Formalin Assay, Recycling and Buffering

(Modified from CBG Biotech, Ltd. Formalin Recycler Installation and Operating Instructions)

1. SCOPE AND APPLICATION

This Standard Operating Procedure (SOP) details the proper method for recycling and assaying waste formalin. Formalin preservative is collected during the preparation of macroinvertebrate samples with Laboratory Information Management System (LIMS) test codes beginning with MI-.

2. SUMMARY OF METHOD

2.1 Waste formalin is collected in properly labeled waste containers until the container is ¾ full (fill line). Once a container is full, the waste formalin must either be recycled or disposed of within three days. Dispose waste formalin following SOP IZ-10.

2.2 Waste formalin is recycled and buffered. Recycled, buffered formalin is assayed to determine the concentration, diluted to 10%, if necessary, and stored in properly labeled containers.

2.3 Formalin recycling, assay, cleaning cycle and repair information is documented in the Formalin_Log in the LIMS Laboratory Logbook module.

3. APPARATUS AND EQUIPMENT

3.1 Recycling

3.1.1 CBG Biotech, Ltd. Formalin Recycler FMA-99(L) and filters - located in Boat Storage

3.1.2 Formalin Waste Container

3.1.3 Fill Container (with spigot)

3.1.4 Recycled Formalin Container

3.1.5 Waste Recovery Container

3.1.6 500 mL container

3.1.7 Glass Wool

3.1.8 Funnel

3.2 Buffering

3.2.1 350 mL container

3.2.2 pH strips

3.2.3 Carboys (for storage)

3.3 Assay

3.3.1 Magnetic stirrer with stir bar

3.3.2 Erlenmeyer flasks, 125 mL or 200 mL beakers, 2 each

3.3.3 Volumetric pipettes, 1.0 mL, 2 each

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3.3.4 Burette with stand, 25 ml

3.3.5 Pipets

3.4 Safety equipment (see section 7.2)

3.5 Laboratory cart

4. REAGENTS AND CHEMICALS

4.1 Recycling

4.1.1 forma-Neut 1A

4.2 Buffering

4.2.1 Sodium phosphate concentrate (Buffer)

4.3 Assay

4.3.1 Assay reagents, #1, #2, #3 and pH indicator - provided by the equipment manufacturer.

4.3.2 Deionized (DI) water

5. METHODS

5.1 When a waste formalin container is ¾ full (fill line), either recycle or dispose of waste formalin (SOP IZ-10) within three days. Allowing the container to become completely full increases the risk of spill or injury during disposal. Proper safety procedures (such as proper lifting techniques) should be followed when dealing with chemicals and when lifting heavy objects.

5.2 Recycling

Note: Moving waste containers of formalin and formalin recycling should be performed by two people. Place the waste formalin container on a cart and take it to Boat Storage. Bring and use proper safety equipment.

Note: Formalin concentrations exceeding 20% should not be put through the recycler without diluting with water. See page 5 in the manual located with the machine in Boat Storage. A second copy of the manual is located with the formalin assay reagents in B205.

5.2.1 Place spigotted Fill Container in upper bracket and secure the spigot on the fill line.

5.2.2 Place siphon tube into Waste Formalin Container. Press and hold red pump button until all the formalin has been pumped into the Fill Container.

Note: Remember when pumping waste formalin into the Fill Container, the lid of the Fill Container needs to be on tight.

Note: You may choose not to use the syphon tube and instead place glass wool into a funnel and pour the waste formalin through that and into the Fill Container.

5.2.3 When siphoning is complete, detach siphon tube from machine and rinse in the sink with hot water.

5.2.4 Place Recycled Formalin Container on the bottom shelf.

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5.2.5 Pour one container of forma-Neut 1A into empty Waste Recovery Container and add 500 mL of hot tap water. Mix thoroughly.

Note: The forma-Neut solution will neutralize up to two gallons of waste formalin. This reaction takes approximately two to four hours to complete.

