches refrigerated or frozen at temperatures ≤8°C. 2. Remove the required number of plates. Return unused plates to pouch. Seal by folding the end of the pouch over and taping shut. Place the pouch into a resealable container. 3. Opened pouches may be stored at room temperature for up to 30 days. 4. For longer term storage, store opened pouches of Petrifilm in a freezer that does not have an automated defrost cycle until the expiration date listed on the package. 5. Do not refrigerate resealed pouches.
6. Quality Control	1. For quality control purposes, the required information is documented on the appropriate data sheets (see section 6.3). 2. Perform quality control procedures described in the PetriScan® User

	Guide, pages 28 and 29, section 3.8 to verify the count accuracy and consistency at both low and high colony counts of the PetriScan® system on an annual basis. a. The electronic data generated during these quality control procedures are stored in a database entitled *Quality Control*. Once per year, archive the quality control data. 3. Sterility assessment: a. On each test day, select one plate, spot it with 1 mL of sterile diluent, and incubate at 36±1°C with the test system alongside inoculated plates. Record results (e.g., growth or no growth). b. The lack of red colonies indicates a sterile plate while the presence of one or more red colonies indicates contamination. c. If contamination is noted in the sterility assessment, investigate the potential source.
7. Interferences	1. Do not use Petrifilm plates that show discoloration. 2. Do not use diluents containing citrate, bisulfite or thiosulfate with Petrifilm plates; these compounds can inhibit growth. 3. Since the colonies grow suspended in a gel matrix, the application of substantial pressure to the plate will cause the colonies to shift; thus avoid the application of pressure to the plate after spreading. 4. High concentrations of colonies on the Petrifilm plates will cause the entire growth area to become red or pink. Occasionally, on overcrowded plates, the center may lack visible colonies, but many small colonies can be seen on the edges. When any of these occurs, do not count the plates using the scanner – record the results as too numerous to count (TNTC). 5. Dust particles may be picked up by the PetriScan® and counted as colonies – analysts should evaluate each film after the program performs its **Auto Count** function.
8. Non-conforming Data	1. Management of non-conforming data will be specified in the study protocol; procedures will be consistent with SOP ADM-07, Non-Conformance Reports. 2. If any colonies are detected on the uninoculated films used for quality control procedures, visually inspect the film for defects or particles. If no particles are present, it may be necessary to clean the scanner (refer to the PetriScan® User Guide, page 41, section 5). 3. For further information involving problems encountered in operating the

	PetriScan®, refer to the PetriScan® User Guide, page 43, section 6 (Troubleshooting).
9. Data Management	1. Data will be archived consistent with SOP ADM-03, Records and Archives. 2. PetriScan® software creates an FDA/GLP-compliant database, which contains every saved plate, including its image and the settings used in its analysis. This allows images to be recalled and re-analyzed at a later date. 3. PetriScan® tracks changes made to the database, in compliance with Code of Federal Regulations, Title 21, Part 11, Electronic Records; Electronic Signatures, and Code of Federal Regulations, Title 21, Part 58, Good Laboratory Practice for Non-Clinical Laboratory Studies. These changes can be seen in the Audit View by clicking the **Audit View** button (refer to the PetriScan® User Guide, page 35, section 4.4 for information regarding Audit View).
10. Cautions	1. Hold the pipette perpendicular to the plate to dispense the sample. 2. Immediately after spotting the sample on the Petrifilm, press the sample with the spreader to distribute the sample. 3. Press the spreader straight down – do not twist or slide the spreader across the plate.
11. Special Apparatus and Materials	1. Petrifilm™ Aerobic Count Plate 2. PetriScan® 3. Computer loaded with PetriScan® software 4. Hewlett-Packard Scanjet 8200
12. Procedure and Analysis	Allow plates to come to room temperature before use.
12.1 Use of Petrifilm	a. Mark the plate identification information within the top ¾ inch portion of the film. b. Prepare dilutions to be plated using appropriate sterile diluents (e.g., PBDW, Luria-Bertani broth, letheen broth, or distilled water). c. For plating, use dilutions that will yield between 0-300 CFU per plate. d. Place the Petrifilm plate on a flat, level surface. e. Lift the top film and hold the pipette perpendicular to dispense the appropriate amount (800, 900 or 1000 µL, depending upon the total volume of the dilution) of sample suspension onto the center of the

