BUREAU OF RECLAMATION

Standard Operating Procedure: Preparation and Analysis of Water Samples for Dreissenid Mussel Veliger Detection: Microscopy

Laboratory Standard Operating Procedure (SOP)
Version 7 (Date Revised: 2024)
Document No. EcoLab-F436A-2024-07

Bureau of Reclamation
Ecological Research Laboratory

The Ecological Research Laboratory

Mission Statements

The Department of the Interior (DOI) conserves and manages the Nation's natural resources and cultural heritage for the benefit and enjoyment of the American people, provides scientific and other information about natural resources and natural hazards to address societal challenges and create opportunities for the American people, and honors the Nation's trust responsibilities or special commitments to American Indians, Alaska Natives, and affiliated island communities to help them prosper.

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

Peer Review

Bureau of Reclamation
Technical Service Center

EcoLab-F436A-2024-07

Standard Operating Procedure: Preparation and Analysis of Water Samples for Dreissenid Mussel Veliger Detection: Microscopy

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Prepared by:

Bureau of Reclamation
Technical Service Center
Hydraulic Investigations and Laboratory Services
Ecological Research Laboratory (86-68560)

Previous Versions:

Lab SOP Version 1	6/2007
Lab SOP Version 2	6/2010
Lab SOP Version 3	2/2011
Lab SOP Version 4	7/2013
Lab SOP Version 5	5/2019
Lab SOP Version 6	4/2022

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1. ACRONYMS / DEFINITIONS

CoC: Chain of Custody
DI water: Deionized water
Dreissenid: Genus of freshwater mussel
SDS: Safety Data Sheet
PCR: Polymerase Chain Reaction; technique used to amplify DNA
Quagga mussel: *Dreissena rostriformis bugensis,* invasive mussel species
Eco Lab: Ecological Research Laboratory (Reclamation Technical Service Center)
Field Blank: DI water used to rinse the net and cod-end in the field
Reclamation: Bureau of Reclamation
Settling cone: Modified Imhoff cone used to measure the volume of solids in water
SOP: Standard Operating Procedure
Veliger: Larval zebra/quagga mussel
Venoset: Plastic tubing with roller wheel system to control flow out of settling cone
Zebra mussel: *Dreissena polymorpha,* invasive mussel species

2. SCOPE AND APPLICABILITY

This Standard Operating Procedure (SOP) is used to establish a uniform format for duties performed by laboratory staff. This method is applicable to dreissenid mussel early detection water samples that are prepared and analyzed by laboratory staff at the Bureau of Reclamation, Technical Service Center, Denver, CO. The goal of this SOP is to standardize how each task in the laboratory is performed by every lab technician. This SOP is not a replacement for training.

3. INTERFERENCES

The main interference in early mussel detection is cross-contamination from other samples. To mitigate this interference, glassware is decontaminated overnight in 5% acetic acid. A new disposable pipette and decontaminated petri dish is used for every sample. Separate pipette bulbs are used for positive and negative samples. Settling cones are decontaminated overnight with 5% acetic acid, scrubbed with a dedicated cone brush, and thoroughly rinsed with DI water.

4. HAZARDS

A variety of chemicals are used in the preparation, analysis, and disposal of water samples collected for the early detection of dreissenid mussels. While most of these chemicals may cause minimal injury or irritation, lab personnel should refer to the Laboratory Job Hazard Analysis (JHA) (Appendix A) and Safety Data Sheets (SDS) for detailed information regarding hazards, handling, storage, and disposal of specific chemicals. Prior to beginning work, all lab personnel will be familiar with the different chemicals and will be prepared to deal with spills and exposures appropriately. Current SDS are in the microscopy lab documentation binder, located in the lab next to the computer.