5.2.6 Place Waste Recovery Container on bottom shelf with drain tube inserted through the hole in the black lid. Insert vent tube into the small hole on top of the container. Make sure small vent tube is in the container and the lid is secure.

5.2.7 Make sure spigot of Fill Container is on fill line which goes into the recycling machine. Open spigot. When starting the recycler, loosen the lid of the Fill Container one quarter of a turn.

5.2.8 Follow prompts to start formalin cycle. Press **Enter** or **Next** to proceed, press **No** for options.

Note: With each cycle, 20% waste per total volume recycled should be obtained at the end of each run. If you acquire too little waste, an Abnormal end of cycle message will appear on the screen. A NaOH cleaning should be performed to remove any buildup of biological material. See Manual.

5.2.8.1 "Formalin to be recycled" press **Enter**. Press **Enter**. Press **Enter.**

5.2.8.2 "Are all containers empty and in position?" Press **Enter.**

5.2.8.3 "Is fill container in place and spigot open?" Press **Enter.**

5.2.8.4 "Check antifoam reservoir." Press **Enter.**

Note: Make sure antifoam is primed before use. Do not let antifoam reservoir run dry or the pump will need to be re-primed. See manual for adding more antifoam and for priming the pump.

5.2.8.5 "How many gallons do you want to process?" Press **Enter.**

5.2.8.6 "Gallons selected: 2." Select desired gallons to be recycled. Select Next to Increase and No to Decrease. Press **Enter.**

Note: Minimum of 2 gallons, maximum of 5 gallons.

5.2.8.7 "Is number of gallons correct?" Press **Enter** or **No.**

5.2.8.8 "Press Enter when ready to fill." Press **Enter** or **No**. There is an audible "Click" when fill valve opens.

5.2.9 "Unit filling press Enter when done." Wait until the container is empty. Close spigot. Press **Enter**.

5.2.10 "System ready press enter to start." Press **Enter**. "Unit operating, Elapsed time. Cycle has begun."

Note: If you have any problems, call Technical Support Representative (CBG BIOTECH). The recycler will prompt the user to clean the system every 10 cycles if each cycle ended with the proper amount of waste (20% of the total volume). Do not forget to adjust finish filter control to the Bypass mode prior to loading the cleaning agent and follow prompts for cleaning tank.

5.2.11 When cycle is completed, the display will read "End of Cycle, Total Time". Hours and minutes of cycle time will be displayed.

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5.2.12 Detach drain and vent tubes from Formalin Waste Container. Following appropriate safety procedures, empty the contents of the Formalin Waste Container into the formalin waste barrel in Chemical Waste Storage (C217). Replace container on Recycler and reattach drain and vent tubes.

Note: The content of the Formalin Waste Container does not contain formalin and has been neutralized.

5.2.13 Press **[Reset]** to return to the beginning of the program.

5.2.14 At the end of every run, measure the amount of formalin recovered and the amount of waste recovered. A 20% waste volume should be collected at the end of each cycle (e.g. 1 gallon of waste per 5 gallons run).

Example: 12.3 L formalin recovery + 7.7 L waste recovery = 20 L initial volume

5.2.15 Buffer the recycled formalin in the Formalin Recovery Container following step 5.3.

5.3 Buffering

5.3.1 Measure amount of formalin to be buffered.

5.3.2 For 12 L of recycled formalin, mix 1 packet of sodium phosphate buffering powder with 350 mL of hot water. Add solution to the 12 L of recycled formalin. Mix thoroughly.

Note: The amount per volume to add is listed in the manual page 25.

5.3.3 Test the buffered, recycled formalin with pH paper. The pH should be 7.5-8.0.

5.3.4 Carefully transfer the buffered, recycled formalin from the Formalin Recovery Container into carboys. Take the buffered, recycled formalin in carboys to Chemical Storage (C215) and transfer into one of the black 55 gallon storage drums using the safety procedures outlined in SOP IZ-11.