	bottom film. f. Drop the top film down onto the sample. Do not roll the top film down. g. Place the plastic spreader with the recessed side down on the center of the plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire area of the recessed side of the spreader on the Petrifilm plate growth area before the gel is formed. h. Remove the spreader and leave the plate undisturbed for at least one minute to permit the gel to form. i. Incubate plates in a horizontal position with the clear side up in stacks of no more than 20 plates at 36±1°C for 24-48 hours (depending on the organism being evaluated). An incubation time of 48 hours is recommended for *Pseudomonas aeruginosa* as well as for microorganisms damaged by exposure to disinfectants or sporicides. j. Petrifilm can be counted manually or using the PetriScan®. k. Count all red colonies regardless of size or intensity.
12.2 Confirmation	a. Colonies may be isolated for further identification by lifting up the top film and picking up growth from the gel; these colonies can be Gram stained or plated.
12.3 PetriScan	a. Turn on the computer and double click the PetriScan® icon on the desktop. The sign-on dialog box will be displayed. Enter the appropriate user name and password. b. PetriScan® data are stored and maintained in database files. After signing on, create a new database or open an existing database. To create a new database, refer to the PetriScan® User Guide, page 13, section 2.2.1. To open an existing database, refer to the PetriScan® User Guide, page 14, section 2.2.2. c. To count colonies using the PetriScan®, lift the scanner lid and place 1-4 Petrifilms face-down within the cutouts on the alignment grid. d. Do not count plates that contain any of the following conditions: i. High concentrations of colonies on the Petrifilm plates that cause the entire growth area to become red or pink. ii. Overcrowded plates where the center lacks visible colonies, but the edges contain colonies.

iii. Excessive spreader growth (>50% of the film area).

e. If liquefied gel interferes with counting, an estimated count can be made by counting the unaffected areas.

f. Close the lid and click **Scan**. When the scan is complete, the images will be displayed in the grid view (see Attachment 1).

g. After scanning, enter in an **ID** and **Dilution** factor (if applicable) for each film to be counted.

h. Click **Select All**, or select each film individually by placing a ✓ mark in the box in the lower left-hand corner of the film.

i. Click **Auto Count**. PetriScan® will mark each counted colony with a green circle and display the number of counted colonies and the CFU/mL or CFU/gm. The CFU/mL or CFU/gm represents the calculated colony forming units per milliliter or gram of the original sample, taking into account the dilution factor.

j. To count film manually, place a ✓ mark in the box in the lower left-hand corner of the film to be counted and click **Manual Count**. As necessary, add or delete colonies using the left and right mouse buttons, respectively. Click **Exit**. Select **Yes** when asked if the data should be saved.

k. After automatic counting, click **Save Data**. Films marked with ✓ marks will be saved as JPEG (.jpg) image files, and data for the selected films will be saved to the open database and displayed in the Data View. The grid will be cleared of images/data and will be ready for the next scan.

   i. NOTE: Data are not saved to the database until **Save Data** is clicked. If the **Scan** button is clicked for the next set of Petrifilm to be scanned before saving data, the images and data will be lost.

