

**U.S. GEOLOGICAL SURVEY
NATIONAL WATER QUALITY LABORATORY
SOP — Laboratory Analytical Method or Procedure**

SOP # BS0334.0	EFFECTIVE DATE: April 7, 2000	PREPARING BENTHIC MACROINVERTEBRATE MISCROSCOPE SLIDES
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WRITTEN BY: S.R. Moulton, II and T.L. Morman	APPROVED BY: Merle W. Shockey	

1. Scope, Application and Summary

- 1.1. Certain groups of benthic macroinvertebrates (BMIs) (for example, Chironomidae larvae and Oligochaeta) are mounted on microscope slides prior to taxonomic identification.
- 1.2. These procedures are used by anyone responsible for preparing BMI microscope slides.
- 1.3. Organisms are oriented in mounting media (for example, CMC-10™) on a microscope slide that is labeled with a unique sample identifier and slide number, covered with a coverslip, and dried for approximately 24 hours at 55°C.

2. Reasons for Revision and Summary of Changes: This is a new SOP.

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. Wear an apron, rubber gloves, and protective eyewear during sample preparation.
- 3.1.3. Know the location of the nearest eyewash and shower stations.
- 3.1.4. Do not eat or drink in the laboratory.
- 3.1.5. Follow other safety procedures described in the 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 BG supervisor if the ventilation systems are not working properly.
- 3.2.2. Use the preservative waste pump system to transfer preservative waste from the fume hood to the storage barrel. Contact the BG Supervisor if the system is not functioning properly. Contact the BG Safety Committee representative when the storage barrel is full and needs to be replaced.
- 3.2.3. Know the location of and be familiar with the Material Safety Data Sheets (MSDS) for each chemical used in the laboratory.
- 3.2.4. Know how to report and handle chemical and sample spills using procedures described in the NWQL Chemical Hygiene Plan (available from the Safety Program).

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

4. Procedure and Responsibilities

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

4.1.1. Supplies

- 4.1.1.1. Forceps (fine-tipped)

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- 4.1.1.2. Probes (fine-tipped)
- 4.1.1.3. Microscope slides
- 4.1.1.4. 18 X 18 mm (2 mm thickness) square cover slips (preferred)
- 4.1.1.5. 18 X 18 mm (1.5 mm thickness) square cover slips (acceptable)
- 4.1.1.6. 22 X 22 mm (1.5 mm thickness) square cover slips (acceptable)
- 4.1.1.7. Watch glasses
- 4.1.1.8. Pasteur pipettes with suction bulbs
- 4.1.1.9. Paper towels
- 4.1.1.10. Slide labels according to project
- 4.1.2. Chemicals
 - 4.1.2.1. CMC-10™ mounting medium
 - 4.1.2.2. 70-percent ethanol
- 4.1.3. Equipment
 - 4.1.3.1. Dissecting microscope
 - 4.1.3.2. Fiber-optic illuminator
 - 4.1.3.3. Slide warmers (set to 55°C)
- 4.2. Interferences
 - 4.2.1. Poor slide mounts often prevent a taxonomist from making identifications to the genus or species level.
 - 4.2.2. Factors contributing to poorly prepared slides include:
 - 4.2.2.1. improper orientation of the organism on the slide
 - 4.2.2.2. mounting too many organisms for one cover slip
 - 4.2.2.3. mounting an organism too large for one coverslip
 - 4.2.2.4. improper clearing action
 - 4.2.2.5. high-viscosity of the mounting medium
 - 4.2.2.6. air bubbles forming beneath cover slips
- 4.3. Obtain Paperwork and Vials
 - 4.3.1. Every Monday morning, obtain a paperwork packet for each sample placed in the “Big Bugs Complete” bin located in the BG laboratory. Each packet should include a Slide Preparations—Identification and Enumeration Worksheet.
 - 4.3.2. Obtain vials of organisms associated with each paperwork packet.

