

U.S. Geological Survey
National Water Quality Laboratory

Preparing Benthic Macroinvertebrate Microscope Slides

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Preparing Benthic Macroinvertebrate Microscope Slides

1. Scope, application, and summary

Certain groups of benthic macroinvertebrates (BMIs) (for example, Chironomidae larvae and Oligochaeta) may need to be mounted on microscope slides to achieve the requested level of taxonomic identification (for example, Moulton and others, 2000).

The use of trade, product, or firm names in this procedure is for descriptive purposes only and does not imply endorsement by the U.S. Government.

These procedures are used by anyone responsible for mounting BMIs on microscope slides.

Specimens are oriented in mounting medium (for example, CMC-10™) on a labeled microscope slide, covered with a cover glass, and the mounting medium is allowed to dry.

Interferences

1.1.1. Poor slide mounts often prevent a taxonomist from making identifications to the requested level (for example, genus or species).

1.1.2. Factors contributing to poorly prepared slides include:

1.1.2.1. Improper orientation of the specimen on the slide

1.1.2.2. Mounting a specimen too large to be mounted under the cover glass

1.1.2.3. Improper clearing of the specimen(s) prior to mounting or by the mounting medium after mounting

1.1.2.4. Viscosity of the mounting medium

1.1.2.5. Air bubbles beneath the cover glass

2. Reasons for and summary of changes

This SOP is being updated to the new format as per NWQL SOP No. QUAX0001.x.

Links to NWQL Technical/Policy Memoranda are being deleted

Reference to a document that is not utilized is being deleted

A grammatical error was corrected in section 10.1.2.

A statement was added to the 'Deviations' section of this SOP to allow for deviations from this SOP at the request of a customer.

NWQL SOP BIOP0334.3	Analytical Procedure	Controlled copy
SOP title: Preparing benthic macroinvertebrate microscope slides		
Authors: Scott Grotheer and Robert Hood		Contact: Rob Hood
Effective date: 7 June 2011		
Approved by and date: Duane S. Wydoski, 06/07/2011		Revise or reapprove by: 06/07/2014
<i>A paper copy of this document is only valid if its revision number matches the controlled copy posted on the intranet.</i>		
<i>It is the user's responsibility to verify that these revision numbers match.</i>		

The analyst is not required to submit a new demonstration of capability based upon the revised SOP. The analyst is not required to submit a document training form for the revised SOP.

3. Health, safety, and waste disposal information

Applicable Health and Safety Issues

- 3.1.1. Wear long pants and closed-toed shoes at all times when working in the laboratory.
- 3.1.2. Know the location of the nearest eyewash and shower stations.
- 3.1.3. Do not eat or drink in the laboratory.

Waste Disposal and Environmental Compliance

- 3.1.4. Only work in the laboratory when the room ventilation system and fume hoods are working properly. Leave the laboratory and contact the Biological Group (BG) supervisor or appropriate Support Services section representative if the ventilation systems are not working properly.
- 3.1.5. Know the location of and be familiar with the Material Safety Data Sheets (MSDS) for each chemical used by the BG.
- 3.1.6. Know how to report and handle chemical and sample spills using procedures described in the National Water Quality Laboratory (NWQL) Chemical Hygiene Plan (available from the NWQL Safety, Health, and Environmental Compliance (SHE) section).
- 3.1.7. Empty bottles of mounting medium and used Pasteur pipettes can be disposed of as solid waste with the gloves and detritus from other laboratory processes in properly labeled solid waste containers.

All NWQL personnel will follow the guidance outlined in the following policy memoranda, accessible on the NWQL Home page (internal USGS access only) > Technical > Policy Memoranda. Supervisors will ensure that employees submit completed training forms, documenting that they have read and understood the information contained in these policy memoranda.