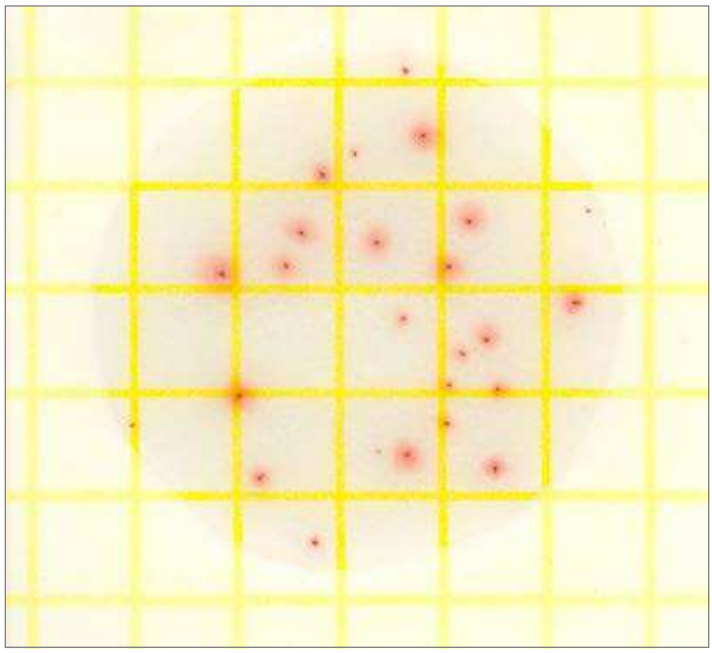
l. It is recommended that after scanning and saving the data, the analyst reviews each film to ensure it was counted correctly. To review each scanned film, double-click on an individual entry to bring up the image of the scanned film. Observe the film to make sure all colonies have been counted (e.g., encircled in blue) and to make sure that all spots that were counted are colonies (occasionally a dust particle will be counted as a colony).

m. It is also recommended that the **ID** and **Dilution Factor** entered by the analyst be checked against the ID and Dilution Factor noted on the label of the Petrifilm.

13. Data Analysis/ Calculations	1. To edit colonies (this option is only available after saving), double-click on the row listed in the Data View. a. To remove marked colonies, click with the right mouse button on the green circles. The circles will turn red to show that they will not be used in the count. b. To add colonies that were not marked as counted, click with the left mouse button. A blue circle will mark the colony, indicating that it will be added to the count. To exclude a newly-marked circle, simply click on it again with the right mouse button. c. Click **Exit** and select **Yes** when asked if the data should be saved. d. Enter the reason for editing the data and click **OK**. The saved data will replace the row in the Data View, and a flag will be assigned (refer to the PetriScan® User Guide, page 27, section 3.6.2 for information regarding flags). The previous data, flag notations, and reason for change will be archived to Audit View.
14. Forms and Data Sheets	1. Attachment 1: Sample Film with Growth 2. Attachment 2: Sample Data Spreadsheet 3. Attachment 3: Sample Audit View
15. References	1. PetriScan® User Guide, Spiral Biotech, Inc., Rev3 110104. 2. Hewlett-Packard Scanjet 8200 Series Scanners User's Manual, Hewlett-Packard, 2003. 3. 3M Petrifilm™ Aerobic Count Plate Pamphlet, 3M, 2004 (38-9018-1246-1). 4. 3M Petrifilm™ Aerobic Count Plate Interpretation Guide, 3M, 2005 (70-2008-4572-8). 5. Storage and Shelf Life of 3M™ Petrifilm™ Plates, 3M Technical Bulletin, October 2008 (TB.002.02). 6. Nelson, M.T., LaBudde, R.A., Tomasino, S.F., and Pines, R.M. (2013) *J. AOAC Int.* 96, 717-722.

Attachment 1

Sample Film with Growth



Attachment 2

Sample Data Spreadsheet

Name QC
Title Quality Control
Reference
High Count Limit 300
Low Count Limit 0
Created by MLB

