Non-SCI Macroinvertebrate Sample Preparation

1. SCOPE AND APPLICATION

1.1 This method details the rinsing, sorting, and storing of benthic macroinvertebrate composite samples collected using a #30 D-frame dipnet or a dredge or coring device that require sub-sampling to a specific target number of individuals.

1.2 This method is not used for the samples collected for the calculation of the Stream Condition Index (SCI).

1.3 This method is to be used for samples with Laboratory Information Management System (LIMS) test code MI-EV-QLDC and MI-FW-QNLC.

2. SUMMARY OF METHOD

2.1 A biological sample, consisting of surface water, leaves, stems, sand and other detritus with formalin as a preservative, is emptied in a U.S. #30 mesh sieve over a discard bucket to catch the waste formalin and all material smaller than #30 mesh.

2.2 The sieved sample is placed into a bucket of water to homogenize the sample. Floating material and macroinvertebrates are again poured through and retained in the #30 mesh sieve.

2.3 After all floatable material has been removed, large material, such as rocks, twigs, and leaves, is then removed and washed over the #30 mesh sieve until the material remaining in the bucket is mostly shell hash and/or sand.

2.4 The large material, shell hash, and sand are examined under a magnifying lamp to find any remaining organisms. Any organisms found in this step are added to the material in the #30 mesh sieve. The sieve is placed in a pan containing enough water to cover the material on the screen and gently agitated to remove any remaining particles and material smaller than #30 mesh.

2.5 The material remaining after sample rinsing is transferred to a gridded tray where sections are randomly removed and placed into a second gridded tray. Sections are then randomly removed from the second tray and placed into a third, small, gridded tray which is divided into 16^{ths}.

2.6 The material in the small, gridded tray is placed into a petri dish 1/16 at a time. This material is examined under a dissecting microscope to search for macroinvertebrates. Organisms are transferred to an ethanol-filled glass vial. Material is examined in 1/16 portions until a target of 100-110 macroinvertebrates is reached.

3. APPARATUS AND EQUIPMENT

3.1 Waterproof paper and pencil
3.2 Fine tip permanent black marker
3.3 U.S. #30 mesh sieve
3.4 U.S. 5/16-inch sieve
3.5 Large diameter funnel
3.6 Bucket

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3.7 Shallow pan
3.8 Luxo® lighted magnifier
3.9 2 trays marked with a grid of 24 equal-sized, 5 cm squares
3.10 1 tray marked with 16 equal-sized squares
3.11 List of random numbers
3.12 2 jars (250 mL)
3.13 Dissecting microscope
3.14 Petri dishes (100 x 15 mm), 10 per sample
3.15 Forceps
3.16 1 vial for picked organisms (1 or 2 dram)
3.17 Laboratory counter
3.18 Squeeze bottle of 80% Ethanol
3.19 Safety equipment (see section 9.1)

4. REAGENTS AND CHEMICALS

4.1 Ethanol (80%) – prepared following SOP IZ-09

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1 Samples are collected, preserved, and delivered to the lab per SOP FS 7000.

5.2 Samples are checked into the laboratory per SOP IZ-01.

6. SAMPLE PREPARATION PROCEDURE

6.1 Select a sample. If two or more containers are taped together, check that all containers are part of the same sample.

6.1.1 Make sure you know how many containers in which the sample is stored (there may be several jugs). The entire sample must be included in this reduction and homogenization process.

6.1.2 If two different samples are accidentally taped together, there is no way to separate them once homogenization occurs. Both samples will be lost.

6.2 Check out the sample using the Laboratory/Receiving/Custody utility in the LIMS. Create a new prep worksheet using the Laboratory/Technical/Prep Worksheet Manager utility in the LIMS.

6.3 Using the Laboratory/Receiving/Print Preparation Labels utility in LIMS, print out 10 dish labels, 2 vial labels, 2 tray labels, and a bench sheet. All labels are printed from the Sato printer.

6.3.1 To print labels, go to the sample Log-in station, open LIMS and select “Laboratory”, “Receiving”, “Print Preparation Labels.” To print dish labels, go to “Invert_Labels”, “Labels for Dishes”, scan the sample bar code, select “File”, “Print”, select the “LBL19” printer, and print 10 copies. To print vial and jar labels, select “Invert_Labels”, “Vial Labels” and print 2 copies. To print tray labels, select “Invert_Labels”, select “Additional Text Small Labels”, select bullet next to “Additional Text 1”, scan the sample, type in additional text (Tray 1 and analyst’s

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initials) and print 1 copy. Repeat this process for Tray 2. To print a bench sheet, select bullet next to "Sample ID", scan sample, go to "BenchSheets", "Macroinvertebrate", select "File", "Print", and select the "EAR-LABS-PRN9" printer, print 1 copy.

