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 Bench Biology, Taxonomy, and Applied Biology Workgroups are scheduled in rotation to participate in the weekly Wash Party. A Wash Party Scheduler prepares a monthly Wash Party Schedule and designates a Wash Party Lead person for each week from the Laboratory Tech Workgroup. It is the responsibility of each person to find a replacement or someone to swap with if they are unable to participate in a Wash Party for which they are scheduled.

2.2 The designated Wash Party Lead individual collects and organizes the glassware on carts in the order that it is going to be washed. This person is also responsible for checking the supply of soap, acid, acetone, bleach, sodium thiosulfate and aluminum foil.

2.3 Each staff member in the Wash Party chooses one of 7 stations or steps to perform in the glassware cleaning process. One person should not always perform the same step. Ideally, everyone in the Wash Party rotation will take turns performing the different steps. If this becomes a problem and if participants are unable to equitably share tasks, the Wash Party Scheduler will assign stations as fairly as possible.

2.4 Glassware is stored, dried, and/or sterilized. The Taxonomy Laboratory Techs are responsible for emptying the drying oven(s) prior to Wash Party so that space is available in the oven for newly washed glassware.

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

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 (lower grade than HPLC; if possible, use pesticide grade which is required for bioassay)
4.4 Bleach Solution, 10%, Add 100 mL bleach to 900 mL DI water
4.5 Sodium Thiosulfate (Na2S2O35H2O), 0.025 N, ACS grade, 20%, Add 200 mL sodium thiosulfate to 800 mL DI water

5. GLASSWARE COLLECTION AND HANDLING

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5.1 The Wash Party Scheduler designates a Wash Party Lead person for each week from the Laboratory Tech Workgroup. The Wash Party Lead person is responsible for collecting glassware from the Algal, Toxicity, Microbiology and Invertebrate Labs on the morning of the wash party. This person is also responsible for checking the supply of soap, acid, acetone, bleach, sodium thiosulfate and aluminum foil and preparing any dilutions necessary. If any supplies are low or will be running low soon, this person should then notify the Lab Tech responsible for overseeing Wash Party supplies to ensure that an order has been or will be made before supplies are depleted.

5.2 Glassware is placed on carts in the order that it is to be washed. All glassware is inspected for chips or cracks that pose a potential risk of breakage or sharp edges. This glassware should be properly disposed of and recorded in the broken glassware log (see Step 9.5). Items where liquid can be poured from one container into another (e.g. AGP flasks, graduated cylinders) usually are washed first. Next, small items and items that need to be dipped (e.g. test tubes, caps, funnels) are washed. PCR glassware is washed using a modified procedure.

5.3 Before the Wash Party begins, the Foil Station person cuts foil in squares for glassware that will be autoclaved and/or dried.

6. GENERAL 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 #1Station: 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 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.

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

6.5 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: Any grade of acetone may be used, however, HPLC grade should be avoided as it is more expensive than lower grades. Pesticide grade acetone is required for bioassay so it may be available for use.

6.6 DI Water Rinse Station: The item gets rinsed thoroughly with De-ionized 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 stored, dried, and/or sterilized (Table 1).

Note: Before foiling, dump out excess water. 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.

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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 beige autoclave indicator tape (blue for PCR 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).

Table 1.

Type of Glassware	Foiled ?	Autoclaved?	Oven?	Special Instructions
Plastic micro funnels (magnetic) and bases	Yes	Yes	Yes	Place funnel base inside of funnel and foil together before autoclaving. Rubber stoppers should not be autoclaved.
Plastic micro funnels (wide mouth) and bases	Yes	Yes	Yes	Place funnel base inside of funnel through the bottom of the funnel and foil together before autoclaving. Rubber stoppers should not be autoclaved.
Plastic chlorophyll funnels (magnetic)	Yes	Yes	Yes	Wrap funnel and base separately in foil before autoclaving. Rubber stoppers should not be autoclaved.
Plastic Rinse bottles	Yes	No	Yes	Foil the top of the bottle and the tip and place in the drying oven with the lid off. Replace lid after dry and remove from drying oven.
Safety- Coated Graduated Cylinders	Yes	No	Yes	
Glass BOD bottle stoppers	Yes	No	Yes	Transfer stoppers to a dry plastic bin lined with paper towels and cover bin with foil before placing in a drying oven.
Glass Amber Jars	Yes	Yes	Yes	Place the lid on loosely and completely wrap in foil before autoclaving.
Glass Milk Dilution Bottles	Yes	Yes	Yes	Place the lid on loosely and cover lid with foil before autoclaving.
300 mL 'Tox' Beakers	No	No	Yes	
Large 'Tox" Jars	No	No	Yes	Designated with alphanumeric numbering system
Small 'Tox' Jars	No	No	Yes	
AGP Flask (250 mL Erlenmeyer)	No	No	No	Washed in Dishwasher
Plastic Graduated Cylinders	No	No	No	Put on cart to dry

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Plastic or Glass Carolina Dishes	No	No	No	Put on cart to dry
Plastic Funnels	No	No	No	Put on cart to dry
Plastic AGP Bottles	No	No	No	Put on cart to dry
PCR square screw cap bottles	Yes	Yes	Yes	Place the lid on loosely and wrap lid with foil before autoclaving. Use blue autoclave tape to indicate that the funnels have been washed following the PCR glassware washing procedure in Step 7.
PCR magnetic funnels and bases	No	Yes	Yes	These have letters etched in the funnel instead of numbers. Wrap funnel base and funnel separately in foil before autoclaving. Use blue autoclave tape to indicate that the funnels have been washed following the PCR glassware washing procedure in Step 7.
PCR autoclavable wash bottles	Yes	Yes	Yes	Place the lid with tip on loosely and completely cover lid and tip with foil before autoclaving. Use blue autoclave tape to indicate that the funnels have been washed following the PCR glassware washing procedure in Step 7.

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 The Taxonomy Laboratory Techs are responsible for emptying the drying oven(s) prior to Wash Party so that space is available in the oven for the newly washed glassware. This responsibility will rotate among Laboratory Techs and be assigned in the monthly schedule by the Wash Party Scheduler.

6.10 If a piece of glassware breaks, follow proper safety and reporting procedures (see Step 9.5).

7. PCR GLASSWARE WASHING PROCEDURE

7.1 Wash Station: Follow Step 6.1.

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

7.3 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.

7.4 Tap Water Rinse #2 Station: Follow Step 6.4.

7.5 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.

7.6 DI Water Rinse Station: Follow Step 6.6.

7.7 Foil/Autoclave/Drying Station: Follow Step 6.7-6-10 with the exception that blue autoclave tape is used instead of the beige autoclave tape.

8. QUALITY CONTROL

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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 a flask, cylinder and beaker to represent all autoclave cycles in the Wash Party. Add TSB2X to each piece of glassware and incubate for 24 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 Applied Biology/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.

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.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

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 The 10% hydrochloric acid waste and 10% bleach 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. Waste acetone should be added to the Acetone Waste bottle.

9.5 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.

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10. REFERENCES

10.1 SOP TA-10-10 Preparation of Stock HCl (Acid) Solution

10.2 SOP TA-06.18 AMSCO Renaissance Series 3021 Autoclave Calibration and Use

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

10.4 Broken Glassware Logbook, form BG-BROKEN-GLASSWARE-(version #), \\fldep1\DEAR\Labs\Lab5\Biology\InvertGroup\Logbooks and Forms

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 #).

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