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 sample is then transferred into a bucket of water to homogenize the sample. Floating material and macroinvertebrates are again poured through and retained on the #30-mesh sieve.

2.3 After all floatable material has been removed, large material, such as rocks, twigs, and leaves, are 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 the #30-mesh.

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

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

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3.6 Bucket
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. Make sure to include all containers for the sample in the reduction and homogenization process.

NOTE: 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 SAMPLE CHECK OUT

6.2.1 Log into LIMS. Select Laboratory → Receiving → Custody → CheckOut.

6.2.2 Scan the barcode of each sample. Be sure that the number of samples corresponds to the total number scanned, shown at the bottom of the screen.

6.2.3 Click OK after scanning the barcodes. When prompted, the analyst will scan their ID badge then click OK. The analyst must be a custodian in LIMS to be able to check out/in samples.

6.3 PREP WORKSHEET

6.3.1 Log into LIMS. To create new prep worksheet select Laboratory → Technical → Prep Worksheet Manager. Select the first shortcut button to “Create a prep worksheet”.

6.3.2 In the panel to the left, under “Preparation ID...”, select SOP-IZ39. Then, select the analyst's name from the module below under “Assign to...”. Ensure “INVERTZOO” is

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selected in the drop-down list box. In the panel labeled "Sample/Tests"…, highlight the invertebrate sample/s that will be added to a worksheet.

6.3.3 To print bench sheet, select the "Print prep labels" shortcut button from the main menu. Scan or key in sample ID. From the top panel, select "BenchSheets" and select "Print macroinvertebrate" from the drop-down menu.

NOTE: If the lab printer is not working you can use the PRN1 printer located in the office.

6.4 PRINT LABELS

6.4.1 Log into LIMS. Select Laboratory → Receiving → Print Preparation Labels. From the top panel, select "Invert_Labels" and from the drop-down menu select labels to be printed. See the table below:

LIMS Label Name	# labels	Used for	Printer
Labels for Dishes	10	Dishes	EAR-Labs-LBL19
Vial Labels	4	Vials/jars	EAR-Labs-LBL19
Additional Text Small Labels: (type "Tray 1"/"Tray 2" and analyst's initials)	2	Trays	EAR-Labs-LBL19

6.5 Using waterproof paper and pencil make clearly written labels to go inside the 2 vials of picked bugs. The labels should include: LIMS sample ID#, project, station identification, and date sampled. Place the waterproof label inside the vial and the corresponding vial label on the outside of the vial. Using permanent marker, label the lid with the same information. Check to ensure that all label information on each vial represents the same replicate number.

6.6 Wear proper safety equipment (see section 9.1). 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 material. The formalin will pass through the sieve and collect in the bucket. After it has finished draining, pour the waste formalin into the designated waste disposal container.

6.6.1 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.

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

6.7 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.

6.7.1 Check the sample back in using LIMS Laboratory → Receiving → Custody → CheckIn.

6.7.2 The LIMS Custody utility tracks the sample bottle, not the contents. Empty containers will be retained for a period of time, designated by the Lab Data Support (LDS) staff, after the job is authorized in LIMS. After this period of time, the invert group discards sample containers from LIMS and stores the empty bottles in a designated location on the fourth floor.

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6.8 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 full with tap water. Gently stir the mixture. Place a U.S. 5/16-inch sieve on top of the #30-mesh sieve. Pour the mixture through the 5/16-inch sieve to separate floating debris. Repeat this process until the majority of the floatable material is removed from the sample.

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

6.9 Begin removing some of the large material (leaves, twigs, rocks, etc.) by continuously picking large pieces out of the 5/16-inch sieve and washing them over the bucket or sieve. Do not discard this material; place it into a shallow pan. When the bucket is full of water, pour the suspended material through the 5/16-inch sieve and the #30-mesh sieve. 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.

6.9.1 Gently rinse the sample material with tap water making sure the water pressure does not damage the organisms (a small hose attached to the faucet works best).

6.9.2 It has been shown that 80 to 95% of the individuals in the sample are found in the refined light material contained in the #30-mesh sieve.

6.10 When the rinsing process is complete, there will be three portions of the sample. The first is the large material (leaves, twigs, rocks, etc.) that was picked out of the sample. The second will be primarily coarse sand, but may also be shell hash and other similarly sized material. The third is the refined light material in the #30-mesh sieve. Examine the first two portions under the lighted magnifier for organisms that were not removed by the rinsing process. If any organisms are found, randomly place them in the #30-mesh sieve which contains 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.

6.10.1 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 O.C. White® lighted magnifier.

6.10.2 Use a waterproof label with the name of the person sorting the sample 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.

6.11 Place the #30-mesh sieve containing the refined material into a pan. Fill the pan with enough tap 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 at least five times. This step also helps distribute individuals evenly in the sieve.

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

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6.12.1 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., 12, 18, 21, etc.). Avoid smearing the material into the sides and corners of the trays.

6.13 Using a number randomizer, 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 analyst's initials, on Tray 2.

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

6.14 Using a number randomizer, select a square from Tray 2 and place it into a smaller tray (Tray 3), which has been subdivided evenly into 16ths. Place a Rep 1 vial sticker on Tray 3.

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

6.14.2 If the sample is particularly dirty, carefully place the Tray 2 square into a small U.S. #30-sieve and repeat step 6.11 to remove small particles before transferring the material to Tray 3.

6.15 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, systematically scan back and forth or up and down picking every organism from the Petri dish and placing it into the appropriately labeled, ethanol-filled vial.

6.15.1 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.

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

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

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

6.16.1 Petri dishes should be saved until there are 10 or the target number of individuals has been reached. At that point another analyst should recheck one of the dishes. If organisms are found, the information is recorded in the LIMS Prep Worksheet Manager, 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 waste container and the material may be discarded. All dishes should be rechecked during training (see SOP IZ-40).

6.16.2 The ethanol waste container is located under the sinks in the main laboratory (B205).

6.16.3 Additional subdivision can be done at any point during the sorting. For example, a whole 16^{th} may be sorted yielding 70 organisms, and then the next 16^{th} could be subdivided into 2 parts to presumably yield 35 organisms for a total of 105 organisms.

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

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6.17 If the target count has not been reached with the entire contents of Tray 3, use a number randomizer to randomly select another square from Tray 2 and repeat steps 6.14-6.16 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.

6.17.1 If the time taken to pick one replicate is nearing 12 hours, notify the Taxonomy Coordinator and Supervisor.

6.17.2 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.

6.18 If all of 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 organisms from the material, go back to Tray 1 and repeat the entire process from Step 6.13.

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

6.19.1 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).

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

6.19.3 After the target number of individuals has been reached, record at the top of the bench sheet the sorter, date of completion, and duration of this part of the analysis. Tally and record the number of individuals picked.

6.19.4 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

6.20 Place a Vial Label sticker onto 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).

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

6.20.2 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.

6.21 Randomly select one additional square from Tray 2 and place it in a separate 250 mL jar labeled with a Vial Label sticker.

6.21.1 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.

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6.22 Using the Laboratory/Technical/Prep Worksheet Manager utility in the LIMS, complete the prep worksheet. Record completion of preparation and QC on the Cumulative Sort QC Excel document located at X:\Labs\Lab5\Biology\InvertGroup\Sample_Prep\ Cumulative_Sort_QC_March_2024

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.

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.

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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

10.4 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*

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.

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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
02/07/2025	Updated multiple sections to align with wording in IZ-02; removed IZ-SORT from References – the information recorded in IZ-SORT is now recorded in Prep Worksheet Manager and the Cumulative_Sort_QC spreadsheet.

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