Invertebrate Core/Grab/Dredge Sample Preparation

(Modified from *Standard Methods 10500C*)

1. SCOPE AND APPLICATION

1.1 This method details the rinsing, sorting, and storing of core and grab/dredge samples collected with a coring device, a petite ponar, or a Van Veen sampler.

1.2 This method is used for samples collected per DEP Standard Operating Procedure (SOP) FS 7000 – *General Biological Community Sampling* in environments deemed to have acceptable conditions for calculating macroinvertebrate community and diversity measures per SOP LT 7000 – *Biological Indices*.

1.3 This method is to be used for all quantitative samples with Laboratory Information Management System (LIMS) test codes ending in QNCC, QNCP, 3COR, QNNC, VNUK, or VNSG.

1.4 This Standard Operating Procedure (SOP) incorporates previous versions of SOP IZ-05, *Invertebrate Grab/Dredge Sample Preparation*.

2. SUMMARY OF METHOD

2.1 These samples are sieved and preserved in the field according to FS-7000, *General Biological Community Sampling*. For quantitative samples, the entire sample or an appropriate subsample is completely picked of macroinvertebrates. When the sample is processed in the laboratory, the sample is emptied into a U.S. #30-mesh sieve over a discard bucket to discard the waste formalin and catch all material smaller than the #30-mesh in the sieve.

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 examined under a dissecting microscope to search for macroinvertebrates. Depending on the requirements of the project subsampling can be performed on samples that contain large numbers of individuals. All organisms are transferred to an ethanol-filled glass vial for identification.

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3. APPARATUS AND EQUIPMENT

3.1 Waterproof paper, pencil, and black pen
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
3.7 Shallow pan
3.8 Luxo® lighted magnifier
3.9 Glass jars, 100 to 250 mL
3.10 Dissecting microscope
3.11 Petri dishes (100 x 15 mm), 10 per sample
3.12 Forceps
3.13 Vial for picked organisms (1 or 2 dram)
3.14 Laboratory counter
3.15 Squeeze bottle of 80% Ethanol
3.16 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

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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-IZ04. Then, select the analyst's name from the module below under "Assign to...". Ensure "INVERTZOO" is 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	2	Vials/jars	EAR-Labs-LBL19

6.5 Using waterproof paper and pencil make clearly written labels 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 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 has been transferred to the sieve. Do not remove the labels. Clean the containers and place them to dry. Check the sample in using the Laboratory/Receiving/Custody utility in the LIMS.

6.7.1 The LIMS Custody utility is tracking the sample bottle, not the contents. Empty containers will be kept for a period of time, designated by the Lab Data Support

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(LDS) staff, after the job is authorized in LIMS. After this period of time, the designated staff will collect the empty bottles and discard them from LIMS.

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

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6.12 Using an ethanol-filled (80% ethanol) squeeze bottle, rinse the organisms plus detritus matrix from the sieve into the smallest practical container (usually a 100-mL to 250-mL glass jar). Place one printed vial label with the sample information on the jar.

6.13 Place a small amount of detritus plus organism mixture into a Petri dish and add ethanol until the material moves around easily with forceps.

6.13.1 Do not overload the petri dish with too much material, as this may result in organisms being overlooked.

6.13.2 The top portion of the petri dish may later be placed over the sample to prevent desiccation if you must leave the sample overnight.

6.14 Under a dissecting microscope, systematically scan back and forth or up and down, picking every organism from the Petri dish and placing it into an 80% 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 the Petri dish will help in the systematic removal of organisms.

6.15 Continue this process until the sample is finished.

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 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).

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

6.16 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

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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 an 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 Material Safety Data Sheets (MSDS) for information about reagents and chemicals in this SOP.

9.7 Refer to the Laboratory Health and Safety Plan for all other safety and waste management information.

10. REFERENCES

10.1 DEP Laboratory Quality Assurance 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 Standard Methods 10500 C, *Benthic Macroinvertebrates: Sample Processing and Analysis*

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

10.5 SOP LT-7000, *Biological Indices*

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

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

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

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

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

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

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

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

Appendix of Changes

12/01/2009	Reformatted the layout following SOP LB-001, added extra quality assurance checks (6.1, 6.1.1, and 6.1.2), modified the creation and completion of the prep worksheet in LIMS (6.2 and 6.15.1), added changes in use of the LIMS custody utility (6.6) and moved rinsing to step 6.7.
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.
11/19/2015	Removed reference to the Log In logbook and corrected form name to IZ-SORT.
02/22/2017	Standardized SOP Naming Conventions.
03/13/2018	Updated DEP Laboratory Quality Manual link
03/06/2019	Changed “Receiving staff” to “designated staff” in Section 6.6.1.
02/25-2020	Edited Summary of Method to better represent the actual process—process is similar to, but not identical to—SCI. Added Standard Methods to the Reference Section.
02/10/2021	changed van veen to van Veen; updated link to Laboratory Health and Safety Plan
01/05/2022	Updated printer names.
02/06/2025	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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