Biology Wash Party Procedures

1. SCOPE AND APPLICATION

1.1 This procedure details the responsibilities and general procedure for cleaning and sterilization of glassware in the Biology lab.

2. SUMMARY OF THE METHOD

2.1 Members of the Taxonomy Laboratory Tech Workgroup are scheduled to participate in each weekly Wash Party. Other members of the Biology Program are scheduled in rotation to participate in Wash Party. The Wash Party Scheduler sends out an availability request and prepares a monthly Wash Party Schedule. A Wash Party Lead person is designated for each week from the Laboratory Tech Workgroup. It is the responsibility of each person to (1) notify the Wash Party Scheduler of your availability when asked, (2) participate when scheduled, (3) if your schedule changes, find a replacement/swap and obtain appropriate approval for the switch, and (4) if the absence is unexpected, communicate the change to the Wash Party Scheduler or supervisor.

2.2 The Wash Party Lead, as designated per schedule, is responsible for Wash Party preparation per Section 5. The Lead collects and organizes glassware on carts, clears work surfaces, and checks washing and chemical supplies. They also load and run autoclaves and ovens; although another person may be designated.

2.3 Each weekly participant chooses one of six sink stations or steps to perform in the glassware cleaning process. The Wash Party Lead is responsible for the seventh station, Foil/Autoclave/Drying Station. One person should not always perform the same step at the sinks. Ideally, everyone in the Wash Party rotation will take turns performing the different steps. If participants are unable to equitably share tasks, the Wash Party Scheduler will assign stations as fairly as possible.

2.4 Glassware is washed, dried and/or sterilized, and stored.

3. APPARATUS AND EQUIPMENT

3.1 Washing bucket
3.2 Brushes
3.3 Aluminum foil
3.4 Autoclave indicator tape (beige and blue colored)
3.5 Cart
3.6 Safety equipment: gloves, safety glasses, lab coat or apron, oven mitt
3.7 Drying mats, prepared with adsorbent cloth and rubber mesh, or paper towels for lining carts

4. REAGENTS AND CHEMICALS

4.1 Liqui-Nox detergent (or any other suitable non-phosphate detergent)
4.2 Hydrochloric Acid, 10%, prepared per SOP TA-10-10
4.3 Acetone (pesticide grade is required for bioassay)
4.4 Bleach Solution, 10%, Add 100 mL 100% bleach to 900 mL deionized (DI) water.

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4.5 Sodium Thiosulfate (Na2S2O35H2O), 0.025 N, ACS grade, 20%, Add 200 mL sodium thiosulfate to 800 mL DI water.

5. PREPARATION FOR WASH PARTY

5.1 Members of each workgroup are responsible for placing glassware on a cart. The Wash Party Lead, as designated per schedule, is responsible for collecting carts from the Algal, Toxicity, Microbiology, Invertebrate and Molecular Labs on the morning of the Wash Party.

5.2 The Lead arranges the glassware on carts in the order it is to be washed. The Lead inspects all glassware for chips or cracks that pose a potential risk of breakage or sharp edges. Compromised glassware is properly disposed and recorded in the broken glassware log (see Step 9.4). Glassware used in the Molecular Lab for Polymerase Chain Reaction (PCR) should be kept together as it is washed first using a modified procedure. Items where liquid can be poured from one container into another (e.g. beakers, graduated cylinders) usually are washed next. Washed last are small items and items that need to be dipped (e.g. test tubes, caps, funnels).

5.3 Before the Wash Party begins, the Lead is responsible for:

- Checking the supply of soap, acid, acetone (amount available and grade), bleach, sodium thiosulfate, autoclave tape and aluminum foil. If any supplies are low or will be low soon, the Lead notifies the Laboratory Tech responsible for overseeing Wash Party supplies to ensure that an order has been or will be made before supplies are depleted.
- Emptying the drying oven(s) so that space is available in the oven for newly washed glassware. Glassware with a Tox label or as described in Table 1 as belonging to the Tox group should be removed from the drying ovens and taken to the Tox Lab on a cart.
- Cutting foil in squares for glassware that will be autoclaved and/or dried.
- Removing empty invertebrate sample containers or other items from the sink area and puts them away.
- Properly purging and setting up the autoclave for use (refer to SOP BG-08, *STERIS AMSCO Series 250LS Autoclave Calibration and Use*).