- 3.1.8. Safety, Health and Environmental (SHE) Compliance Section's Policy Memorandum 2006.01, *General laboratory health, safety, and waste disposal requirements*
- 3.1.9. NWQL Policy Memorandum 2008.01, *Conducting chemical and other potentially hazardous operations at the NWQL*
- 3.1.10. NWQL Policy Memorandum 04.01, *Pollution prevention and waste minimization policy*
- 3.1.11. NWQL Policy Memorandum 2010.02, *NWQL hours of operation: High, Moderate, and Low Risks*, pp. 3–4

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All NWQL personnel will follow the guidance outlined in the NWQL SOP SHEX0355.x, *Waste disposal at the National Water Quality Laboratory*.

4. Sample preservation, handling, containers, analytical processing/holding times, and disposal

Refer to NWQL SOP No. BIOB0332.x and NWQL SOP No.. BIOB0333.x.

5. Preparation of reagents, standards, and solvents

CMC-10™ mounting medium

70-percent ethanol

6. Apparatus

Supplies

6.1.1. No.1.5 18 x 18 mm square cover glasses with a thickness range of 0.163 to 0.196 mm are preferred, but others may be used if necessary

6.1.2. No.2 18 X 18 mm square cover glasses with a thickness range of 0.17 to 0.25 mm.

6.1.3. Colored labeling tape (“time tape”)

6.1.4. Forceps (fine-tipped)

6.1.5. Microscope slides

6.1.6. Paper towels

6.1.7. Pasteur pipettes with suction bulbs

6.1.8. Probes (fine-tipped)

6.1.9. Slide Boxes

6.1.10. Slide labels according to project

6.1.11. Watch glasses or other similar glass dishes

Equipment

6.1.12. Binocular microscope

6.1.13. Fiber-optic illuminator

6.1.14. Slide warmers, if needed, should not be set to a temperature in excess of 55° Celsius.

7. Sample preparation, analysis, and instrument operation and shutdown

Produce consecutively numbered slide labels for each sample.

- 7.1.1. Obtain the paperwork packet for each sample(s) and the specimen(s) to be mounted from their designated locations.
- 7.1.2. Determine the number of specimens to be mounted from each sample.
 - 7.1.2.1. For qualitative samples processed using NWQL Lab Code 2176, produce nine (9) labels per sample.
 - 7.1.2.2. For chironomidae in quantitative samples, determine the number of labels needed by dividing the total number of specimens in each life stage by 8, round up each result to the next highest integer, and add the results from each life stage together. For example, if 226 larvae and 6 pupae are to be mounted, generate 30 labels (i.e. $226 \div 8 = 28.25$, round-up to 29; $6 \div 8$ is 0.75, round-up to 1; $29 + 1 = 30$).
 - 7.1.2.3. For oligochaetes simply divide the total number of specimens by 8 and round up.
- 7.1.3. Generate the labels using the following general procedure. Due to changes in the NWQL computer systems, this procedure may vary slightly. If you have any questions, consult with the BG Production Coordinator to determine the current procedure. These labels will include the sample identification code, the first initial and last name of the taxonomist that identified the unmounted taxa, the date the labels were printed, and a slide number.
 - 7.1.3.1. Open Exceed™, enter your user ID and password, and change directory to “/home/invert” by typing “cd /home/invert” at the prompt.
 - 7.1.3.2. To run the label program, type “midge” at the prompt and follow the instructions.
 - 7.1.3.3. When the program is complete, open Framemaker™, open ‘/home/invert/MIDGE.label.blank’, import the file generated by the program into the label blank, and print the labels on full-sheet white adhesive back paper using a laser jet printer.
- 7.1.4. Cut the labels into strips according to sample identification code; paperclip the labels to their corresponding Slide Preparations—Identification and Enumeration Worksheet (see Attachment 10 in NWQL SOP No. BIOB0335.x).
- 7.1.5. If automated labels cannot be produced, place consecutively numbered labels on each slide with the first initial and last name of the taxonomist that will identify the specimens, the date of mounting, sample identification code, and portion of sample from which the specimens were obtained. Record the dates in a ‘dd-mm-yyyy’ format, using Roman numerals for the month. For example, July 12, 2004 is recorded as 12-VII-2004.