5.3.5 When a 55 gallon drum is full and ready for dispensing, perform the Assay (step 5.4) and generate a lot number for the 55 gallon drum (step 5.4.11). The lot number represents this drum of formalin until it is empty and nothing should be added to this drum until it is empty.

5.3.6 Document the recycling event in the Formalin_Log in the LIMS Laboratory Logbook module. This is also the location to document assay, cleaning cycle or repair information, changing field entries as appropriate.

5.3.6.1 Open the LIMS/Laboratory/Technical/Laboratory Logbook module. Select Formalin_Log. For Group ID “Biology”, choose radio button “Enter a new log”. Highlight the previous recycling entry. Click “Enter/Edit”. Enter “Completed” under “Entry Value”. Add a comment if needed. Review information, name and date. Save.

5.4 Assay

5.4.1 Rinse burette b**e**fore use and make sure it is closed before adding reagent. Using a clean 10 mL volumetric pipette, fill up a 25-mL burette with Reagent #3 and place on stand to be used in Step 5.4.8. Note level on burette.

5.4.2 Using a pipette, add 10.0 mL of DI water to a 125 mL Erlenmeyer flask or 200 mL beaker.

5.4.3 Add magnetic stir bar to flask or beaker and place on stirrer. Place a white background behind the flask or beaker. Do not run the stirrer without liquid; the stir bar will cause flask or beaker to break.

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5.4.4 Mix the formalin to be assayed. Using a clean 1.0 mL volumetric pipette, add 0.4 mL of the well-mixed formalin to be assayed to the flask or beaker.

5.4.5 Using the dropper on the reagent bottle, add 10 drops of assay pH indicator. If formalin is buffered, proceed to step 5.4.6. If not buffered, skip to step 5.4.7.

Note: Step 5.4.6 is always done unless formalin has been recycled or is not buffered. (Recycling removes buffers.)

5.4.6 Using a pipette, add drops of Reagent #1 until clear color starts to change blue. Make sure blue color persists for one minute before proceeding.

5.4.7 Using a pipette, add 4.0 mL Reagent #2. Reagent #2 will cause the mixture to turn dark blue.

5.4.8 Titrate Reagent #3 drop wise until blue color changes back to clear. There should be no blue tint. Record the number of milliliters used to remove blue tint and calculate the multiplier factor for a desired final 10% formalin concentration.

multiplier factor = 10 ÷ # mL of Reagent #3

Note: The multiplier factor equation can be found in the manual, which is located with the machine in Boat Storage. Another copy is with the assay reagents in B205.

5.4.9 Calculate the % recycled formalin concentration. Record the value.

% recycled formalin concentration = # mL of Reagent #3 × multiplier factor

Example:

- 5.5 = the # of mL of Reagent #3 used
- multiplication factor for a final 10% formalin solution = 10 / 5.5 = 1.82
- 5.5 mL x 1.82 = 10.01% formalin

5.4.10 Recycling procedure adds 2% to the final concentration. Diluting is necessary to end up with a final formalin concentration near 10%. Dilutions should be made with DI water. For the amount of DI water to add, see formalin dilution table (section 10) or in the manual page 23. Record the amount of DI water added.

5.4.11 Generate a lot number for the full, assayed 55 gallon drum and label the drum. The lot number must contain the percentage concentration, the month and year, and the initials of the person generating the batch of formalin. The lot number represents this drum of formalin until it is empty and nothing should be added to this drum until it is empty.

Example: If the percent concentration is 10.6%, the present month and year are January 2015, and the person generating the batch of formalin is H. Neeley, then the lot number will be 10.60115HN.

5.4.12 Label containers filled with this batch of formalin with the lot #, % buffered formalin, and container contents (formalin). Store containers in appropriate area.

5.4.13 Document the assay information in the Formalin_Log in the LIMS Laboratory Logbook module. This is also the location to document recycling, cleaning cycle or repair information, changing field entries as appropriate.

5.4.13.1 Open the LIMS/Laboratory/Technical/Laboratory Logbook module. Select Formalin_Log. For Group ID “Biology”, choose radio button “Enter a new log”.