5. MATERIALS

Glassware

Plastic test tube with screw on lid (Falcon, 352097, Falcon 15 mL Conical Centrifuge Tubes Polypropylene; 15 mL; In Rack:Tubes, | Fisher Scientific)

Petri dishes (Pyrex, 3160-60, Corning PYREX Reusable Petri Dishes: Complete 60 x 15 mm:Dishes, Quantity: | Fisher Scientific)

2 mL Transfer pipettes (Fisherbrand, 13-678-20B, Fisherbrand Disposable Borosilicate Glass Pasteur Pipets Approx. length: | Fisher Scientific)

Watch glasses, 12-cm diameter (Fisherbrand, 02-612E, Corning PYREX Watch Glass/Beaker Cover with Fire-Polished Edges 125 mm:Dishes, | Fisher Scientific)

Microscopes

Compound: Spencer AO, 40X – 100X magnification

Inverted: Zeiss Axio Observer A1, 100X – 400X magnification

Research: Olympus SZH10, 10.5X – 210X magnification

Micrometrics 318CU digital camera mounted on research scope

Screening: Meiji EMZ-5D, 7X-45X magnification

Screening: Meiji EMZ-8TR, 7X-45X magnification

Miscellaneous Items

1.5-mL Eppendorf tube (USA Scientific, 1615-5510, Seal-Rite 1.5mL Microcentrifuge Tubes - USA Scientific, Inc

Cone brush for washing settling cones (Fisher, 03-562, Fisherbrand Imhoff Settling Cone Brush Overall Length: 26 in.:Facility | Fisher Scientific)

Cooler login binder

Designated wash bottles for DI water (Fisher, 03-409-22C, Fisherbrand Translucent White Wash Bottles | Fisher Scientific)

Fisherbrand labeling tape, rainbow pack (Fisher, 15-901-R, Fisherbrand Colored Labeling Tape, Rainbow Pack:Facility Safety and Maintenance:Labels | Fisher Scientific)

Kimwipes (Fisher,06-666A, Kimberly-Clark Professional Kimtech Science Kimwipes Delicate Task Wipers, | Fisher Scientific)

Labels (Label Value: LV-30334R, LV-30334POLY) Dymo LV-30334 Removable Labels 2-1/4 x 1-1/4”

Dymo LV-30334 Durable Polypropylene Labels 2-1/4 x 1-1/4"

Lens paper (Fisherbrand, 11-996, Fisherbrand Lens Paper, Quantity: Pack of 12 | Fisher Scientific)

Micro pipette (Fisherbrand Finnpipette, 20-200 uL, Fisherbrand Elite Adjustable-Volume Pipettes:Pipette Products:Pipettes | Fisher Scientific)

Micro pipette tips (Fisherbrand SureOne, 02707422, Fisherbrand SureOne Beveled Pipette Tips, Universal Fit Yellow; Sterile; | Fisher Scientific)

Modified Imhoff settling cones (Fisher, 15-438, Thermo Scientific Nalgene Polycarbonate Imhoff Settling Cone 1000mL:Water | Fisher Scientific) (Appendix B)

pH strips (MColorpHast, 1.09533.0001) MilliporeSigma pH-Indicator Strips range 5-10

Rubber bulbs (Fisher, 03-448-26, Fisherbrand Dropper Bulb Capacity: 3mL:Pipet Products, Quantity: Pack of | Fisher Scientific)

Safety gloves, nitrile (small, medium, and large) (USA Scientific, 4914-4400,Cobalt® Nitrile Exam Gloves - Box/100 - Clearance - USA Scientific, Inc)

Specimen counters (Counting Devices, Inc)

Technician notebooks

Venoset (C.I.A Medical, C.I.A. Medical, CIA7024977, Wholesale Medical Supplies & Surgical Equipment - CIA Medical, Contact Company by email to place order))

1-gallon plastic jugs for 5% acetic acid solutions

6. SAMPLE RECEIVING / RETURN

All coolers will be opened as soon as possible after receipt and the following tasks completed. Care should be taken that samples are not intermixed when opening multiple coolers at the same time. Chain of Custody (CoC) or data sheet forms should remain with samples until login is complete.

- Open cooler and remove all tape, stickers, and labels related to shipping. **Do not discard shipping label until cooler is prepared for return. Do not discard the Limited Quantity label.**
- Remove all contents of cooler, including packing materials, to ensure nothing is overlooked.
- Enter shipping information into the Cooler Login binder. ALL samples, including those collected by Eco Lab staff, will be recorded in the Login binder.
- On a self-stick note, write date received, where the samples are from, and number of samples received. Attach this **with tape** to one of the sample bottles.
- Store samples and applicable documentation on designated shelf in refrigerator, with self-stick note in front until they're logged in.
- Drain cooler, then replace all packing materials, including ice packs, bubble wrap, newspaper, Ziplock bags, unused diapers, etc. **Include the Limited Quantity label if you were able to remove it intact**. Empty cardboard boxes are not returned – only coolers and cooler boxes or boxes with other materials in them.
- Prepare FedEx request with return shipping information on the CoC. Coolers will be returned to the person listed on the CoC. If no CoC is provided, return to the address on the original shipping label. Return the cooler via ground 4-5-day shipping. Tape cooler securely closed and attach FedEx request to the lid.
- Enter return information into Cooler Login binder.
- Coolers can be set aside for the mailroom to collect. If not collected within 2 days, take coolers to the mailroom in building 67 or email mailroom to arrange for pickup.