6. PCR GLASSWARE WASHING PROCEDURE

6.1 Wash Station: A 5 gallon washing bucket is filled ¾ with tap water and a cap-full of Liqui-Nox detergent. The Wash person chooses an appropriate brush for each item of glassware and then scrubs the item on both the inside and outer surfaces with the soapy water.

6.2 Tap Water Rinse #1 Station: The item gets rinsed in tap water until all soap has been removed from all surfaces. The rinse person inspects the glassware for any dirt or residue missed during the washing process and sends it back if anything is found.

6.3 Bleach Rinse Station: The rinsed glassware is washed with 10% bleach solution. Bleach should be carefully poured from one item into the next or poured into a bowl so that it can be re-used during the Wash Party.

6.4 Tap Water Rinse #2 Station: The item is rinsed inside and out with tap water.

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6.5 Sodium Thiosulfate Rinse Station: The item gets washed with 20% sodium thiosulfate. Sodium thiosulfate should be carefully poured from one item into the next or poured into a bowl so that it can be re-used during the Wash Party.

6.6 DI Water Rinse Station: The item gets rinsed thoroughly with DI water as a final rinse. The final rinse person inspects the glassware for any dirt or residue missed during the washing process. If dirt or residue is found, the item is sent through the complete process again (Steps 6.1-6.6).

6.7 Foil/Autoclave/Drying Station: Glassware is sterilized and/or dried, then stored. (Table 1)

Note: Before foiling, dump out excess water.

Note: For items covered with foil, make sure that items are foiled completely and that vacuum holes and other openings are covered.

Note: Items needing to be autoclaved must have a small piece of blue autoclave indicator tape (beige for general glassware) attached to them to verify that the item went through the autoclave process and that it reached a temperature high enough to trigger the color change in the tape (i.e. to kill bacteria).

6.8 Autoclave using Cycle 3: Gravity, 15 min, at 121°C. See SOP TA-06.18 for autoclave maintenance and use. Drying ovens are set to 90°F.

6.9 If a piece of glassware breaks, follow proper safety and reporting procedures (see Step 9.4).

7. GENERAL GLASSWARE WASHING PROCEDURE

7.1 Wash Station: Follow Step 6.1.

7.2 Tap Water Rinse #1 Station: Follow Step 6.2.

7.3 Acid Rinse Station: The rinsed glassware is washed with 10% hydrochloric acid. Acid should be carefully poured from one item into the next or poured into a bowl so that it can be re-used during the Wash Party. No more than 400ml of acid should be needed during the Wash Party.

7.4 Tap Water Rinse #2 Station: Follow Step 6.4

7.5 Acetone Rinse Station: The item gets washed with acetone. Acetone should be carefully poured from one item into the next or poured into a bowl so that it can be re-used during the Wash Party. No more than 400ml of acetone should be needed during the Wash Party.

Note: Pesticide grade acetone is required for bioassay so it may be available for use.

7.6 DI Water Rinse Station: Follow Step 6.6 with the exception that if dirt or residue is found, the item is sent through the complete process again following Steps 7.1-7.6. All acetone should be thoroughly rinsed out at this station so that the “waft” check in 7.7 is clear.

7.7 Foil/Autoclave/Drying Station: Follow Step 6.7-6.9 with the exception that beige autoclave tape is used instead of the blue autoclave tape.

Note: The person at this station should also do the “waft” check to make sure there is no residual acetone before sealing it. If there is residual acetone, the item should be sent back to the DI Water Rinse Station for more rinsing.

Note: Small items can be placed in a metal bin during the autoclave and drying oven steps.

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Table 1. Foil, Autoclave, and Drying Oven Instructions for Each Type of Glassware.