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Highlight the previous assay entry. Click “Enter/Edit”. Enter the # mL of Reagent #3 and amount DI water used for dilution into the white text box under “Entry Value”. Enter the lot number generated. Add a comment if needed. Review information, name and date. Save.

6. QUALITY CONTROL AND DOCUMENTATION

6.1 Write date received, date opened, and the analyst’s initials on all reagent bottles.

6.2 Document all recycling, assay, cleaning cycle and repair information in the Formalin_Log of the LIMS Laboratory Logbook module.

7. SAFETY

7.1 CAUTION: The recycler is not to be used with waste streams containing picric acid, Bouin’s Fixative, mercury compounds, acetone, or other azide materials.

7.2 Lab coat, latex or vinyl gloves, and eye protection are required when working with formalin.

7.3 Avoid breathing formalin fumes or exposing co-workers to them. Use proper safety equipment, a lab hood or chemical ventilation system, and keep face away from open containers of this chemical. Work in a well-ventilated area.

7.4 Refer to the Material Safety Data Sheet (MSDS) for information about reagents and chemicals in this SOP.

7.5 Refer to the DEP Laboratory Health and Safety Plan.

7.6 Refer to the DEP Laboratory Quality Manual.

8. POLLUTION PREVENTION AND WASTE MANAGEMENT

8.1 Use caution when handling formalin. Should a spill occur, **immediately** notify the Biology Safety Officer, Laboratory Safety Officer, Taxonomy Supervisor, and Biology Program Administrator. Proper clean-up and reporting procedures must be followed.

8.2 Refer to the DEP Laboratory Health and Safety Plan.

9. REFERENCES

9.1 DEP Laboratory Quality Manual, http://www.dep.state.fl.us/labs/library/lab_sops.htm

9.2 DEP Laboratory Health and Safety Plan, https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

9.3 SOP IZ-10, *Preparation and Proper Disposal of Formalin*

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10. TABLES

10.1 Dilution Table

Percent formalin to be diluted:	Volume of D.I. water added per gallon to return recycled formalin to 10% concentration:
10.1%	38 mL
10.2%	76 mL
10.3%	114 mL
10.4%	152 mL
10.5%	190 mL
10.6%	228 mL
10.7%	266 mL
10.8%	304 mL
10.9%	342 mL
11.0%	380 mL
11.1%	418 mL
11.2%	456 mL
11.3%	494 mL
11.4%	532 mL
11.5%	570 mL
11.6%	608 mL
11.7%	646 mL
11.8%	684 mL
11.9%	722 mL
12.0%	760 mL
12.1%	798 mL
12.2%	836 mL
12.3%	874 mL
12.4%	912 mL
12.5%	950 mL
12.6%	988 mL
12.7%	1026 mL
12.8%	1064 mL
12.9%	1102 mL
13.0%	1140 mL
13.1%	1170 mL
13.2%	1216 mL
13.3%	1254 mL
13.4%	1292 mL
13.5%	1330 mL

Appendix of Changes

02/28/2009 Reformatted the layout following SOP LB-001. Moved Assay instructions after the Buffering instructions.

03/10/2010 Added option of using 200 mL beaker for assay

03/09/2011 Updated disposal of waste formalin, step 5.2.12.

03/07/2012 Added steps 5.3.5 and 5.4.11. Re-worded step 5.4.12.

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02/05/2015 Added new instructions for calculating the correction factor in step 5.4.9, which is not a static number. Added new instructions for using LIMS Laboratory Logbook module. Reformatted for 508 compliance.

02/11/2016 Updated links. Updated names of and moved the FDEP Laboratory Quality Manual and FDEP Laboratory Health and Safety Plan links to the reference section.

02/28/2017 Corrected SOP IZ-11 to IZ-10 as the two SOPs were combined into one. Added the use of glass wool. Removed references to distilled water and inaccurate summary steps for following the prompts during recycling.

04/02/2018 Added a note to section 5.2.2 mentioning that a glass wool filter can be used in place of the syphon tube.

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