Invertebrate Taxonomic Identification Quality Control

Initial and Continuing Proficiency Demonstration

1. SCOPE AND APPLICATION

1.1 This method details the procedure for training a new analyst in macroinvertebrate identification and for determining when the new analyst is considered proficient and able to identify samples without constant Quality Control (QC) of samples.

1.2 This method also details the procedure for assessing continuing proficiency of macroinvertebrate taxonomists who have already been deemed proficient.

1.3 This method applies to all macroinvertebrate samples with a Laboratory Information Management System (LIMS) test code beginning with MI- and not ending in -SORT.

2. SUMMARY OF METHOD

2.1 Macroinvertebrate taxonomists who are new to the Florida Department of Environmental Protection (DEP) Biology Program, Invertebrate Taxonomy Workgroup begin training on macroinvertebrate identifications by identifying samples that have been previously identified by a proficient taxonomist. This procedure occurs regardless of the prior training of the new taxonomist, although a taxonomist with prior training and/or experience may proceed through the process faster than one with less training and/or experience.

2.2 Initial proficiency is determined by giving the new analyst periodic “test” samples, which consist of combinations of a variety of macroinvertebrate species found in Florida. The analyst continues to analyze previously identified samples between test samples until a test sample is identified ≥ 95% correctly at the species level.

2.3 Once the new analyst achieves ≥ 95% correct identifications on the test samples to the species level and ≥ 95% correct identifications on three consecutive samples, the new analyst is considered proficient, and an Initial Documentation of Proficiency is generated in LIMS.

2.4 Proficient analysts keep a running list of the samples they identify and have 1 out of every 20 of those samples (5%) re-identified by another proficient analyst. A minimum of 1 sample must be reidentified independently per quarter per analyst. If disagreements are encountered in the original identification and the re-identification, those differences are addressed using available references and expertise. Outside expertise is used in cases where a resolution cannot be reached internally. The final resolution is noted on the original and QC bench sheet, and copies of both bench sheets are stored for future reference.

2.5 All QC results are tracked in a spreadsheet so that initial proficiency and continuing proficiency can be monitored by the Taxonomy Supervisor.

3. APPARATUS AND EQUIPMENT

3.1 Dissecting and compound microscopes
3.2 Identification references
3.3 Squeeze bottle of 80% Ethanol
3.4 Forceps

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3.5 Petri dishes (100 x 15 and/or 60 x 15 mm) or Syracuse watch glass (2-5/8" diameter)
3.6 Laboratory counter (optional)
3.7 List of random numbers
3.8 Previously identified samples
3.9 Test Quality Control (QC) samples

4. REAGENTS AND CHEMICALS

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

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1 Samples are collected, preserved, and delivered to the lab according to SOP FS 7000: *General Biological Community Sampling*, SCI 1000: *Stream Condition Index Methods*, or BRN 1000: *Biological Reconnaissance Field Methods*.

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

6. SAMPLE PREPARATION PROCEDURE

6.1 Samples are prepped per one of the following SOP IZ-02, IZ-03, IZ-04, or IZ-39, depending on the type of sample, and checked for QC following SOP IZ-40.

6.2 The Test QC samples for proficiency determination are prepared by a proficient analyst. Organisms are pulled from previously identified samples and placed into individually labeled scintillation vials. Each vial is labeled with a code that relates to an answer key prepared at the same time. An effort is made to include a variety of organisms found throughout Florida in different types of samples. The Statewide Biological Database (SBIO) can be used to find samples with enough of a specific organism so that specimens are not as difficult to find.

7. SAMPLE ANALYSIS PROCEDURE

7.1 Initial Demonstration of Proficiency

7.1.1 When a trainee begins identifying organisms in previously identified samples following SOP IZ-06, all their identifications are checked continually by a proficient analyst. The trainee is encouraged to review reference collection materials at any point during training but not while identifying a test QC sample.