Mounting Procedure

- 7.1.6. Guidelines
 - 7.1.6.1. Use no more than two (2) cover glasses per slide.
 - 7.1.6.2. Mount only one life stage under each cover glass.

- 7.1.6.3. Do not mount more than four (4) specimens under a cover glass.
- 7.1.7. Obtain the paperwork, labels (see Section 4.3), and vial(s) for the sample to be mounted. Cut the label strips into individual labels of suitable size. Peel the backing off and apply the necessary number of labels to slides. Labels are applied to the left hand side of each slide.
- 7.1.8. Transfer specimens into a dish of 70-percent ethanol.
 - 7.1.8.1. Inspect the vial, cap, and label for attached/residual specimens.
 - 7.1.8.2. If necessary, rinse the vial, cap and/or label into the dish with the other specimens.
- 7.1.9. Sort specimens by size and/or morphology into similar groups using forceps and a dissecting microscope.
- 7.1.10. Mount the Specimens
 - 7.1.10.1. Produce a thin film of mounting medium by placing one drop of mounting medium on the slide using a Pasteur pipette; spread the mounting medium to approximate the area of a cover glass
 - 7.1.10.2. Blot four (4) or fewer specimens on a paper towel to reduce the amount of excess ethanol.
 - 7.1.10.3. Orient the specimens in the mounting medium according to procedures specific to certain taxonomic groups (see Sections 7.4 and 7.5). This will generally result in optimal viewing of diagnostic structures.
 - 7.1.10.4. Add additional mounting medium as necessary to compensate for the size and/or number of specimens being mounted, so as to minimize the number of air bubbles that may form under the cover glass.
 - 7.1.10.5. Place a cover glass on the slide by dropping it over the specimens and mounting medium parallel to the plane of the slide. Placing the cover glass over the specimens at a slight angle may aid in properly orienting the specimens.
 - 7.1.10.6. Apply slight downward and/or directional pressure to the cover glass, as necessary, until the orientation of the specimens is appropriate (see Sections 7.4 and 7.5) and all air bubbles have been expelled from under the cover glass.
 - 7.1.10.7. Add a small bead of mounting medium around the perimeter of the cover glass.
- 7.1.11. Place slides on a drying rack and dry for no less than 24 hours at no more than 55° Celsius; longer drying times may be needed at lower temperatures.
- 7.1.12. Check slides periodically for empty spaces where the mounting medium has evaporated or where re-expansion of the specimens has formed air bubbles. If necessary, add mounting medium to the edge of the cover glass. Capillary action will often draw the mounting medium beneath the cover glass to fill the void(s). In rare cases the cover glass may need to be removed and the specimen(s) re-mounted.

- 7.1.13. Check slides prior to removing them from slide dryer by applying slight directional pressure to the cover slip. If the mounting medium is dry, the cover slip will not move when this pressure is applied.

Clean-Up

- 7.1.14. Rinse and clean all tools with 70-percent ethanol.
7.1.15. Wipe up spilled mounting medium and clean workstation.
7.1.16. Put away supplies and equipment.

Procedures Specific to Chironomidae

- 7.1.17. Group Chironomidae according to life stage (larva or pupa), size, and/or Subfamily/Tribe.
7.1.18. Mount larvae first, followed by pupae.
7.1.19. Position a larva vertically on the slide with its head (ventral side up) to the top.
7.1.20. When mounting the majority of specimens, the whole specimen should be mounted.
7.1.20.1. When necessary, mount only the head, thorax, and last three abdominal segments of a large larva (for example, Diamesinae) under a single cover glass. Remaining abdominal segments are discarded.
7.1.20.2. When necessary, separate heavily sclerotized larval heads (for example, some Tanypodinae) from the rest of the body and mount so that each head is oriented above its respective body.
7.1.21. Position pupae vertically with the dorsal side or the abdomen up and the lateral side of the head/thorax up. If present, mount attached larval head capsules, ventral side up, next to their respective pupae.