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- 4.3.3. Obtain the number of organisms (for example, Chironomidae larvae) to be mounted for each sample from the bench data sheet.
- 4.4. Produce Slide Labels
- 4.4.1. Consecutively numbered labels are prepared for each sample.
- 4.4.2. Labels are printed on white adhesive back paper using a laser jet printer.
- 4.4.3. Open Exceed, and change directory to "/home/invert".
- 4.4.4. Type "midge" at the prompt and follow instructions.
- 4.4.5. Qualitatively processed samples: produce nine labels per sample.
- 4.4.6. Quantitatively processed samples: Determine the number of labels needed by dividing the total number of organisms for each group to be prepared by 8, round up to the next highest integer, and add 2.
- 4.4.7. Cut labels and group according to sample identification code; place labels with slide preparation worksheet.
- 4.5. General Procedure
- 4.5.1. Prepare one sample at a time.
- 4.5.2. Verify that the sample identification codes on the organism vial(s), slide labels, and worksheet are identical.
- 4.5.3. Cut labels to size and peel the back off. Apply the necessary number of labels to slides. Labels are applied to the left hand side of each slide.
- 4.5.4. Pour organisms into a watch glass.
- 4.5.4.1. Inspect vial and label for organisms.
- 4.5.4.2. Rinse vial and cap with 70 percent ethanol.
- 4.5.5. Sort organisms into morphologically similar groups using forceps and a dissecting microscope.
- 4.5.6. Place one drop of CMC-10 mounting media on a slide using a Pasteur pipette; spread mounting medium to approximate the area of a cover slip (maximum two cover slips per slide).
- 4.5.7. Blot organisms on a paper towel to remove excess ethanol.
- 4.5.8. Orient the organisms in the mounting medium according to procedures specific to certain taxonomic groups (see below). This will generally result in optimal viewing of diagnostic structures.
- 4.5.9. Do not mount more than four organisms under each cover slip.
- 4.5.10. Add mounting medium to completely fill where necessary to compensate for the size and number of organisms being mounted.

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- 4.5.11. Place a cover slip over the organisms by laying one side against the slide and carefully lowering it over the organisms.
 - 4.5.12. Apply slight downward and lateral directional pressure to the cover slip, as necessary to orient organisms or to remove air bubbles in the mounting medium.
 - 4.5.13. Ring each cover slip by applying a small bead of mounting medium to the outer edges of each cover.
 - 4.5.14. Place slides on a drying rack and dry for approximately 24 hours at 55°C.
 - 4.5.15. Complete information on the Slide Preparations—Identification and Enumeration Worksheets.
 - 4.5.15.1. Record the number of organisms mounted per cover slip.
 - 4.5.15.2. Record the life stage of organisms mounted per cover slip.
 - 4.5.15.3. Record the subsampling correction factor per cover slip.
 - 4.5.15.4. Record the date prepared and total preparation time.
 - 4.5.16. Record the date prepared and total preparation time on the BMI Identification and Enumeration Bench Data Sheet.
 - 4.5.17. Check slides periodically for empty spaces where the mounting medium has evaporated; if necessary, add mounting medium to the edge of the cover slip. Capillary action will draw the mounting medium beneath the cover slip.
 - 4.5.18. Store dried slides on their sides in boxes grouped by project according to the sample identification code (Note: slide numbers for each sample must be in consecutive order).
- 4.6. Procedures Specific for Chironomidae Larvae
- 4.6.1. Group Chironomidae according to life stage (larva or pupa), size, and Subfamily/Tribe.
 - 4.6.2. Prepare 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. Mount the head, thorax, and last three abdominal segments of a large larva (for example, Diamesinae) under a single coverslip. Remaining abdominal segments are discarded.
 - 4.6.5. 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.
 - 4.6.6. Apply firm pressure to each cover slip several times over a 5–10 minute period.
 - 4.6.7. Apply downward/lateral directional pressure to cover slips to rotate larvae so that the ventral sides of heads are visible. Larval heads do not always rotate ventral side up.
 - 4.6.8. Position pupae dorsal side up on the slide. Mount attached larval head capsules next to the pupa.

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4.7. Procedures Specific for Oligochaeta

- 4.7.1. Group worms according to Family and size.
- 4.7.2. Orient worms horizontally on the slide with the head region to the left.
- 4.7.3. If necessary, apply slight directional pressure to roll worms on their sides.

4.8. Clean-Up

- 4.8.1. Rinse and clean all tools with 70-percent ethanol.
- 4.8.2. Wipe up spilled mounting medium and clean workstation.
- 4.8.3. Put away supplies and equipment. Restock and organize supplies if necessary.
- 4.8.4. Notify the Production Coordinator if supplies need to be purchased.

5. Quality Control and Quality Assurance

- 5.1. Inspect all dishes before preparing slides; make sure there are no organisms or sample debris remaining from previously processed slides.
- 5.2. Make sure slide and cover slips are not broken.
- 5.3. Check the viscosity of the mounting medium.
- 5.4. Inspect prepared slides to evaluate the orientation of the mounted organisms.

6. Data and Records Management

- 6.1. Place completed paperwork in the "Midges Mounted" bin located in the BG laboratory.
- 6.2. Give box of prepared slides to the taxonomist responsible for performing the identifications of the sample.

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 (IN PRESS).
- 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

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Attachments

1. Slide Preparations — Identification and Enumeration Worksheet
2. BMI Identification and Enumeration Bench Data Sheet