



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

**Standard Operating Procedure for  
Glass Washing and Detergent Residues  
Test**

**Date Revised: 03-17-08**

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

Standard Operating Procedure  
for  
Glass Washing and Detergent Residues Test

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1.0 SCOPE AND APPLICATION:

1.1 This protocol describes procedures for washing laboratory glassware and for shipping glassware to a contract laboratory for analysis. Detergents used in washing glassware may leave residues which are bacteriostatic. If residues are present, glassware may require additional rinsing to remove them (see ref. 15.1 and 15.2).

2.0 DEFINITIONS: None

3.0 HEALTH AND SAFETY: None

4.0 CAUTIONS:

4.1 The plates will be analyzed by a contract laboratory qualified to perform detergent residue testing (for example; QC Laboratories, Southhampton, PA). In preparation for shipment to the contract laboratory, the Petri dishes must be placed in a cardboard box containing shipping peanuts or suitable packing material. Petri plates should be shipped within 36 hours after completion of sterilization.

5.0 INTERFERENCES:

5.1 All glassware will be inspected prior to use. Discard items with chips and etched surfaces.

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 Miele Thermal Disinfector/Laboratory Glassware Washer Model G7783 serial number 16/18344823 located in room B206 (Glassware and Media Preparation Room) of the OPP Microbiology Laboratory at the ESC, Ft. Meade, MD.

7.2 Lancer1600 UP Laboratory Glassware Washer serial number 9G050714 located in room B206 (Glassware and Media Preparation Room) of the OPP Microbiology Laboratory at the ESC, Ft. Meade, MD.

- 7.3 Powder Detergent for Miele dishwasher
  - 7.4 Liquid Detergent for Lancer dishwasher
  - 7.5 Alconox Powdered Precision Cleaner
  - 7.6 Glass Petri Dishes (20 X 100 mm)
  - 7.7 Cardboard box containing shipping peanuts or suitable packing material
  - 7.8 Disposable plastic Petri dishes provided by the contract laboratory
- 8.0 INSTRUMENT OR METHOD CALIBRATION: Not applicable
- 9.0 SAMPLE HANDLING AND STORAGE:
- 9.1 Detergents will be used and stored according to manufacturer's instructions.
  - 9.2 Refer to section 4.0 for cautions associated with handling of Petri dishes.
- 10.0 PROCEDURE AND ANALYSIS:
- 10.1 The Detergent Residues Test will be performed annually or when a new lot or different type of detergent is used. The test bacteria used is *Enterobacter aerogenes*.
  - 10.2 Glassware will be washed in the Miele Thermal Disinfector/Laboratory Glassware Washer and the Lancer1600 UP Laboratory Glassware Washer. Hand-washed items will be analyzed when hand washing is actively being utilized.
  - 10.3 The procedure includes four groups of six Petri dishes. Group A, Group B and Group C contain Petri dishes washed by dishwasher and Group D contains sterile Petri dishes as a reference point. Preparation numbers are assigned to each group of Petri dishes according to SOP QC-15, Media Prep and Sterilization Run Numbers. Each group (Group A, Group B and Group C) is washed separately in each dishwasher. The six glass Petri dishes in Group A are washed and rinsed by the regular procedure used in the laboratory for the particular dishwasher or by hand. The six glass Petri dishes in Group B are washed by the regular procedure used in the laboratory followed by twelve additional rinses. The six glass Petri dishes in Group C are washed with the detergent at the dilution normally used in the laboratory but the Petri dishes are not run through any rinse cycles. The six sterile, plastic Petri dishes in Group D are provided by the testing laboratory as a

reference point.

10.3.1 In the Glass Washing and Detergent Residues Test, laboratory staff treat the Petri dishes as described below and ship them to a vendor (for example, QC Laboratories) for analysis.

10.4 Machine Washed Glassware in the Miele Thermal Disinfector/Laboratory Glassware Washer Model G7783

10.4.1 Group A consisting of six glass Petri dishes

10.4.1.1 Place the Petri dishes in the dishwasher facing down and spaced evenly so the water will run out of the dish. Place three Petri dishes in the lower compartment and three Petri dishes in the upper compartment.

10.4.1.2 Fill the detergent compartment on the door with the amount suggested by the manufacturer of powder detergent (2 scoops) and close the detergent cover. Record the amount of detergent used on the media prep sheet. Use the detergent as specified by the manufacturer. In addition, place one scoop of detergent directly on the washer door and a second one on the base of the dishwasher for the Pre-wash cycle.