Coolers should be returned as soon as possible after opening, to reduce confusion related to return shipping and to avoid congestion in the lab. If there is an issue with the cooler, inform the laboratory manager so that the owner of the cooler can be contacted, and the issue can be addressed.

7. SAMPLE LOGIN

All samples received in the lab will be logged into the Mussels Database and assigned a unique identifying number prior to being set up. This number will be printed on a label that will remain with the sample throughout the analysis process. The sample number will also be written on the top of the bottle.

Samples will be entered into the database in the order received when possible. At least two sets of labels will be printed for each batch: one set on permanent labels which will be affixed to the original sample bottle; one set on removable labels which will be affixed to the corresponding test tube during set up. Samples from priority water bodies will need an additional removable label set printed and will be identified with red tape on the lid.

8. SAMPLE PREPARATION PROCEDURE

This sample preparation method was developed from Hosler (2011), which was adapted from the US Army Corps of Engineers (ZMIS 2002), the Standard Method (Standard Methods 2001), and the US Environmental Protection Agency (USEPA 2003). Appendix B contains instructions for constructing a modified Imhoff cone with a passive venoset system.

Sample Setup

- Arrange Imhoff cones on sturdy countertop with enough room to ensure samples do not fall off or get knocked over (Figure 1A). Samples from waterbodies with known mussel populations will be set up in a separate area using dedicated cones.
- Turn cones so that the graduation lines face front.
- Apply roller clamp and back up clamp to each cone (Figure 1B). Ensure roller clamps are completely locked to prevent leaks.
- Samples will be set up in numerical order unless priority processing has been requested. Samples with known veliger presence may be set up out of order as needed. Note that positive samples are not separated from negative samples during login, so pay attention to the bottles when pulling samples for setup.
- Place sample bottles under the cone in which they will be settled, ensuring that they are in the correct numerical order. ALWAYS double-check that samples are in the correct order!
- Place a watch glass on top of each cone to prevent cross-contamination by splashing.
- Shake sample bottle to homogenize the sample, remove watch glass, and carefully pour sample into designated cone.
- Rinse sample bottle with DI water, using care so the tip of the wash bottle does not come into contact with the sample bottle, and pour contents into cone. Place sample bottle under cone.
- Replace watch glass on top of cone.
- Settle samples overnight.
- Repeat these steps for all samples in the group.

Figure 1: A. Cones are set up with samples. B. Close-up image of the closed roller clamp and the secondary clamp in place on the tubing.

Sample Takedown

- Prior to taking samples down, use a tablet to log into the Mussels Database and input "Quick Add: Sample Prep" data for each sample.
- 15 mL of each sample will be collected into a plastic test tube with a screw top.
- Carefully drain the bottom (heaviest) 15 mL of each sample into a test tube and screw the top on securely.
    - If venoset is plugged with debris, manipulate the roller back and forth to try to dislodge sediment/debris. If needed, use a metal rod (available in lab) to push sediment/debris through the venoset. Do not lift cones and allow to drop back onto rack – this will not dislodge debris, and there are numerous risks for injury, sample loss, and sample contamination.
    - Decontaminate metal rod in 5% acetic acid after each use and rinse with tap water. Dry with a paper towel. **Do not use metal rods in more than one sample without decontaminating in between.**
- Locate the label that corresponds to the sample just collected and affix to the test tube. Do not apply the label prior to collecting the sample – the amount of ethanol in each sample will erase the information on the label if it spills, drips, or leaks. **ALWAYS** compare the test tube label to the label on the sample bottle.
- Drain remaining volume of settling cone into the original sample bottle and rinse cone with DI water. Any excess that does not fit in the original sample bottle can be collected in a separate container and discarded down the sink.
- Place sample bottle into refrigerator in numerical order. Test tubes will be stored in numerical order in a Styrofoam holder.
- **ALWAYS CONFIRM THAT SAMPLES ARE BEING STORED IN THE CORRECT ORDER!!**

Cone Washing and Decontamination

- When all cones are empty, clean and decontaminate following the instructions in Appendix C.
- Once the cone cleaning procedure is complete, return cones and stands to the appropriate setup area and allow to air dry.