6.4 Using waterproof paper and pencil make a clearly written label to go inside the vial of picked bugs. The label should include: LIMS sample ID#, project, station identification and date sampled. Place the waterproof label inside the vial and one vial label on the outside of the vial. Using permanent marker, label the lid with the same information.

6.5 Put on the proper safety equipment. Place a U.S. #30 mesh sieve over a discard bucket. Empty the contents of the sample container into the sieve slowly, making sure not to lose any of the sample. The formalin will go through the sieve and collect in the bucket. After it has finished draining, pour the waste formalin into the proper container.

6.5.1 Material from all the sample containers may not fit in the sieve at the same time. If this is the case, put the material in the sieve in batches and then combine in a bucket.

6.5.2 Formalin disposal containers are located under the sinks in the main laboratory (B205).

6.6 Ensure all organisms and material have been transferred to the sieve. Do not remove the labels from the sample containers. Clean the containers and place them to dry. Check the sample in using the Laboratory/Receiving/Custody utility in the LIMS.

6.6.1 The LIMS Custody utility is tracking the sample bottle, not the contents. Empty containers will be kept for a period after the job is authorized in LIMS. After this period, designated staff will collect the empty bottles and discard them from LIMS. The Invert Workgroup Log-in logbook tracks the sample aliquots.

6.7 Rinse the entire sample with water a few times to remove any remaining formalin from the sample. Place the entire sample into a bucket and fill the bucket about 1/2– 2/3 with water. Place a U.S. 5/16-inch sieve on top of the U.S. #30 mesh sieve. Gently stir the mixture and then pour through the sieves to separate floating debris. Repeat this process until most of the floatable material is removed from the sample.

6.7.1 With the change from formalin to water, many of the small organic particles (including macroinvertebrates) will float to the surface.

6.8 Begin removing some of the large material (leaves, twigs, rocks, etc.) by continuously picking large pieces out and washing them over the bucket or sieves. Do not discard this material but place it into a pan. When the bucket is full of water, pour the elutriate through the sieves. Keep repeating until you have separated the light material from the heavier material (i.e. light organic matter from coarse sand). This process is repeated until only the coarse material (i.e. shell hash and/or sand) is left in the bucket. Rinse the course material into a pan.

6.8.1 It has been shown that 80 to 95% of the individuals in the sample will be found in the elutriate.

6.8.2 Gently rinse the sample material in the U.S. 5/16 inch sieve (while it's still on top of the U.S. #30 sieve) with tap water making sure the water pressure does not damage

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the organisms (a small hose attached to the faucet works best). Place the remaining material from the 5/16-inch sieve into a pan.

6.8.3 In the end there will be a pan full of leaves, twigs, rocks, etc., the sample material in the U.S. #30 sieve, and a pan full of sand, shell hash, etc.

6.9 When the rinsing process is complete, there will be three portions of the sample. The first is the large material that was picked out of the sample and placed in pans. The second will be primarily coarse sand that was placed in a pan. Examine these two portions under the lighted magnifier for organisms that weren't removed by the rinsing process. If any organisms are found, randomly place them into the third and final portion; the elutriate material in the sieve, which will contain the majority of the organisms. Have another analyst scan through the material in each pan before discarding. Any organisms found by the second analyst should be added to the sieve.

6.9.1 This inspection is best accomplished by placing the debris in a pan with enough water to cover the material and then observing it with the Luxo® lighted magnifier. Do not place too much material in a single pan. The debris should be easy to sort through without missing macroinvertebrates.

6.9.2 Use a waterproof label with the name of the person performing the sorting and "Please QC" on opposite sides of the paper for each tray. With many different trays, this helps prevent confusion regarding which step in the process the trays are in.

6.10 Leaving the sample material in the sieve, place the U.S. #30 mesh sieve into a pan. Fill the pan with enough water to cover the top of the sieve screen. With the appropriate up and down and back and forth motion, most of the very small particles will pass through the sieve into the tray. Do this for approximately 2 minutes and then discard the water. Repeat the procedure until the water remains clear or the process has been done five times, whichever is first. This step also helps distribute individuals evenly in the sieve.

6.11 Place the sieved sample into a gridded tray (Tray 1). Each 5 cm x 5 cm grid should have a pre-assigned number, 1 through 24. Place the Tray 1 sticker, including analyst's initials, on Tray 1.

6.11.1 The sample may not cover all 24 grids. It should be spread out evenly across a number of grids that is divisible by 3 (e.g., 3, 6, 12, 18, etc.). Avoid smearing the material into the sides and corners of the trays.