10.4.1.3 Press the button for Program E, the Universal wash program, and then press the Start button (diamond with vertical bar symbol). This program includes a pre-wash and heated main wash (85°C), two tap water rinses and two DI water rinses, one unheated and one heated (70°C). All rinses use recycled rinse water from the washer reservoir. This will constitute the normal treatment that all machine-washed laboratory glassware receives in this dishwasher.

10.4.2 Group B consisting of six glass Petri dishes

10.4.2.1 Place the Petri dishes in the dishwasher facing down and spaced evenly so the water will run out of the dish. Place three Petri dishes in the lower compartment and three Petri dishes in the upper compartment.

10.4.2.2 Fill the detergent compartment on the door with the amount

suggested by the manufacturer of powder detergent (2 scoops) and close the detergent cover. Record the amount of detergent used on the media prep sheet. Use the detergent as specified by the manufacturer. In addition, place one scoop of detergent directly on the washer door and a second one on the base of the dishwasher for the Pre-wash cycle.

10.4.2.3 Press the button for Program E, the Universal wash program, and then press the Start button (diamond with vertical bar symbol). This program includes a pre-wash and heated main wash (85°C), two tap water rinses and two DI water rinses, one unheated and one heated (70°C). All rinses use recycled rinse water from the washer reservoir. This will constitute the normal treatment that all machine-washed laboratory glassware receives in this dishwasher.

10.4.2.4 To achieve the additional twelve rinses for Group B, repeat the following steps twelve times to achieve the twelve additional rinses.

- Turn the dishwasher off at the end of the cycle.
- While pressing the T1 and T2 buttons, turn the dishwasher back on.
- Select Program D. Press the T2 button so that the temperature is set to 60°C. It may be necessary to press the T2 button a few times to set the temperature to 60°C.
- Continuously press the start button until "38" appears in the display. Note: As the display climbs from 1 to 38, you will hear various components in the machine cycle on and off. This is normal.
- Washer will automatically stop at the end of the cycle (approximately 12-15 minutes).

10.4.3 Group C consisting of six glass Petri dishes

10.4.3.1 Place the Petri dishes in the dishwasher facing down and spaced evenly so the water will run out of the dish. Place three Petri dishes in the lower compartment and three Petri dishes in the upper compartment.

10.4.3.2 Fill the detergent compartment on the door with the amount

suggested by the manufacturer of powder detergent (2 scoops) and close the detergent cover. Record the amount of detergent used on the media prep sheet. Use the detergent as specified by the manufacturer. In addition, place one scoop of detergent directly on the washer door and a second one on the base of the dishwasher for the Pre-wash cycle.

10.4.3.3 Press the button for Program E, the Universal wash program, and then press the Start button (diamond with vertical bar symbol).

10.4.3.4 The washer must be stopped after the main wash cycle but before the first tap water rinse, approximately 20 minutes after starting the Universal cycle when the digital display reads 20 minutes, thus eliminating the two tap water rinses and two DI water rinses.

#### 10.5 Machine Washed Glassware in the Lancer 1600 UP Laboratory Glassware Dishwasher

10.5.1 Group A consisting of six glass Petri dishes.

10.5.1.1 Place the Petri dishes in the dishwasher facing down and spaced evenly so the water will run out of the dish. Place three Petri dishes in the lower compartment and three Petri dishes in the upper compartment.

10.5.1.2 Enter 10 on the keypad to select Cycle 10 and press the Start button. Cycle 10 is the designated standard laboratory wash program. This program includes a pre-wash and heated main wash (85°C), two tap water rinses and three DI water rinses, two unheated and one heated (70°C). All rinses use recycled rinse water from the washer reservoir. Liquid detergent is dispensed automatically through a metering pump at the rate of approximately 5 mL/sec in this dishwasher. The water consumption estimated per operation (according to the baskets used) is 30 L. The metering pump during normal wash is set to run for approximately 75 seconds to deliver the appropriate volume of detergent.

