



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

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

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

SOP Number: QC-03-07

Date Revised: 04-16-14

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Title	Glass Washing and Detergent Residues Test
Scope	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 section 15).
Application	To verify that detergent residue is not present in laboratory glassware when cleaned using the dishwashers.

	Approval	Date
SOP Developer:	_____	_____
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1. Definitions	Abbreviations/definitions are provided in the text.
2. Health and Safety	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.
3. Personnel Qualifications and Training	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	Not Applicable.
5. Sample Handling and Storage	<ol style="list-style-type: none"> 1. Use and store detergents according to manufacturer's instructions. 2. Refer to section 10 for cautions associated with handling of Petri dishes.
6. Quality Control	For quality control purposes, the required information is documented on the appropriate form(s) (see section 14).
7. Interferences	<ol style="list-style-type: none"> 1. Inspect all glassware prior to use. Discard items with chips and etched surfaces. 2. Ensure dishwashers are working properly prior to commencing the assay (e.g., scheduled preventive maintenance, if available). 3. Petri plates should be shipped within 36 hours after completion of sterilization.
8. Non-conforming Data	<ol style="list-style-type: none"> 1. Management of non-conforming data will be consistent with SOP ADM-07, Non-Conformance Reports. 2. Any deviation from the protocol will be documented. If the regular wash procedure (Group A plates) is found to be inadequate for removal of inhibitory detergent residues, then the wash procedure will be adjusted and the detergent residue test repeated.
9. Data Management	<ol style="list-style-type: none"> 1. Data will be archived consistent with SOP ADM-03, Records and Archives. 2. The plates are analyzed by a contract laboratory qualified to perform detergent residue testing (for example; QC Laboratories, Southampton, PA). The reports of inhibitory residue analysis received from the contract laboratory and completed forms must be placed in the Quality Assurance of Glass Washing and Detergent Residues Test Book.
10. Cautions	<ol style="list-style-type: none"> 1. Ensure plates are properly packaged in a cardboard box with suitable packing material prior to shipping to contract laboratory.

	<ol style="list-style-type: none"> 2. Laboratory personnel should run full dishwasher loads whenever possible, and consider wash programs that conserve water and produce glassware or labware appropriate for its intended use.
<p>11. Special Apparatus and Materials</p>	<ol style="list-style-type: none"> 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. 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. 3. Powder Detergent for Miele dishwasher 4. Liquid Detergent for Lancer dishwasher 5. Alconox Powdered Precision Cleaner 6. Glass Petri Dishes (20 × 100 mm) 7. Cardboard box and suitable packing material 8. Disposable plastic Petri dishes provided by the contract laboratory
<p>12. Procedure and Analysis</p>	<p>Perform the detergent residues test annually or when a new lot or different type of detergent is used. The test bacteria used is <i>Enterobacter aerogenes</i>.</p> <ol style="list-style-type: none"> 1. All rinses use recycled rinse water from the washer reservoir. 2. Glassware is washed in the Miele Thermal Disinfector/Laboratory Glassware Washer and the Lancer1600 UP Laboratory Glassware Washer. 3. Use the detergent as specified by the manufacturer. 4. Hand-washed items will be analyzed when hand washing is actively being utilized. 5. Wash each group (Group A, Group B and Group C) separately in each dishwasher. 6. The procedure includes four groups of six Petri dishes each. Group A, Group B and Group C contain Petri dishes washed by dishwashers and Group D contains sterile plastic Petri dishes as a reference point. 7. Preparation numbers are assigned to each group of Petri dishes

	<p>according to SOP QC-15, Media Prep and Sterilization Run Numbers.</p> <ol style="list-style-type: none"> 8. Group A (six glass Petri dishes) are washed and rinsed by the regular procedure used in the laboratory for the particular dishwasher. 9. Group B (six glass Petri dishes) are washed by the regular procedure used in the laboratory followed by twelve additional rinses. 10. Group C (six glass Petri dishes) are washed with the detergent at the dilution normally used in the laboratory but the Petri dishes are not run through any rinse cycles. 11. Group D (six sterile, plastic Petri dishes) are provided by the testing laboratory as a reference point.
<p>12.1 Washing Procedure for Miele Thermal Disinfector/Laboratory Glassware Washer (Model G7783)</p>	<p>Group A (six glass Petri dishes):</p> <ol style="list-style-type: none"> a. 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. b. Fill the detergent compartment on the door with the amount suggested by the manufacturer of powder detergent (2 scoops) and close the detergent cover. 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. A total of 4 scoops are used. Record the amount of detergent used on the media prep sheet. c. 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). This will constitute the normal treatment that all machine-washed laboratory glassware receives in this dishwasher. <p>Group B (six glass Petri dishes):</p> <ol style="list-style-type: none"> a. 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.