Plate #*	Plate ID	Dilution	Count	CFU/ml	Flag	Date	Time	User ID
238	Template 1, 03/12	0	30	3.00E+01	ID ED	3/14/2012	2:04:28 PM	MLB
239	Template 2, 03/12	0	200	2.00E+02	ID ED	3/14/2012	2:04:28 PM	MLB
240	Uninoculated 1,03/12	0	0	0.00E+00	ED	3/14/2012	2:04:29 PM	MLB
241	Uninoculated 2,03/12	0	0	0.00E+00		3/14/2012	2:04:29 PM	MLB
242	Template 1, 06/12	0	30	3.00E+01	ID ED	6/28/2012	2:38:02 PM	MLB
243	Template 2, 06/12	0	200	2.00E+02	ID ED	6/28/2012	2:38:03 PM	MLB
244	Uninoculated 1,06/12	0	0	0.00E+00	ID ED	6/28/2012	2:38:04 PM	MLB
245	Uninoculated 2,06/12	0	0	0.00E+00	ID ED	6/28/2012	2:38:04 PM	MLB
246	Template 1, 09/12	0	30	3.00E+01	ID ED	9/26/2012	3:44:11 PM	MLB
247	Template 2, 09/12	0	200	2.00E+02	ID ED	9/26/2012	3:44:11 PM	MLB
248	Uninoculated 1,09/12	0	0	0.00E+00	ED	9/26/2012	3:44:11 PM	MLB
249	Uninoculated 2,09/12	0	0	0.00E+00	ED	9/26/2012	3:44:11 PM	MLB
250	Template 1, 12/12	0	30	3.00E+01	ID ED	12/24/2012	11:26:26 AM	MLB
251	Template 2, 12/12	0	200	2.00E+02	ID ED	12/24/2012	11:26:26 AM	MLB
252	Uninoculated 1,12/12	0	0	0.00E+00	ED	12/24/2012	11:26:26 AM	MLB
253	Uninoculated 2,12/12	0	0	0.00E+00		12/24/2012	11:26:26 AM	MLB

Attachment 3

Sample Audit View

PetriScan Quality Control Procedure Records for 2012 (Audit View)
OPP Microbiology Laboratory

Name QC
Title Quality Control
Reference
High Count Limit 300
Low Count Limit 0
Created by MLB

Plate #	Plate ID	Dilution	Count	CFU/ml	Flag	Mod Date	Mod Time	Mod By	Reason
238	Template 1	0	31	3.10E+01		3/14/2012	2:05:13 PM	MLB	Added date to ID, deleted piece of dust.
239	Template 2	0	202	2.02E+02		3/14/2012	2:05:34 PM	MLB	Added date to ID, deleted piece of dust.
240	Uninoculated 1,03/12	0	3	3.00E+00		3/14/2012	2:05:46 PM	MLB	Deleted pieces of dust.
242	Template 1	0	32	3.20E+01		6/28/2012	2:38:45 PM	MLB	Added date to ID, deleted pieces of dust.
243	Template 2	0	202	2.02E+02		6/28/2012	2:40:24 PM	MLB	Added date to ID, deleted pieces of dust.
244		0	2	2.00E+00		6/28/2012	2:40:51 PM	MLB	Added ID, deleted pieces of dust.
245		0	2	2.00E+00		6/28/2012	2:41:15 PM	MLB	Added ID, deleted pieces of dust.
246	Template 1	0	32	3.20E+01		9/26/2012	3:44:41 PM	MLB	Added date to ID, deleted pieces of dust.
247	Template 2	0	202	2.02E+02		9/26/2012	3:44:57 PM	MLB	Added date to ID, deleted pieces of dust.
248	Uninoculated 1,09/12	0	3	3.00E+00		9/26/2012	3:45:10 PM	MLB	Deleted pieces of dust.
249	Uninoculated 2,09/12	0	2	2.00E+00		9/26/2012	3:45:19 PM	MLB	Deleted pieces of dust.
250	Template 1	0	32	3.20E+01		12/24/2012	11:27:02 AM	MLB	Added date to ID, deleted pieces of dust.
251	Template 2	0	201	2.01E+02		12/24/2012	11:27:23 AM	MLB	Added date to ID, deleted piece of dust.
252	Uninoculated 1,12/12	0	5	5.00E+00		12/24/2012	11:27:50 AM	MLB	Deleted pieces of dust.