9. SAMPLE ANALYSIS

- Remove original sample bottle and associated test tube containing the settled 15 mL sample from refrigerator. Ensure the sample ID on bottle's label matches sample ID on the test tube. Do not use the handwritten number on the lid to confirm matching ID.
- Do not analyze positive and negative samples in the same 24-hour period.
- Remove label from test tube and place in technician notebook.
- Use a new 2 mL glass pipette and a clean glass Petri dish for every sample.
- Use the appropriate rubber bulb for the sample being analyzed. Bulbs marked with an "X" on the top should only be used for known positive samples. Bulbs with no "X" should only be used for non-positive samples.
- If needed, draw a grid on the bottom of the Petri dish with a Sharpie.
- Invert test tube a few times to mix contents.
- Pipette 2 mL of sample from the test tube into the Petri dish, being careful that sample material is not drawn up into the bulb. Dilute with DI water if necessary.

- Analyze contents of the Petri dish thoroughly, using a cross-polarized filter on a stereo/ dissecting microscope. Reference the identification book (prepared by the Eco Lab) to differentiate organisms.
- When analysis of the Petri dish is complete pour contents into the original sample bottle and rinse with DI water. Repeat these steps until all 15 mL from test tube has been analyzed. Then, rinse the pipette inside and out into the Petri dish and analyze the contents.
- When analysis of the full 15 mL is complete, record findings in technician notebook.
- Place pipette into glass recycling container and other glassware into the dishpan for decontamination.
- Technician performing analysis should write their initials on the lid of the sample bottle and place in the appropriate place in the storage refrigerator in no particular order.
  - Priority samples – those marked with red tape – will be placed on PCR shelf.
  - Non-priority samples will be placed on non-priority shelf.
- All efforts should be made to complete analysis of a sample in one sitting. In the event this is not possible, technician should use a Sharpie to write the sample number, date started, and their initials on a beaker. Reseal test tube with screw top lid and place in beaker. Place beaker and test tube with sample bottle on shelf in refrigerator. The same technician should complete analysis on the next business day.
- Soak glassware overnight in 5% acetic acid, then use scrub pad and bottle brush to thoroughly clean, ensuring all sharpie markings are removed. Rinse thoroughly with DI water and allow to air dry.
- At the end of the day, clean work surfaces and microscope with disinfecting wipes. Clean lenses of microscope with Kimwipes. Right after the analysis of a positive sample, clean work surfaces and microscope with disinfecting wipes, and wash hands.
- Once a week, black eyepieces should be removed from the microscope, washed thoroughly with soap and warm water, and allowed to air dry.

10. SUSPECT ORGANISMS AND POSITIVE WATERBODIES

- If a suspect organism is found, request assistance from other lab personnel in confirming identification.
- Remove suspect organism(s) from primary sample with a micro pipette, using a new micro pipette tip for every sample. Place each suspect into a clean Petri dish, preserve with alcohol, and examine under higher magnification microscope, taking digital photographs for further analysis.
- Inform the lab manager of the finding so the reporting process can begin.
- Once pictures of suspect organism(s) have been taken, pipette the organism into an Eppendorf tube with minimal liquid. Label the tube with the sample number. Place the suspect in the rack on the PCR shelf. Inform the molecular biologist so the sample can be analyzed as soon as possible.
- If counting veligers in a known positive sample, use a counter to keep an accurate tally. Subsampling may be used for samples with more than 2000 veligers (Appendix D). Record final veliger count in technician notebook.

11. SAMPLE SPILLS

- When sample spills occur, salvage whatever remains in the test tube to complete analysis. To prevent cross-contamination DO NOT return spilled sample material to tube.

