U.S. GEOLOGICAL SURVEY

Preparing Benthic Macroinvertebrate Microscope Slides

1. Scope, Application and Summary

1.1. 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.

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

1.3. 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.

2. Reasons for Revision and Summary of Changes:

2.1. This SOP is being updated to the new format.

2.2. Detail is being added to sections as needed to better define the procedure.

3. Health and Safety Warnings

3.1. Personal Safety

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.

3.1.4. Follow other safety procedures described in the U. S. Geological Survey (USGS) Occupational Hazards and Safety Procedures Handbook (September 1999).

3.2. Chemical Safety

3.2.1. 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.

3.2.2. Know the location of and be familiar with the Material Safety Data Sheets (MSDS) for each chemical used by the BG.

3.2.3. 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).

3.3. Follow other standard safety guidelines as described in National Research Council (1995).

3.4. All NWQL personnel will follow the guidance outlined in the NWQL Pollution Prevention and Waste Minimization Policy (see NWQL Policy Memorandum 04.01 at http://wwwnwql.cr.usgs.gov/USGS/policy/policy.04-01.html). Refer to NWQL SOP No. TX0035.x, Waste Disposal at the National Water Quality Laboratory, for further information.

3.5. Waste Disposal Requirements

3.5.1. Empty bottles of mounting medium and used Pasteur pipettes can be disposed of in the container designated for broken glass.

4. Procedure and Responsibilities

4.1. Obtain the following supplies, chemical, and equipment before preparing slides.

4.1.1. Supplies

4.1.1.1. #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

4.1.1.2. #2 18 X 18 mm square cover glasses with a thickness range of 0.17 to 0.25 mm.

4.1.1.3. Colored labeling tape ("time tape")

4.1.1.4. Forceps (fine-tipped)

4.1.1.5. Microscope slides

4.1.1.6. Paper towels

4.1.1.7. Pasteur pipettes with suction bulbs

4.1.1.8. Probes (fine-tipped)

4.1.1.9. Slide Boxes

4.1.1.10. Slide labels according to project

4.1.1.11. Watch glasses or other similar glass dishes

4.1.2. Chemicals

4.1.2.1. CMC-10™ mounting medium

4.1.2.2. 70-percent ethanol

4.1.2.3. Equipment

4.1.2.4. Binocular microscope

4.1.2.5. Fiber-optic illuminator

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

4.2. Interferences

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

4.2.2. Factors contributing to poorly prepared slides include:

4.2.2.1. Improper orientation of the specimen on the slide

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

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

4.2.2.4. Improper cleaning of the specimen prior to mounting

4.2.2.5. Viscosity of the mounting medium

4.2.2.6. Air bubbles beneath the cover glass

4.3. Produce consecutively numbered slide labels for each sample.

4.3.1. Obtain the paperwork packet for each sample(s) and the specimen(s) to be mounted from their designated locations.

4.3.2. Determine the number of specimens to be mounted from each sample.

4.3.2.1. For qualitative samples processed using NWQL Lab Code 2176, produce nine (9) labels per sample.

4.3.2.2. 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 ÷ 8 = 28.25, round-up to 29; 6 ÷ 8 is 0.75, round-up to 1; 29 +1 = 30).

4.3.2.3. For oligochaetes simply divide the total number of specimens by 8 and round up.

4.3.3. 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. 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.

4.3.3.1. Open Exceed™, enter your user ID and password, and change directory to "/home/invert" by typing "cd /home/invert" at the prompt.

4.3.3.2. To run the label program, type "midge" at the prompt and follow the instructions.

4.3.3.3. 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.

4.3.4. Cut the labels into strips according to sample identification code; paperclip the labels to their corresponding Slide Preparations—Identification and Enumeration Worksheet.

4.3.5. 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.

4.4. Mounting Procedure

4.4.1. Guidelines

4.4.1.1. Use no more than two (2) cover glasses per slide.

4.4.1.2. Mount only one life stage under each cover glass.

4.4.1.3. Do not mount more than four (4) specimens under a cover glass.

4.4.2. 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.

4.4.3. Transfer specimens into a dish of 70-percent ethanol.

4.4.3.1. Inspect the vial, cap, and label for attached/residual specimens.

4.4.3.2. If necessary, rinse the vial, cap and/or label into the dish with the other specimens.

4.4.4. Sort specimens by size and/or morphology into similar groups using forceps and a dissecting microscope.

4.4.5. Mount the Specimens

4.4.5.1. 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

4.4.5.2. Blot four (4) or fewer specimens on a paper towel to reduce the amount of excess ethanol.

4.4.5.3. Orient the specimens in the mounting medium according to procedures specific to certain taxonomic groups (see Sections 4.6 and 4.7). This will generally result in optimal viewing of diagnostic structures.

4.4.5.4. 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.

4.4.5.5. 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.

4.4.5.6. Apply slight downward and/or directional pressure to the cover glass, as necessary, until the orientation of the specimens is appropriate (see Sections 4.6 and 4.7) and all air bubbles have been expelled from under the cover glass.

4.4.5.7. Add a small bead of mounting medium around the perimeter of the cover glass.

4.4.6. 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.

4.4.7. 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.

4.4.8. 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.

4.5. Clean-Up

4.5.1. Rinse and clean all tools with 70-percent ethanol.

4.5.2. Wipe up spilled mounting medium and clean workstation.

4.5.3. Put away supplies and equipment.

4.6. Procedures Specific to Chironomidae

4.6.1. Group Chironomidae according to life stage (larva or pupa), size, and/or Subfamily/Tribe.

4.6.2. Mount larvae first, followed by pupae.

4.6.3. Position a larva vertically on the slide with its head (ventral side up) to the top.

4.6.4. When mounting the majority of specimens, the whole specimen should be mounted.

4.6.4.1. 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.

4.6.4.2. 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.

4.6.5. 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.

4.7. Procedures Specific to Oligochaeta

4.7.1. Group worms according to Family and size.

4.7.2. Orient worms oriented horizontally on their side with the head region to the left.

5. Quality Control and Quality Assurance

5.1. Mount the specimens from only one sample at a time.

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

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

5.4. Make sure slides and cover glasses are not broken.

5.5. Check the viscosity of the mounting medium.

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

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

6. Data and Records Management

6.1. Record the following information in a laboratory record book for each sample mounted. Information that is redundant need not be recorded. For example, the sample may be may be completely mounted on the same date that it is started.

6.1.1. Sample identification code.

6.1.2. Start date (mm/dd/yyyy) and time (hh:mm).

6.1.3. End date (mm/dd/yyyy) and time (hh:mm).

6.1.4. Problems or errors associated with mounting the sample (for example, the number of specimens not found in a vial).

6.2. Slide Preparations – Identification and Enumeration Worksheet:

6.2.1. Record the life stage(s) of specimens mounted in the 'LS' column of the left-hand section of the worksheet for each slide.

6.2.2. 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.

6.2.3. The individual that mounted the specimens should record their first initial, last name, 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.

6.2.4. 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.

6.3. 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.

6.4. Label the slide box with the appropriate project name and block code (see Section 6.2.2). 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.

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

7. Definitions—none

8. References

8.1. 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.

8.2. National Research Council, 1995, Prudent practices in the laboratory—Handling and disposal of chemicals: Washington, D.C., National Academy Press, 427 p.

8.3. U.S. Geological Survey, 1999, Occupational Hazards and Safety Procedures Handbook: Manual No. 445-2-h, available at http://www.usgs.gov/usgs-manual/handbook/hb/445-2-h.html

9. Key Words

benthic macroinvertebrate, slide preparation, mounting medium, chironomidae

10. Attachments

None