- 10.5.2           Group B consisting of six glass Petri dishes.
- 10.5.2.1       Place the Petri dishes in the dishwasher facing down and spaced evenly so the water will run out of the dish. Place three Petri dishes in the lower compartment and three Petri dishes in the upper compartment.
  - 10.5.2.2       Enter 10 on the keypad to select Cycle 10 and press the Start button. Cycle 10 is the designated standard laboratory wash program. This program includes a pre-wash and heated main wash (85°C), two tap water rinses and three DI water rinses, two unheated and one heated (70°C). All rinses use recycled rinse water from the washer reservoir. Liquid detergent is dispensed automatically through a metering pump at the rate of approximately 5 mL/sec in this dishwasher. The water consumption estimated per operation (according to the baskets used) is 30 L. The metering pump during normal wash is set to run for approximately 75 seconds to deliver the appropriate volume of detergent.
  - 10.5.2.3       To achieve the additional twelve rinses for Group B, run Cycle 30 four times. Cycle 30 consists of 3 cold demineralized rinses. Enter 30 on the keypad to select Cycle 30 and press the Start button.
- 10.5.3           Group C consisting of six glass Petri dishes.
- 10.5.3.1       Place the Petri dishes in the dishwasher facing down and spaced evenly so the water will run out of the dish. Place three Petri dishes in the lower compartment and three Petri dishes in the upper compartment.
  - 10.5.3.2       Enter 20 on the keypad to select Cycle 20 and press the Start button. Cycle 20 includes a pre-wash and heated main wash (85°C) without the two tap water rinses and three DI water rinses. Liquid detergent is dispensed automatically through a metering pump at the rate of approximately 5 mL/sec in this dishwasher. The metering pump during normal wash is set to run for approximately 75 seconds to deliver the appropriate volume of detergent.

10.6 Hand Washed Items (when needed)

- 10.6.1 For Group A, wash six glass Petri dishes in Alconox detergent at the manufacturers' prescribed dilution (1%), rinsed with tap water, and then two times with DI water.
- 10.6.2 For Group B, wash six glass Petri dishes in Alconox detergent at the manufacturers' prescribed dilution (1%), rinsed with tap water, and then two times with DI water. To achieve the additional twelve rinses for Group B, rinse the Petri dishes twelve additional times with DI water (use DI water as it comes from the DI faucet not the DI water from the Barnstead unit).
- 10.6.3 For Group C, wash six glass Petri dishes in Alconox detergent at the manufacturers' prescribed dilution (1%) omitting the tap water or DI rinses.

10.7 Resource Management

- 10.7.1 Water Conservation. Laboratory personnel should be mindful of water consumption, and whenever possible, employ practices that minimize water use.
  - 10.7.1.1 Specifically, laboratory personnel should run full loads whenever possible, and consider wash programs that conserve water and produce glassware or labware appropriate for its intended use.

10.8 Sterilization

- 10.8.1 Wrap each group of dishes in brown paper obtained from room A140 (Region 3), and record group designation and dishwasher type (Miele A, Miele B, Miele C, Lancer A, Lancer B, Lancer C, Handwash A, Handwash B or Handwash C) on outside of paper. Sterilize all plates for each Group in the usual manner (gravity cycle for 25 min). Record the sterilization run number on the appropriate Laboratory Detergent Residue Test Form (see 16.1 and 16.2).

10.9 Shipping

- 10.9.1 Once all plates have been sterilized, place the wrapped plates in a

cardboard box with shipping peanuts or suitable packing material to prevent the plates from breaking during shipment.

- 10.10 Obtain a FedEx airbill from one of the Environmental Science Center's mailroom and complete it. Note the following items:
  - 10.10.1 In section 1, enter laboratory's return address as Environmental Protection Agency, 701 Mapes Road, Ft. Meade, MD 20755-5350.
  - 10.10.2 In section 2, enter "Laboratory Glassware" as the internal billing reference information.
  - 10.10.3 In section 3, enter the contract laboratory's name, address, and phone number (for example, QC Laboratories' address as QC Laboratories, 1205 Industrial Blvd., Southampton PA 18966-0514 and the phone number as 215-355-3900).
  - 10.10.4 In section 4a, check the "FedEx Express Saver" box
  - 10.10.5 In section 5, check the "Other Pkg." box.
  - 10.10.6 In section 6, do not check either of the "HOLD" or "WEEKEND" Delivery boxes. Also, check the "No" box under dangerous goods.
  - 10.10.7 In section 7, check the "Sender" box and fill in the FedEx account number.
  - 10.10.8 Detach the "Sender's Copy" of the airbill, make a copy and place the copy in the Quality Assurance of Glass Washing and Detergent Residues Record Book. Give the original "Sender's Copy" to Jane Ulrich in D105 for billing reconciliation purposes.
- 10.11 Place the airbill inside the airbill holder. Peel off the paper from the backside (larger, lower section of paper) of the airbill holder to reveal an adhesive surface. Attach the airbill holder to the top of the shipping container. Do not peel off the narrow, top strip of paper from the airbill. FedEx must be able to pull out the airbill from the airbill holder.
- 10.12 Take the package to the Region III dock area for pickup by FedEx or as an alternative, the analyst may take the package directly to a local FedEx office for mailing.