	<ul style="list-style-type: none">b. Fill the detergent compartment on the door with the amount suggested by the manufacturer of powder detergent (2 scoops) and close the detergent cover. 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. A total of four scoops are used. Record the amount of detergent used on the media prep sheet.c. 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). This will constitute the normal treatment that all machine-washed laboratory glassware receives in this dishwasher.d. To achieve the additional twelve rinses for Group B, repeat the following steps twelve times:<ul style="list-style-type: none">e. Turn the dishwasher off at the end of the cycle.f. While pressing the T1 and T2 buttons, turn the dishwasher back on.g. 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.h. 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.i. Washer will automatically stop at the end of the cycle (approximately 12-15 minutes). Repeat steps 12.1 i-1 a total of twelve times. <p>Group C (six glass Petri dishes):</p> <ul style="list-style-type: none">a. 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.b. Fill the detergent compartment on the door with the amount suggested by the manufacturer of powder detergent (2 scoops) and close the detergent cover. In addition, place one scoop of detergent directly on the washer door and a second one on the
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	<p>base of the dishwasher for the Pre-wash cycle. A total of four scoops are used. Record the amount of detergent used on the media prep sheet.</p> <ul style="list-style-type: none"> c. Press the button for Program E, the Universal wash program, and then press the Start button (diamond with vertical bar symbol). d. 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.
<p>12.2 Washing Procedure for Lancer 1600 UP Laboratory Glassware Dishwasher</p>	<p>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.</p> <p>Group A (six glass Petri dishes):</p> <ul style="list-style-type: none"> a. 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. b. 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). <p>Group B (six glass Petri dishes):</p> <ul style="list-style-type: none"> a. 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. b. 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). c. To achieve the additional twelve rinses for Group B, run Cycle

	<p>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.</p> <p>Group C (six glass Petri dishes):</p> <ol style="list-style-type: none"> a. 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. b. 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.
<p>12.3 Hard Washing Procedure (when needed)</p>	<ol style="list-style-type: none"> a. 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. b. 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). c. Group C: wash six glass Petri dishes in Alconox detergent at the manufacturers' prescribed dilution (1%) omitting the tap water or DI rinses.
<p>12.4 Petri Plate Preparation and Sterilization</p>	<ol style="list-style-type: none"> a. Wrap each group of dishes in brown paper obtained from room A140 (Region 3). b. 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. c. Sterilize all plates for each Group in the usual manner (gravity cycle for 25 min). d. Record the sterilization run number on the appropriate Laboratory Detergent Residue Test Form (see section 14).

<p>12.5 Shipping</p>	<ol style="list-style-type: none"> a. After sterilization, place the wrapped plates in a cardboard box with suitable packing material to prevent the plates from breaking during shipment. b. Obtain a chain of custody form (COC form) from the contract laboratory's website (for example QC laboratories). Fill the form with all the necessary information. The original COC form is placed inside the box with plates; a copy is retained for internal records. c. Use a shipping company that is authorized by the agency. Obtain shipping company's airbill from MLB authorized personnel and fill with all necessary information. Shipping should be conducted overnight or next day to ensure plates arrive at the contract laboratory within 36 hours after sterilization (see section 7). d. Notify the ESC mailroom personnel of the package to be shipped. e. Ship package to contract laboratory, for example: QC Laboratories, 1205 Industrial Blvd., Southampton PA 18966-0514 and the phone number as 215-355-3900. f. Notify contract laboratory that package is being shipped for Detergent Residue Analysis (for example, QC Laboratory phone number 215-355-3900).
<p>13. Data Analysis/ Calculations</p>	<ol style="list-style-type: none"> 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 (see section 14). 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. 3. 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. 4. Differences between B and D greater than 15% indicate an inhibitory residue.
<p>14. Forms and Data Sheets</p>	<p>Test Sheets. Test sheets are stored separately from the SOP under the following file names:</p>

	Laboratory Detergent Residue Test Form QC-03-07_F1.docx for Machine Washed Items Laboratory Detergent Residue Test Form QC-03-07_F2.docx for Hand Washed Items
15. References	<ol style="list-style-type: none">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.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, 21st Edition. American Public Health Association, Washington, DC.