- Clean work surface with paper towels, then disinfecting wipes.
- If sample was from a known positive waterbody, first decontaminate work surface by soaking for at least 10 minutes with 5% acetic acid. Then wipe away the acid and rinse the area with a wet paper towel.
- Note spill in technician notebook next to appropriate label.
- Analyze remaining sample.

12. TECHNICIAN NOTEBOOK

- Remove label from test tube of sample being analyzed and place in notebook.
- Complete analysis of entire 15 mL of each test tube.
- Make note of organisms other than dreissenid mussels that are present (ostracods, snails, corbicula, seeds, etc.), estimating the number of each in the full 15 mL sample.
  - Less than 25 = Low
  - 25-50 = Medium
  - More than 50 = High
- Record the counted number of suspect/un-confirmed dreissenid mussels – do not estimate. Suspect organisms are NOT entered in the database.
  - For known positive samples record the number of counted dreissenid veligers, or the subsample total - do not estimate.
- When analysis is complete, technician should initial and date next to the appropriate label in the notebook.
- At the end of the workday, technician should log results into the database and place a check mark on the label in the technician notebook to indicate this has been done.

13. DISPOSAL OF SAMPLES

- Non-priority samples may be purged 30 days after analysis. Priority samples (those marked with red tape) and field blanks will be purged at the discretion of the molecular biologist.
- Sample purging procedure:
  - Enter the unique identifying number of samples to be purged into the Mussels Database using the “Quick Add: Purge” tab
  - Pour sample into the sink
  - Recycle empty sample bottles
    - It is not necessary to remove labels, tape, sharpie markings, etc.
  - Make a note in the database if field blanks were not used for analysis

14. CONTACT INFORMATION

Eco Lab	303-445-2498	bor-sha-ecolab@usbr.gov
Sherri Pucherelli	303-445-2015	spucherelli@usbr.gov
Diane Mench	303-445-2050	dmench@usbr.gov
Jacque Keele	303-445-2187	jkeele@usbr.gov
Yale Passamaneck	303-445-2480	ypassamaneck@usbr.gov
Annie Quattlebaum	303-445-2798	rquattlebaum@usbr.gov

Appendix A – Laboratory Job Hazard Analysis (JHA)

ECOLOGICAL RESEARCH LABORATORY
JOB HAZARD ANALYSIS (JHA)

1. **Project Title:** Invasive Dreissenid Mussel Early Detection and Monitoring Program

2. **Purpose:** To prepare and conduct analysis of water samples for the presence of dreissenid mussels within the 17 western states.

3. **Start Date:** May 2024

4. **End Date:** May 2025

5. **Personnel Requirements:** Work will be performed by Bureau of Reclamation (Reclamation) employees and seasonal interns assigned to the lab.

6. **Hazards**

   - **Minor Injuries:** Cuts, scrapes, bruises. A first-aid kit is maintained in the main hallway for small cuts, scrapes, and other minor injuries that might occur.

   - **Serious Injuries:** Serious injuries are those that cannot be treated in the lab with a first-aid kit. Contact local authorities and/or medical personnel immediately for serious injuries. An AED is available in the main hallway.

   - **Heavy Lifting:** Items such as field equipment, outreach items, racks of settling cones, and client coolers can be quite heavy. Use caution when lifting heavy items. Two people should lift items weighing more than 50lbs.

   - **Ergonomics:** Prolonged periods of sitting. Prolonged periods of work on microscopes. Personnel should plan to take several short breaks throughout the day to rest eyes and stretch.

   - **Chemicals:** Glacial acetic acid, which is used to make dilutions, is highly corrosive and flammable and every precaution should be taken when handling this chemical. Most other chemicals used in the lab are relatively harmless and pose little risk of illness or injury. However, all personnel should review the SDS for the specific chemicals used in the lab and understand the risks posed by those chemicals. All chemicals, regardless of associated risk, should be handled, used, stored, and disposed of in accordance with the manufacturer recommendations.