6.12 Using the random number table, select 1/3 of the total squares in Tray 1 (e.g., 8 of 24 squares) and spread those out as much as possible into a second, standard gridded tray (Tray 2). Place the Tray 2 sticker, including the analyst's initials, on Tray 2.

6.12.1 Use a ruler or other straight edge to delineate the edges of a grid while removing the sample.

6.13 Using the random number table, select a square from Tray 2 and place it into a smaller tray (Tray 3), which has been subdivided evenly into 16ths. Place a vial sticker on Tray 3.

6.13.1 These small divisions help avoid going over the target number of individuals.

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6.13.2 If the sample is particularly dirty, carefully place the Tray 2 square into a small U.S. #30 sieve and repeat step 6.10 to remove small particles before transferring the material to Tray 3.

6.14 Select 1/16 to begin the sorting process. Place the 1/16 into a Petri dish and add ethanol until the material moves around easily with forceps. Under a dissecting scope set at low power (approximately 7x or 10x), systematically scan back and forth or up and down picking every organism from the Petri dish and placing it into the labeled, ethanol-filled vial.

6.14.1 Using a pair of forceps in each hand enables you to better tease organisms out of fibrous detritus. Forceps should be sharp and properly aligned.

6.14.2 Use a laboratory counter to keep a running total of the number of organisms picked.

6.14.3 Drawing lines on the bottom of all ten Petri dishes will help in the systematic removal of organisms.

6.15 Continue this process until the target number of organisms is reached. The target number of organisms is 100-110. Once a 1/16 or other subdivision is selected, its entire contents must be sorted.

6.15.1 Petri dishes should be saved until there are 10 or the target number of individuals has been reached. At that point another analyst should randomly pick and recheck one of the dishes. If organisms are found, the information is recorded and the organisms are added to the sample vial and count toward the total number of individuals sorted. After a dish has been checked, the ethanol in the remaining dishes is emptied into a collection container and the material may be discarded. All dishes should be rechecked during training (see SOP IZ-40).

6.15.2 The ethanol disposal container is located under the sinks in the main laboratory (B205).

6.15.3 Additional subdivision can be done at any point during the sorting. For example, a whole 16th may be sorted yielding 72 organisms, and then the next 16th could be subdivided into 2 parts to presumably yield 36 organisms for a total of 108 organisms.

6.15.4 If more than the target number of organisms is sorted, additional subsampling in SOP IZ-06 will reduce the number of organisms to be identified.

6.16 If the target count has not been reached with the entire contents of Tray 3, randomly select another square from Tray 2 using a random number table and repeat steps 6.13-6.15 until the target number of organisms has been reached. Note that as before, once a 1/16 or other subdivision is selected, its entire contents must be sorted.

6.16.1 If the time taken to pick through the material is nearing 12 hours, notify the Taxonomy Coordinator and Supervisor.

6.16.2 If 1/3 of the squares in Tray 2 have been picked, and each square is consistently giving a low number of organisms such that the material will not yield the target number of organisms, notify the Taxonomy Coordinator and Supervisor.

6.17 If all the material in Tray 2 has been sorted and the target number of individuals has not been reached, sorting has not exceeded 12 hours, and it is possible to get the target number of

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organisms from the material, go back to Tray 1 and repeat the entire process from Section 6.13.

6.18 Record the number of squares and subdivisions sorted. This information is necessary to calculate the portion of the fraction of the sample sorted.

6.18.1 Example: Subsampling data: 8/24 x 2/12 x 1.1875/2 =. Where 8 is 1/3 of the original 24 squares from Tray 1, 12 is the number of squares the 1/3 was spread across in Tray 2, 2 is the number of squares selected from Tray 2, and 1.1875 the amount of material sorted to obtain the target number of individuals (1 entire square from Tray 2 and an additional 3/16 from a second Tray 2 square).

6.18.2 Failure to record the subsampling data compromises the usefulness of the data.

6.18.3 If the target number of individuals cannot be met, notify the Taxonomy Coordinator and Supervisor. Before the sample is given to the taxonomists, the Coordinator or Supervisor will notify the customer and try to identify the cause for the low number of organisms.

6.19 Transfer the vial sticker from Tray 3 to a 250 mL jar and place the remaining 16^{ths} (from Tray 3) in the jar, making a note on the sticker of the amount of material in the jar (e.g. 4/16).

6.19.1 After QC, discard any remaining material from the Petri dishes.

6.19.2 Occasionally, the actual number sorted is mistakenly lower than the target count; additional organisms can be sorted from this material in order to reach the target count. If more organisms are needed, update on the bench sheet the amount of material sorted and the extra time spent.

6.20 Transfer the Tray 2 sticker to a second 250 mL jar. Randomly select one additional square from Tray 2 and place it in this jar.