Type of Glassware	Foiled?	Autoclaved?	Oven?	Special Instructions
PCR Orange-Cap Bottles	Yes	Yes	Yes	Place cap on bottle loosely and wrap completely in foil. Use blue autoclave tape.
PCR Plastic Rinse Bottles	Yes	Yes	Yes	Place cap on bottle loosely and wrap completely in foil. Use blue autoclave tape.
PCR Small Bottles	Yes	Yes	Yes	Place cap on bottle loosely and wrap completely in foil. Use blue autoclave tape.
PCR Filters and Bases	Yes	Yes	Yes	Wrap base and filter separately before stacking together. Use blue autoclave tape.
Erlenmeyer and Volumetric Flasks (Non-AGP)	Yes	Yes	Yes	
Beakers	Yes	Yes	Yes	
Diatom Beakers (1000 mL)	Yes	No	Yes	
'Tox' Beakers (350 mL)	No	No	Yes	
Glass Graduated Cylinders	Yes	Yes	Yes	
Safety-Coated (Amber) and Plastic (Clear) Graduated Cylinders	No	No	No	Place on cart lined with drying mats or paper towel to dry in Toxicity Lab
Large and Small 'Tox' Mason Jars	No	No	Yes	
Plastic/Glass Micro Filters and Bases	Yes	Yes	Yes	Wrap base and filter separately in foil.
Plastic Chlorophyll Filters and Bases	Yes	Yes	Yes	Wrap base and filter separately in foil.
Plastic Rinse Bottles	Yes	No	Yes	Place foil on all the openings. Place the lid on loosely over foil before placing in the drying oven.
Plastic White Funnels	No	No	No	Place on cart lined with drying mats or paper towel to dry in Toxicity Lab
Plastic AGP 'Tox' Bottles (square)	No	No	No	Place on cart lined with drying mats or paper towel to dry in Toxicity Lab

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Type of Glassware	Foiled?	Autoclaved?	Oven?	Special Instructions
Glass Amber Jars and Bottles	Yes	Yes	Yes	Place cap on bottle loosely and wrap completely in foil.
Glass Milk Dilution Bottles	Yes	Yes	Yes	Place cap on bottle loosely and wrap completely in foil.
Glass Carolina Dishes and Plastic Bowls	No	No	No	Place on cart lined with drying mats or paper towel to dry in Toxicity Lab
Glass Test Tubes	No	No	Yes	
Glass BOD Bottle Stoppers	Yes	Yes	Yes	Transfer stoppers to an instant sealing sterilization pouch or wrap each one in aluminum foil completely.
BOD Bottles	Yes	Yes	Yes	Place foil on the opening.
AGP Flasks	Yes	No	Yes	Place cap on bottle loosely and wrap in foil.

8. QUALITY CONTROL

8.1 The people working the Tap Water Rinse #1 and DI Water Rinse Stations inspect the glassware for any dirt or residue missed during the washing process. Dirty glassware is sent through the process again.

8.2 The Microbiology group performs a pH check for every Wash Party. Add bromothymol blue to a randomly selected piece of glassware after it has completed the full wash/dry cycle. If the solution stays green, then the glassware has a neutral pH and the QC check passes. If the solution changes to blue or yellow, the pH is not neutral, and the QC check has failed. If the QC check fails, notify supervisor. Record pH check in the Glassware_QC_Log in LIMS laboratory logbook.

8.3 The Microbiology group performs a sterility check for every glassware autoclave cycle. Randomly select one piece of glassware per cycle that is representative of the autoclave cycle it came from in terms of size and type. Add double strength Tryptic Soy Broth (TSB2X) prepared per SOP MB-02.33 to each piece of glassware and incubate for 24 +/- 2 hours at 35ºC. The QC check passes if media is clear and no growth is observed after incubation. If media is turbid (bacterial growth), the QC check has failed. If the QC check fails, notify supervisor. Record the sterility check in the Glassware_QC_Log in LIMS laboratory logbook.

8.4 The Molecular Biology group performs a chlorine check for every Wash Party with PCR glassware. Add 1 mL of DI water to a test PCR square bottle with screw cap after it has completed the full wash/dry cycle. Cap and shake the bottle to make sure the DI water contacts the entire inner surface. Check the water for chlorine with a test strip. The QC check passes if no chlorine residual is detected. If any chlorine residual is detected, the QC check has failed. If the QC check fails, notify supervisor. Record the chlorine check in the Glassware_QC_Log in LIMS laboratory logbook.

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8.5 The Microbiology group must keep on file a Certificate of Analysis and Inhibitory Residue Test Result for each lot of detergent. Wash Party Scheduler should notify Microbiology group each time a new lot of detergent is received. The certificate and test results are obtained online on the detergent website, and are placed in the folder located at this file path: \\floridadep\Data\DEAR\Labs\Lab5\Biology\BenchCommon\COA\Cleaning, wash party

8.6 The Microbiology group must use a NIST Traceable Max Thermometer in at least one autoclave cycle per week that the autoclave is in use. The NIST thermometer is always used with laboratory waste or media cycles, which are recorded in the Autoclave_Log in LIMS laboratory logbook. For gravity cycles and the purposes of Wash Party, the maximum temperature from the autoclave receipt can be recorded along with the maximum pressure and total cycle time in the Autoclave Use Log located next to the autoclave.