10.13 Notify contract laboratory that package is being shipped for Detergent Residue Analysis (for example, QC Laboratory phone number 215-355-3900).

11.0 DATA ANALYSIS/CALCULATIONS:

11.1 When the report of inhibitory residue is received from the contract laboratory, record the testing results legibly and in indelible ink under the “Calculations and Conclusions” column.

11.2 Differences in averaged counts on plates in Groups A through D should be less than 15% if there are no toxic or inhibitory effects. Differences in averaged counts of less than 15% between Groups A and B and greater than 15% between Groups A and C indicate that the cleaning detergent has inhibitory properties that are eliminated during routine washing. Differences between B and D greater than 15% indicate an inhibitory residue.

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 The reports of inhibitory residue analysis and completed forms (section 16.0) must be placed in the Quality Assurance of Glass Washing and Detergent Residues Test Book.

12.1 Data will be recorded promptly, legibly, and in indelible ink on the forms. Completed forms are archived in notebooks kept in secured file cabinets in file room D217. Only authorized personnel have access to these files. Archived data are subject to OPP’s official retention schedule contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 The Detergent Residues Test will be performed at least once yearly or when a new lot or different type of detergent is used. The test bacteria used is *Enterobacter aerogenes*.

13.2 The OPP Microbiology Laboratory conforms to 40CFR Part 160, Good Laboratory Practice Standards. Appropriate quality control measures are integrated into each SOP.

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

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Any deviation from the protocol will be documented. If the regular wash procedure (Group A plates) is found not to be adequate for removal of inhibitory detergent residues then the wash procedure will be adjusted and the detergent residue test repeated until the wash procedure has confirmed that all inhibitory residues have been removed.

15.0 REFERENCES:

15.1 Bordner, R. H., J. A. Winter and P. V. Scarpino. eds. 1978. Microbiological Methods for Monitoring the Environment, Water and Wastes. EPA-600/8-78-017, Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.

15.2 Eaton, A. D., Clesceri, L. S., Rice, E. W., Greenberg, A. E. and Franson, M. A. H. eds. 2005. Standard Methods for the Examination of Water and Wastewater, 21<sup>st</sup> Edition. American Public Health Association, Washington, DC.

16.0 FORMS AND DATA SHEETS:

16.1 Laboratory Detergent Residue Test Form for Machine Washed Items

16.2 Laboratory Detergent Residue Test Form for Hand Washed Items

Laboratory Detergent Residue Test Form for Machine Washed Items  
 OPP Microbiology Laboratory

Test Information			
Washer Name/Model		Date Test Performed	
Detergent Name		Organism	
Lot No.			
Detergent Control #			
Glassware Preparation			
	Group A*	Group B*	Group C*
Date Washed			
Date Sterilized			
Sterilization Run #			
Prep No.			

Calculations and Conclusions			
	A\B	A\C	B\D
% Difference $\perp$			
Comments			

\* The treatments/groups are defined as follows, A is normal wash, B is normal wash followed by twelve additional DI rinses, C is washed but not rinsed, D is sterile disposable Petri dishes used as controls and supplied by the contract laboratory.

$\perp$  Differences in colony counts of more than 15% in any of the three comparisons indicate inhibitory residues.

Laboratory Detergent Residue Test Form for Hand Washed Items  
 OPP Microbiology Laboratory

Test Information			
Washer Name/Model		Date Test Performed	
Detergent Name		Organism	
Lot No.			
Detergent Control #			
Glassware Preparation			
	Group A*	Group B*	Group C*
Date Washed			
Date Sterilized			
Sterilization Run #			
Prep No.			

Calculations and Conclusions			
	A\B	A\C	B\D
% Difference $\perp$			
Comments:			

\* The treatments/groups are defined as follows, A is normal wash, B is normal wash followed by twelve additional DI rinses, C is washed but not rinsed, D is sterile disposable Petri dishes used as controls and supplied by the contract Laboratory.

$\perp$  Differences in colony counts of more than 15% in any of the three comparisons indicate inhibitory residues.