6.20.1 Occasionally, the actual number sorted is mistakenly lower than the target count; additional organisms can be sorted from this material to reach the target count. If more organisms are needed, update on the bench sheet the amount of material sorted and the extra time spent.

6.21 Using the Laboratory/Technical/Prep Worksheet Manager utility in the LIMS, complete the prep worksheet. Record completion of preparation and QC on the form IZ-SORT-(version #), *Macroinvertebrate Sample Sorting.*

7. SAMPLE ANALYSIS PROCEDURE

7.1 Samples are analyzed per SOP IZ-06.

8. QUALITY CONTROL AND DOCUMENTATION

8.1 Use a pen with black ink for all bench sheet and form entries.

8.2 Macroinvertebrate Sample Sorting form

8.3 To correct raw data entries, place a single line through the incorrect entry, write the corrected entry near the error, and initial and date the correction. If the correction requires an explanation, write a comment and reference the error.

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8.4 If a problem occurs during sample processing, submit a nonconformance report (NCR) describing the problem and the corrective action. To create a NCR, open LIMS and go to Laboratory/Administrative/Non-Conformance Report. For a decision tree regarding issuance of NCR's, refer to SOP LB-002.

8.5 QC of Sorting is performed per SOP IZ-40.

8.6 QC of Taxonomic Identification is performed per SOP IZ-13.

8.7 Refer to the Laboratory Quality Manual.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

9.2 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.

9.3 When rinsing samples preserved with formalin, catch the "first flush" and transfer the waste formalin into a hazardous waste storage container.

9.3.1 Formalin disposal containers are located under the sinks in the main laboratory (B205).

9.3.2 Handling of waste formalin is performed per SOP IZ-10 and SOP IZ-38.

9.4 When discarding material from petri dishes, collect and transfer the waste ethanol into a hazardous waste storage container.

9.4.1 The ethanol disposal container is located under the sinks in the main laboratory (B205).

9.5 Squeeze bottles must be labeled with the name and concentration of the reagent.

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

9.7 Refer to the Laboratory Health and Safety Plan.

10. REFERENCES

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

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

10.3 Form IZ-SORT-(version #)

10.4 SOP FS-7000, *General Biological Community Sampling*

10.5 SOP IZ-01, *Sample Arrival/Log-in Procedures*

10.6 SOP IZ-06, *Invertebrate Enumeration and Taxonomic Analysis*

10.7 SOP IZ-09, *Preparation of 80% Ethanol*

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

10.9 SOP IZ-13, *Invertebrate Taxonomic Identification Quality Control*

10.10 SOP IZ-38, *Formalin Assay, Recycling and Buffering*

10.11 SOP IZ-40, *Invertebrate Sorting Quality Control (QC)*

10.10 SOP LB-002, *Non-conformance Reporting System*

Appendix of Changes

12/01/2009	Reformatted the layout following SOP LB-001.
12/01/2009	Added an extra quality assurance check (6.1, 6.1.2) and to the creation and completion of the prep worksheet in LIMS (6.2 and 6.22.1).
12/01/2009	Added changes in use of the LIMS custody utility (6.6) and an additional sieving to step 6.13 for randomly chosen individual squares.
12/01/2009	Added rinsing to step 6.7 and labeling of trays to step 6.9.
12/01/2009	Added circumstances for which to notify Supervisor and Manager (6.16.1, 6.16.2, 6.18.3).
12/01/2009	Added a directive to update sorting documentation (6.19.2, 6.20.1) and moved what is now 6.15.3 and 6.15.4 from 6.16.
11/10/2011	Added ethanol handling and disposal to section 9. Updated name for the Laboratory Safety Manual.
11/04/2013	Removed formalin respirator requirement. Updated sorting QC and formalin SOP #s
10/27/2014	Formatted for 508 compliance.
11/05/2015	Updated SOP references. Included reference to form IZ-LOG.
11/17/2015	Removed reference to the Log In logbook and corrected form name to IZ-SORT.
02/28/2017	Standardized SOP naming conventions. Re-titled to include “Sample Preparation” in the title instead of a subtitle and to specify that this is for use with “Non-SCI” sampling methods.
03/14/2018	Added information about not overloading pans with debris. Updated DEP Laboratory Quality Manual link.
03/08/2019	Clarified the use of sieves and pans in sections 6.5-6.10. Replaced outdated references to step numbers to reflect the current SOP section numbers.
02/17/2020	Updated the printer name. Updated the benchsheet we use.
02/10/2021	Updated link to Laboratory Health and Safety Plan
01/05/2022	Updated printer name. Added that dishes need to be randomly picked for QC.
02/08/2024	Changed numbers to more commonly used numbers in 6.11.1

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