Note: New analysts will be assigned a variety of samples (e.g. wetland, springs, etc.) that have previously been identified by a proficient analyst to make certain they have seen most of the organisms likely to be encountered in future samples.

7.1.2 After each training sample has been identified, the new analyst should go back and review the original bench sheet and discuss any issues with the original identifier, if available, or another proficient taxonomist. Then, the electronic taxonomic Internal Resolution form IZ-QC-(version 1.1), should be filled out as described in 7.1.3.4 to track training progress. This form is then saved the copy in a created training folder labeled with the new analyst's name.

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7.1.3 Test QC Samples: The purpose of the test samples is to gauge the trainee's progress.

7.1.3.1 After approximately 4-6 weeks of training, a trainee will be given a test QC sample. The first test sample will usually contain a variety of taxa and the subsequent test samples may be tailored more toward the taxa with which the trainee has difficulty. For the first test, the trainee will record the Family as well as the lowest practical level of identification. The target for the test sample is ≥ 80% correct identifications at the Family level. If the trainee achieves the target level, they will continue to identify previously identified samples for approximately another two weeks.

Note: The timing of the first test samples will depend on the previous taxonomic experience of the trainee and the recommendation of a proficient analyst.

Note: The trainee may take a preliminary test QC evaluating the trainee's ability to sort invertebrates into the correct Order, given before the Family test.

7.1.3.2 At 6-8 weeks, the trainee will be given another test QC sample. The trainee will record the Genus as well as the lowest practical level of identification. The target for this sample is ≥ 90% correct identifications at the Genus level. Upon successful completion, the trainee will continue to analyze previously identified samples.

7.1.3.3 At 10-12 weeks, the trainee will be given a third and final test sample. The trainee will be expected to achieve ≥ 95% correct identifications at the Species level. If the target is achieved, the trainee will analyze two samples to the final target level of ≥ 95% correct identifications at the Species level. When a new analyst achieves ≥ 95% correct identifications at the Species level on 3 consecutive samples, the analyst will have demonstrated an initial proficiency.

Note: The two samples can be new samples, not identified previously, and these two samples must both be examined for Quality Control by another proficient taxonomist.

7.1.3.4 For each test sample, complete the electronic taxonomic Internal Resolution form, IZ-QC-(version 1.1) located in the Invert Group's Internal QC folder, currently located in:
DEAR\Labs\Lab5\Biology\InvertGroup\Quality_Control\Internal_QC\Resolutions

The proficiency rate is automatically calculated on the Internal Resolution Excel file, IZ-QC-(version1.1).

% Proficiency = 100 * [(Total # Individuals – Total # Errors) / Total # Individuals]

Save the file with name "LIMS#-Rep[replicate#]_initials of first identifier_initials of second identifier" in the Invertebrate Workgroup's Internal QC Resolutions folder, currently located in

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DEAR\Labs\Lab5\Biology\InvertGroup\Quality_Control\Internal_QC\Resolutions

7.1.4 After an analyst has successfully passed the Initial Demonstration of Proficiency, the analyst will begin working on samples independently, being certain to ask questions as they arise. The reference collection should be used routinely by all analysts to verify their identifications

Note: Test sample results and Initial Demonstration of Proficiency are recorded in an Excel workbook located in the Invert Group's Internal QC Taxonomy Exam folder, currently located in DEAR\Labs\Lab5\Biology\InvertGroup\Quality_Control\Internal_QC\Taxonomy_Exams . The date proficiency was achieved should be recorded in LIMS under Laboratory/Administrative/Training by the Invertebrate Taxonomy Supervisor and in SBIO by the Database Administrator

7.1.5 If a trainee fails to meet a target for a test QC sample, an effort will be made to provide additional training in deficient groups. If, after approximately 12 weeks of training, a trainee has not successfully demonstrated an Initial Proficiency, the Taxonomy Supervisor and the Program Administrator will decide the future course of action.