     - **100% Acetic Acid, Glacial**
       Handling: Never add water to this product; when diluting, always add acid to water. Always prepare in a fume hood. Keep away from heat. Keep away from sources of ignition. Do not ingest. Do not breathe gas/fumes/vapor/spray. In case of insufficient ventilation, immediately contact a physician or Poison Control Center and bring the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, reducing agents, metals, acids, and alkalis.
       Storage: Store separate from other chemicals and in an approved area, under a fume hood or in a fire or corrosives cabinet. Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame). Room temperatures below 63° F could cause acid to freeze. If a glass bottle of acetic acid freezes, wearing protective gloves, gently place bottle inside a

plastic bucket and place in fume hood or fire cabinet to allow to thaw. Freezing and thawing does not affect product quality.
Personal Protective Equipment: When handling, wear splash goggles, synthetic apron, and rubber or latex gloves. Ensure that eyewash stations and safety showers are proximal to the workstation.
Spills/Leaks: Absorb small spills (less than 500 mL) with inert material (e.g., vermiculite, sand, or earth), then place material in a 5-gallon bucket and contact Lise Pedersen or Robert (Bob) Allen for HazMat disposal. Wash area with water. Remove all sources of ignition. Provide ventilation. Use water spray to cool and disperse vapors, protect personnel, and dilute spills to form nonflammable mixtures. Control runoff and isolate discharged material for proper disposal.

Lise Pederson, Civil Engineer
lpederson@usbr.gov, 303-445-3095

Robert (Bob) Allen, Civil Engineer
rallen@usbr.gov, 720-879-6133

Due to its high flammability, fumes, and explosive potential, if a bottle of glacial acetic acid breaks and/or spills, regardless of the volume, do not attempt to clean. Evacuate the building – immediately leave the lab through the nearest exit and pull the fire alarm on the way out. Locate Janet White or Connie Svoboda (or acting) and notify them of the type, location, and volume of the spill so they can direct emergency services.

Janet White (Chief, Engineering and Laboratory Services Division), 303-594-2749

Connie Svoboda (Group Manager, Hydraulic Investigations and Laboratory Services), 303-524-0285

- **Acetic Acid, 5% (Distilled White Vinegar)**
  Handling: Use with adequate ventilation. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale. May cause eye and skin irritation. May be harmful if absorbed through the skin. Harmful if swallowed, causes gastrointestinal tract irritation. May cause respiratory tract irritation if inhaled.
  Storage: Store in a cool location. Provide ventilation for containers. Avoid storage near extreme heat, ignition sources or open flame. Store away from oxidizing agents. Store in cool, dry conditions in well-sealed containers. Keep containers tightly sealed. Protect from freezing.
  Personal Protective Equipment: Splash goggles, synthetic apron, vapor and rubber or latex gloves. Ensure that eyewash stations and safety showers are proximal to the workstation.
  Spills: Collect liquids using vacuum or by use of absorbents. Place into properly labeled containers for disposal. Remove from all sources of ignition. Soak with inert material. Use spark-proof tools and explosion-proof equipment.

- **Disinfecting Wipes**
  Handling: Avoid contact with eyes, skin, and clothing. Do not eat or drink when using this product. Exposure to vapor or mist may irritate respiratory tract. Liquid may cause irritation to eyes and skin. Ingestion of liquid may cause slight irritation to mucous

membranes and gastrointestinal tract. Ensure adequate ventilation. Ensure that eyewash stations and safety showers are proximal to the workstation. Wash hands after use.
Storage: Keep containers tightly closed in a dry, cool, and well-ventilated place.
Personal Protective Equipment: No special protective equipment is required under normal use conditions.
Spills: Liquid may be absorbed with paper towels and discarded in the trash.

- **Tris-HCl (4M, pH 7.5)**
  Handling: Wash hands after use. Do not eat or drink in work areas.
  Storage: Keep away from food, drink, drains, surface, and ground water. Use in well-ventilated areas.
  Personal Protective Equipment: No special protective equipment is required under normal use conditions.
  Spills/Leaks: Wipe up with absorbent material (e.g., cloth, paper towel).

- **Alcohol (Isopropyl or Ethyl)**
  Handling: Avoid contact with eyes, skin, and clothing. Empty containers retain product residue (liquid and/or vapor) and can be dangerous. They should be left with the lid off to off-gas in the fume hood for at least 24 hours before being placed in mixed recycling container. Take precautionary measures against static discharges. Avoid breathing vapor or mist. Ensure adequate ventilation. Wash hands thoroughly after handling. Ensure that eyewash stations and safety showers are proximal to the workstation.
  Storage: Keep away from sources of ignition. Store in a tightly closed container. Keep from contact with oxidizing materials. Store in a cool, dry, well-ventilated area away from incompatible substances. Ethanol should not be stored in the same cabinet as glacial acetic acid.
  Personal Protective Equipment: Wear appropriate protective eyewear, gloves, and clothing to prevent exposure.
  Spills/Leaks: If spilled on clothing, remove clothing and wash before reuse. Absorb spill with inert material (e.g., vermiculite, sand, or earth), then place in suitable container. Use water spray to dilute spill to a non-flammable mixture. Clean up spills immediately, observing precautions in the Personal Protective Equipment section (Section 7). Remove all sources of ignition. Provide ventilation.