9. SAFETY

9.1 Safety glasses, lab coats, and Neoprene gloves must be worn by all Wash Party participants. Long pants and closed toed shoes must be worn by all Wash Party participants. Aprons are also available which are more water and chemical resistant than lab coats.

9.2 Autoclave gloves must be worn when removing items from the autoclave or ovens.

9.3 Carts should be used to transport the glassware to minimize the chance of breakage.

9.4 Should a piece of glassware break, the glass pieces should be carefully picked up while wearing gloves or swept into a dust pan and the shards should be placed in a broken glass disposal box. Forceps may also be used to pick up shards. The sink and drain should be carefully inspected for broken glass. Broken glassware should be recorded in the Broken Glassware Logbook so that it may be quickly re-ordered**.** Toxicology or Bench staff will check the broken glassware logbook on a regular basis (~2 weeks). Should injury occur, immediately notify the Biology Safety Officer, appropriate Supervisor, and/or Program Administrator.

9.5 Refer to the DEP Laboratory Health and Safety Plan.

9.6 Refer to the DEP Laboratory Quality Manual.

10. POLLUTION PREVENTION AND WASTE MANAGEMENT

10.1 The 10% hydrochloric acid waste, 10% bleach solution, and 20% sodium thiosulfate solution may be discarded down the drain after the Wash Party has been completed. Tap water should be turned on as the acid is being dumped to further dilute it.

10.2 Any remaining acetone should be poured into the acetone waste container after the Wash Party has been completed. Used acetone waste containers are transferred to the waste room after they are full.

10.3 Refer to the DEP Laboratory Health and Safety Plan.

11. REFERENCES

11.1 DEP Laboratory Quality Manual, https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

11.2 DEP Laboratory Health and Safety Plan, https://www.floridadep.net/resources/health-safety-manual

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11.3 SOP TA-10, *Preparation of Stock HCl (Acid) Solution*

11.4 SOP BG-08, *STERIS AMSCO Series 250LS Autoclave Calibration and Use*

11.5 SOP MB-02.33, *Preparation of Dehydrated Culture Media*

11.6 Standard Methods Online Edition (1997), Sections 9020B

11.7 Broken Glassware Logbook, form BG-BROKEN-GLASSWARE

11.8 Autoclave Use Log, form BG-AUTOCLAVE

Appendix of Changes

07/10/2012 Changed Taxonomy Laboratory Tech Supervisor to Wash Party Scheduler. Added notification of low supplies to Step 5.1.

06/28/2013 Added PCR glassware washing procedure and required Microbiology QC.

07/01/2015 Formatted for 508 compliance. Created form BG-BROKEN-GLASSWARE-(version #).

10/26/2016 Included molecular laboratory references. Moved PCR Glassware Washing Procedure in front of General Glassware procedures to reflect the order of Wash Party. Renamed Section 5. Bulleted list created for Wash Party Lead's duties. Reordered and named Table 1. Corrected names of glassware in Table 1. Added reference for *Preparation of Dehydrated Culture Media*. Modified Safety section and added Pollution Prevention and Waste Management section.

12/19/2019 Changed a section of Table 1 to show that Plastic/Glass Micro Filters and Bases are now wrapped separately, not together.

01/13/2020 Added note about BOD bottles going through wash party instead of dishwasher. Also, changed their Foiling? and Autoclaved? from No to Yes; updated glass test tubes table information (no autoclaving required). Underlined and changed the font size of the Table 1's header row and added a header row to the second page of the table. Moved note from Sec 7.7 to Sec 5.3; added Maximum Temperature documentation requirement to QC section; updated the autoclave SOP number and title in the References section

02/05/2021 Updated link to Health and Safety Manual.

01/07/2022 No changes.

02/20/2024 Updated section 8.3 to specify that the piece of glassware selected for QC must be a representative of the cycle it came from and added the proper analysis window for TSB. Added the file path for COAs and IRTs in section 8.5. Updated section 8.6 to specify when to use a NIST Traceable thermometer and mentioned the appropriate use of the Autoclave Use Log, which has also been added to the list of references. Updated Table 1. Foil, Autoclave, and Drying Oven Instructions for Each Type of Glassware.

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