7.2 Continuing Demonstration of Proficiency

7.2.1 Once 20 samples or sample replicates have been identified, the analyst, using a list of random numbers, will select one of the 20 vials filled with organisms previously identified and give that sample to another proficient analyst to re-identify (QC). A minimum of 1 sample per quarter per analyst will be reidentified in this manner.

7.2.2 The QC analyst will identify the organisms to the lowest practical taxonomic level using the most appropriate taxonomic key for each group and then return all organisms to the sample vial. If a large number of same taxa are present, a laboratory counter should be used to keep a running total.

Note: Removing organisms as they are counted will help avoid counting errors. In addition to ID manuals, remember to check the reference collection, the organism's geographic range, and other taxonomists if needed.

7.2.3 Record the name and number of each taxon on a blank Bench Sheet.

7.2.4 Obtain the original list of taxa for that sample and complete the electronic taxonomic Internal Resolution form, IZ-QC-(version1.1). If there is a disagreement regarding an identification, consult with the original identifier until a consensus is reached.

Note: When warranted, bring another taxonomist into the discussion. If a consensus cannot be reached, leave the organism(s) at the lowest taxonomic level agreed upon by both identifiers. An appropriate follow-up may involve sending the specimen(s) to an expert for verification. Make a note on the bench sheet when an organism(s) is sent off for verification.

Note: Invertebrate identification Internal QC files are located in the Invert Group's Internal QC folder, currently located in DEAR\Labs\Lab5\Biology\InvertGroup\Quality_Control\Internal_QC

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7.2.5 Any changes on the original bench sheet should be crossed through once, initialed and dated. In addition, make any necessary changes to data if it has already been entered into SBIO. For quality control purposes, the SBIO Database Manager must be notified to make corrections if the sample has been marked complete for greater than 3 months.

Note: If it is determined (through expert verification or changes in taxonomy) that an incorrect or obsolete name is given to an organism, appropriate changes to data sheets and in computer databases are made to reflect the corrected nomenclature. These changes are limited in scope to a given study.

7.2.6 The proficiency rate is automatically calculated on the Internal Resolution Excel file, IZ-QC-(version 1.1). Save the file with name "LIMS#-Rep[replicate#]_initials of first identifier_initials of second identifier" in the Invertebrate Workgroup's Internal QC Resolutions folder, currently located in DEAR\Labs\Lab5\Biology\InvertGroup\Quality_Control\Internal_QC\Resolutions

7.2.7 The Taxonomy Supervisor reviews each Internal Resolution form, records the proficiency in a spreadsheet, and moves each completed Internal Resolution form to a storage folder.

7.2.8 For **continued** demonstration of proficiency, a cumulative average of ≥ 95% correct identifications for organisms for each quarter must be maintained.

7.2.8.1 The corrective action for dropping below the continued demonstration is to go back onto training status until able to demonstrate the required proficiency.

7.2.8.2 The corrective action for a single QC proficiency rate below 95% correct ID for individuals, is to have a follow-up QC performed by another taxonomist on another randomly chosen sample from the same list used to choose the original QC sample.

7.2.8.3 Corrective action for taxonomic deficiencies is approved by the Taxonomy Supervisor. External sources that initiate corrective action include knowledge gained through training workshops and new reference material, and nomenclatural verification by experts. Laboratory personnel are notified of problems and corrective action through discussion with the Taxonomy Supervisor, and in conjunction with performance evaluation procedures.

7.3 Initial Demonstration of Proficiency – identification of non-mounted midges

7.3.1 A taxonomist follows the same procedure for mounted midge identifications using a compound microscope. Once the taxonomist demonstrates continued proficiency for mounted midge identifications, that taxonomist can then attempt initial demonstration of wet midge identifications.

7.3.2 A taxonomist that demonstrates the ability to meet the DEP 95% correct taxa identification quality control criteria for wet Chironomidae larvae identifications on a minimum of 10 samples (replicates of SCI) is not required to mount Chironomidae larvae for which he or she feels an accurate identification can be made. Routine

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internal quality control of 5% completed samples will include both the mounted and non-mounted portions of the samples.