- **Glassware**
  Clean, unbroken glass can be placed in the mixed recycle container. In the case of broken glassware, use a dustpan and broom or a paper towel to sweep up the pieces. Discard in the container marked "Glass Disposal," located across from the main door to the lab. Do not handle broken glass by hand if it can be avoided. Glass pipettes are rinsed and disposed of in the "Glass Disposal" container after each sample. Contact Lise Pedersen (lpederson@usbr.gov) or Bob Allen (rallen@usbr.gov) to empty Glass Disposal container.

7. **Personal Protective Equipment (PPE):** Personnel are responsible for maintaining their own PPE to ensure adequate protection. Safety glasses, gloves, and lab coats are provided by the lab.
   - **Footwear:** All footwear will be closed toe and have nonskid soles. Due to the high volume of water used in the lab and the associated risk of slipping, high-heeled shoes are not permitted in the lab.

- **Clothing:** Flowing or loose long-sleeved shirts are not permitted in the lab due to the high volume of chemicals and glassware used, as well as the risk of sample cross-contamination. When working with acetic acid, both glacial and 5% dilutions, nitrile gloves and safety glasses will be worn. When washing dishes, a lab coat or apron will be worn to protect clothing and skin. A lab coat may be worn only when washing dishes. To minimize the risk of sample cross contamination, lab coats will not be permitted at any other time.

- **Rubber or Nitrile Gloves:** Disposable gloves should be worn while handling chemicals and cleaning glassware and cones.

8. Training Requirements

Training on safety, sample handling, and lab hygiene will be conducted by experienced personnel.

9. Security Requirements

Due to the sensitive nature of the work being conducted in the lab all personnel, including Reclamation employees and seasonal interns, will have a DOI Access Card prior to beginning work. All visitors/tours will be escorted by an Eco Lab employee.

10. Emergency Contact Numbers

Personnel should be aware of local emergency contact numbers such as law enforcement, fire/medical response, and Poison Control.

Emergency number: 911 or the GSA Denver MegaCenter: 877-437-7411

Non-emergency number: Connie Svoboda, 303-524-0285, Diane Mench, 303-445-2050

Emergency room services: St. Anthony's Hospital, 11600 W 2nd Pl, Lakewood, CO 80228
(720) 321-0000

11. Acknowledgement Signatures

All personnel assigned to the lab will read and acknowledge the above information prior to beginning work.

I have been briefed on the details of this JHA, and what my role and responsibilities will be during the project. My signature below indicates that I have read and understand the requirements.

Signature_____ Date_____

Appendix B - How to Make a Modified Imhoff Cone

1. Materials

- Imhoff Cone
- Large animal venoset
- 3/16” 1D flexible tubing
- Plumber’s tape
- Clamp

2. Tools

- Drill with 3/16” drill bit
- Pliers
- Flathead screwdriver
- Silicone gel lubricant
- Scissors
- Razor blade
- Ruler

3. Instructions

1) Pull out the small tube at the opening of the venoset using pliers (Figure 1).
2) Enlarge the opening of the venoset using a 3/16” drill bit and remove any plastic pieces still inside (Figure 1).
3) Apply silicone gel to the outside of the venoset opening, coating the entire bottom portion to the shoulder (Figure 1).
4) Attach the tubing by sliding over the outside of the venoset. Push the tubing as far as it will go; with some pressure it should slide all the way up (Figure 2).
5) Cut off the hard-plastic parts of the venoset using razor blade (Figure 1).
6) Trim the tubing so it is long but not touching the stand when attached to the cone (Figure 1).
7) Wrap the collar of the cone with a liberal amount of plumber’s tape and check for leaks using water (Figure 2).
8) Slide a clamp onto venoset and over the collar of the cone. The collar of the cone should sit on the line on the venoset with the clamp sitting just above the collar (Figures 1 and 3).
9) Tighten clamp firmly using screwdriver. It should not wiggle or slide in any direction.
10) Test for leakage using water. Apply more tape and tighten clamp if there are any leaks and test again.
11) Place the fully assembled cone in the stand (Figure 4).