Procedures Specific to Oligochaeta

- 7.1.22. Group worms according to Family and size.
7.1.23. Orient worms oriented horizontally on their side with the head region to the left.

8. Data acquisition, processing, and evaluation

None

9. Quality control (QC) and quality assurance (QA)

Mount the specimens from only one sample at a time.

Verify that the sample identification codes on the specimen vial(s), slide labels, and worksheet are identical.

Inspect all dishes before preparing slides; make sure there are no specimens or sample debris remaining from previously processed samples.

Make sure slides and cover glasses are not broken.

Check the viscosity of the mounting medium.

Ensure that the expiration date on the bottle of mounting medium has not passed.

During the drying process check the slides for air bubbles, improper specimen orientation, or other problems and repair as necessary.

10. Data management and records

Record the following information on the Slide Preparations – Identification and Enumeration Worksheet (see Attachment 10 in NWQL SOP No. BIOB0335.x):

- 10.1.1. Record the life stage(s) of specimens mounted in the 'LS' column of the left-hand section of the worksheet for each slide.
- 10.1.2. Write and "X" through all cells corresponding to positions without a mounted specimen for slides that have at least one specimen mounted on them.
- 10.1.3. Draw a complete line through the row immediately following the last slide with mounted specimens used for the sample.
- 10.1.4. Record the Block Code for the week that the non-midge identifications are completed. For example, if the unmounted identifications from the sample were completed during the week ending Friday, October 1, 2004, record 20041001.
- 10.1.5. Record the first initial and last name of the individual that mounted the specimens along with the date (mm/dd/yyyy) and the time (hh:mm) that the mounting was completed. The time is critical to determine when the slides can be removed from the slide dryer.
- 10.1.6. Record problems or errors associated with mounting the sample (for example, the number of specimens not found in a vial). Attach additional sheets as needed.
- 10.1.7. Place completed worksheet, original label(s) from the vial(s), and other paperwork for the sample in the bin designated by the BG Production Coordinator.

Using a separate slide box for each project, store the completed slides on their sides grouped by sample identification code. Slides for each sample must be in consecutive order.

Label the slide box with the appropriate project name and block code (see Section 10.1.4). Do not place more than one project in a box. If multiple boxes are needed for a project, in a given block, label them consecutively (for example, 1 of 3, 2 of 3, and 3 of 3), so that the total number of boxes in the series can be determined.

Place the labeled box of prepared slides after the rack(s) of identified unmounted taxa completed during the corresponding week.

11. Definitions, abbreviations, and acronyms

None

12. Deviations

NAWQA is asking the Biological Group (BG) to evaluate modifications to this procedure to gain efficiencies that may reduce the amount of time and/or supplies required to process samples. Certain sections of OFR 00-212 (<http://nwql.usgs.gov/Public/pubs/OFR00-212.pdf>) require that specific procedures, equipment, and/or supplies be used. Since the BG does not have access to samples that can be used for evaluation, actual field samples must be used to evaluate process and/or equipment modifications. As a result, the BG may deviate from the requirements specified in this SOP to evaluate potential procedural modifications. Deviations that are isolated to a single sample will be documented on the appropriate sample paperwork. Documentation of deviations that are more global in nature will be kept in a laboratory notebook dedicated for that purpose. All samples to which this statement applies will have a sticker affixed to them referring to this section of the appropriate SOP. In any case, the BG staff will use its best professional judgement to ensure that deviations do not violate the intent of OFR 00-212. If there is concern regarding whether or not the deviation will violate the intent of the OFR, BG staff will consult with the customer and the supervisor of the BG.

13. References

Moulton, S.R., II, Carter, J.L., Grotheer, S.A., Cuffney, T.F., and Short, T.M., 2000, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory — processing, taxonomy, and quality control of benthic macroinvertebrate samples. U.S. Geological Survey Open-File Report 00-212.

14. Key Words

benthic macroinvertebrate, chironomidae, mounting medium, slide preparation,

15. Attachments

None