See report "FINAL – Wet Chironomidae Demonstration Study 2-10-10.dox", DEAR\Labs\Lab5\Biology\InvertGroup\Quality_Control\Internal_QC\Wet Chironomidae QC

8. QUALITY CONTROL AND DOCUMENTATION

8.1 Internal QC Resolution form, IZ-QC-(version 1.1)

8.2 Appropriate bench sheet for sample being re-identified.

8.3 Use pen with black ink for all bench sheet entries.

8.4 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.

8.5 DEP Laboratory Information Management System (LIMS)

8.6 Refer to the Laboratory Quality Manual.

9. SAFETY

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

9.2 Refer to the MSDS for information about reagents and chemicals in this SOP.

9.3 Refer to the Laboratory Health and Safety Plan.

10. POLLUTION PREVENTION AND WASTE MANAGEMENT

10.1 Refer to the Laboratory Health and Safety Plan.

11. REFERENCES

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

11.2 DEP Laboratory Health and Safety Plan, https://floridadep.sharepoint.com/dear/SitePages/Home.aspx

11.3 Form IZ-QC: *DEP Macroinvertebrate Taxonomic Internal QC Resolution*

11.4 SOP FS-7000: *General Biological Community Sampling*

11.5 SOP SCI-1000: *Stream Condition Index Methods*

11.6 SOP BRN-1000: *Biological Reconnaissance Field Methods*

11.7 SOP IZ-01: *Sample Arrival/Log-in Procedures*

11.8 SOP IZ-02: *Stream Condition Index (SCI) Sample Preparation*

11.9 SOP IZ-03: *Invertebrate Hester-Dendy Sample Preparation*

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11.10 SOP IZ-04: *Invertebrate Core/Grab/Dredge Sample Preparation*

11.11 SOP IZ-06: *Invertebrate Enumeration and Taxonomic Analysis*

11.12 SOP IZ-09: *Preparation of 80% Ethanol*

11.13 SOP IZ-39: *Macroinvertebrate Subsampling Sample Preparation*

11.14 SOP IZ-40: *Invertebrate Sorting Quality Control (QC)*

Appendix of Changes

02/28/2009	Reformatted the layout following SOP LB-001.
03/10/2010	Updated the Invert Group link; changed a "Note" in step 7.2.6 and included 7.3 Initial Demonstration of Proficiency – identification of non-mounted midges
03/08/2011	Renamed the Quality Manual and updated the link
03/07/2012	Added 7.2.7, continued proficiency rate and corrective action.11/01/2012 Included additional corrective action details
11/04/2013	Updated sorting SOP #; clarified process regarding electronic resolutions
10/27/2014	Formatted for 508 compliance.
11/05/2015	Updated links and SOP references. Included reference to form IZ-QC, an electronic log of taxonomic identification QC.
11/19/2015	Included using electronic log for IDOC test samples.
12/20/2016	Updated links for Laboratory Quality Manual and Invertebrate QC spreadsheet locations and standardized SOP naming conventions; Removed paper bench sheet references in initial demonstration of proficiency.
01/14/2020	Corrected syntax and grammar throughout areas of the text. Added a note in section 7 to explain the Order QC test given to trainees prior to the Family test. Updated file pathways.
12/15/2020	Section 2.4. and 7.2.: added minimum number of samples to be QCed per analyst per quarter.
11/14/2022	Section 7.1.1: to read: The trainee is encouraged to review reference collection materials at any point during training, but not while identifying a test QC sample.
11/16/2023	Updated broken links
11/08/2024	Added version numbers to IZ-QC Invertebrate Taxonomic Resolution Form where mentioned in document. Updated location of Internal Resolution folder in Section 7.1.3.4. Removed "Also save a copy of IZ-IDQC-(version 3-2011#) with the same name as a .pdf file" comment from Sections 7.1.3.4 and 7.2.6.

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