Mounting of Worms, Midges, and Aquatic Mites

(Based on Epler, 2001, Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida. And Pluchino, 1984, Guide to the common water mite genera of Florida)

1. SCOPE AND APPLICATION

1.1 This method details the slide mounting of macroinvertebrate specimens, such as worms, midges and aquatic mites (if necessary), or parts of specimens, such as labrums, mouthparts, parapodia, etc., for taxonomic identification.

1.2 This method is to be used for all samples with Laboratory Information Management System (LIMS) test codes beginning with MI- and not ending in -SORT.

2. SUMMARY OF METHOD

2.1 Specimens are mounted on glass microscope slides under glass coverslips using CMC-1019 Mounting Media. Slides are then either air dried or dried in an oven at 85° to 95° C for 1-2 hours. Slides can be dried for 2-4 hours at 50° C if there are problems, such as boiling media or air pockets forming under the coverslips, at the higher temperatures.

3. APPARATUS AND EQUIPMENT

3.1 Dissecting microscope
3.2 Forceps
3.3 Glass microscope slides, 25 x 75 mm
3.4 Cover slips, round, 12 and 18 mm diameter
3.5 Labels for slides

4. REAGENTS AND CHEMICALS

4.1 Mounting medium (CMC-1019)
4.2 1N Potassium Hydroxide (KOH)

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1 Samples are collected, preserved, and delivered to the lab per 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 Standard Operating Procedure (SOP) IZ-01.

6. SAMPLE PREPARATION PROCEDURE

6.1 Samples are prepared per SOP IZ-02, IZ-03, IZ-04, or IZ-39

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7. SAMPLE ANALYSIS PROCEDURE

7.1 Samples are analyzed per SOP IZ-06 and IZ-07.

7.2 Using the Laboratory/Receiving/Print Preparation Labels utility in LIMS, print out slide labels. All labels are printed from the Sato printer.

7.2.1 To print slide labels, go to the sample Log-in station, open LIMS and select "Laboratory", "Receiving", "Print Preparation Labels." Go to "Invert_Labels". For Stream Condition Index (SCI) samples with replicates, choose either "Invert Slide Labels Rep 1" or "Invert Slide Labels Rep 2" as appropriate. For all other samples, choose "Invert Slide Labels". Scan the sample bar code, select "File", "Print", select the "LBL19" printer, and print the number of labels needed.

7.3 Affix a slide label to each slide by wrapping the label around the right edge of a slide, or on the left side if you are left-handed.

7.4 Starting on the far left of the slide, place a drop of CMC on the slide for each specimen being mounted. Use enough CMC to form a seal around the coverslip when the specimen is pressed down.

7.4.1 Mount 4 specimens per slide under 12 mm cover slips. In the case of very large specimens, two are mounted per slide under 18 mm coverslips.

7.5 Using a needle or a pair of forceps, place the specimen in the CMC. Midges and mites (if necessary) should be mounted ventral side up and upside down.

7.5.1 Mounting of hard-bodied aquatic mites or other large specimens requires more CMC, otherwise air will accumulate under the coverslip and the necessary structures will not be visible. If necessary, mites can be cleared in a 1N KOH solution and then rinsed in DI water before mounting.

7.6 Using forceps, place the coverslip over the specimen.

7.6.1 Place the coverslip over the specimen at an angle to reduce air bubbles and roll the specimen into position.

7.7 Using forceps or the eraser of a pencil, press the coverslip onto the specimen. At this point, orient the specimen by pushing or pressing the coverslip in various ways.

7.7.1 Usually, midges are "C" shaped when preserved, and can be rolled into position when the coverslip is placed over the specimen. Sufficient pressure must be applied to spread the mandibles and labrum. Be careful not to break the coverslip, as the pressure required to flatten the specimen may be great.

7.8 Place the slides on a slide tray and place in the drying oven at 85° to 95° C for 1-2 hours or at 50° C for 2-4 hours if there are problems at higher temperatures, such as boiling media or air pockets forming under the coverslips. Alternatively, the slides may be set aside to dry at room temperature. (Note: It may take 1 to 2 days for the slides to dry completely at room temperature). Following either method, the slides should be left on the tray to dry completely for 12-24 hours before putting them away in a slide holder for archiving. This will prevent the slides from sticking together, which in turn will make performing a QC on the slides much easier.

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7.8.1 Station identifiers along with the total number of slides, the initials of the mounter, and the date mounted can be written on a small piece of paper and attached to the tray on which the slides are placed to dry. This is often helpful when identifying the organisms.

7.9 Record the mounting information on the top of the sample bench sheet.

8. QUALITY CONTROL AND DOCUMENTATION

8.1 Department of Environmental Protection (DEP) Laboratory Freshwater, Marine Invertebrate, or Macroinvertebrate bench sheet. Use a pen with black ink for all bench sheet entries.

8.2 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.

8.3 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.

8.4 Quality Control (QC) of Sorting is performed per SOP IZ-40.

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

8.6 Refer to the Laboratory Quality Manual.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

9.1 CMC-1019 Mounting Media can cause irritation to eyes and skin. Avoid contact with eyes and skin and avoid inhaling or ingesting. For skin, wash affected areas with soap and water (MSDS says for at least 15 minutes).

9.2 Triple rinse empty containers with water before disposing.

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

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

10.4 SOP SCI-1000, *Stream Condition Index Methods*

10.5 SOP BRN-1000, *Biological Reconnaissance Field Methods*

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

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10.7 SOP IZ-02, *Stream Condition Index (SCI) Sample Preparation*

10.8 SOP IZ-03, *Invertebrate Hester-Dendy Sample Preparation*

10.9 SOP IZ-04, *Invertebrate Core/Grab/Dredge Sample Preparation*

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

10.11 SOP IZ-07, *Subsampling of Worms and Midges*

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

10.13 SOP IZ-39, *Non-SCI Macroinvertebrate Sample Preparation*

10.14 SOP IZ-12, *Invertebrate Sorting Quality Control (QC)*

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

10.16 Epler, 2001, *Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida*

10.17 Pluchino, 1984, *Guide to the common water mite genera of Florida*

Appendix of Significant Changes

12/21/2009	Reformatted the layout following SOP LB-001.
11/10/2011	Updated date for Epler's taxonomic key. Clarified slide drying process. Updated name for the Laboratory Safety Manual.
11/04/2013	Revised scope to apply to any specimen or part of specimen from any type of macroinvertebrate sample.
10/27/2014	Formatted for 508 compliance.
11/05/2015	Updated SOP references.
11/19/2015	Included step 7.9, record mounting information on the bench sheet.
03/01/2017	Standardized SOP naming conventions. Added potassium hydroxide to the reagents section.
03/14/2018	Updated DEP Laboratory Quality Manual link
02/17/2020	Added Reference: Based on Epler, 2001, Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida and Pluchino, 1984, Guide to the common water mite genera of Florida)
02/08/2021	Updated link to the DEP Laboratory Health and Safety Plan. Added the recommendation of allowing time for slides to dry before archiving.
01/05/2022	Updated printer name.
02/16/2023	Updated CMC-10 to CMC-1019 to reflect the new formula.

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