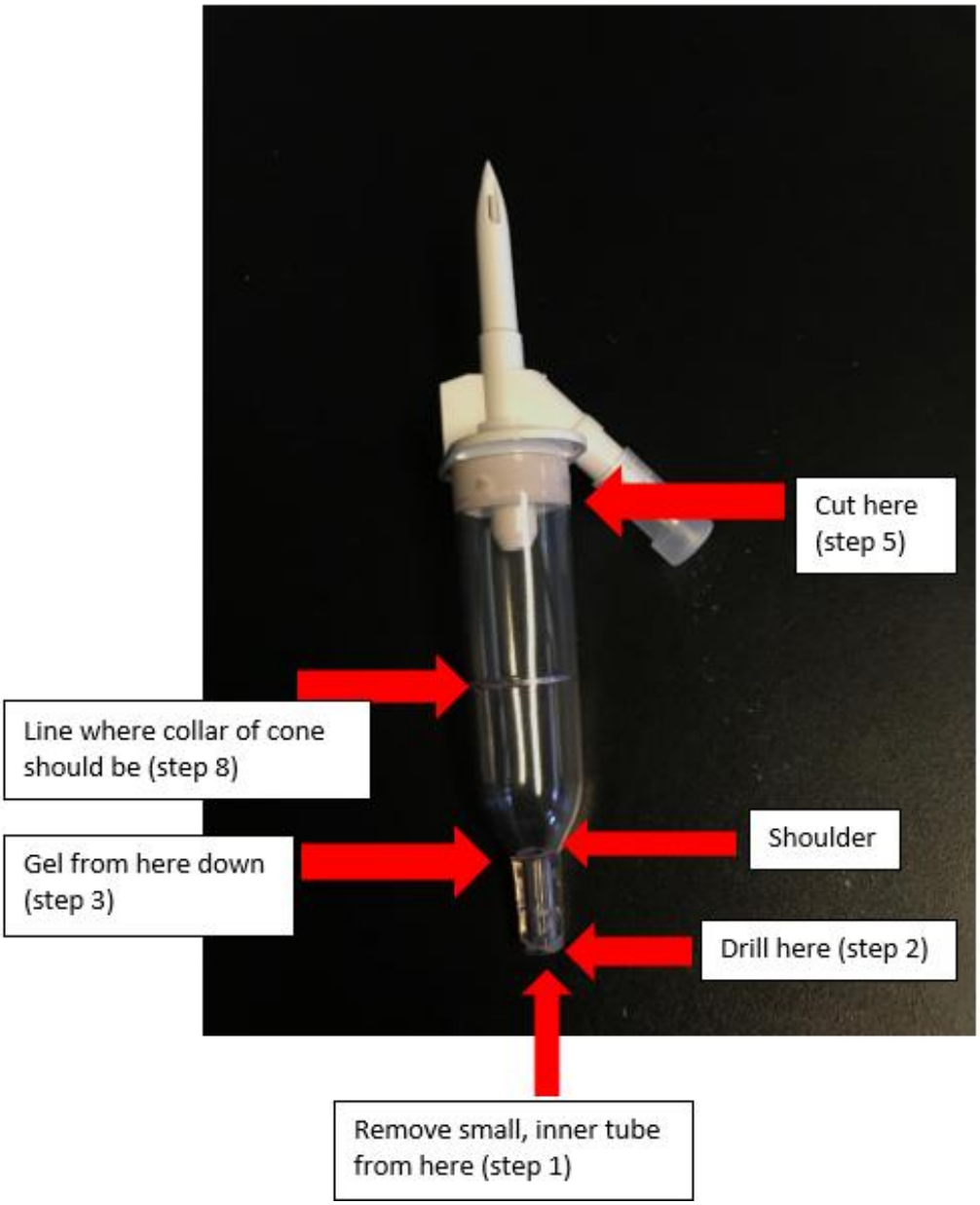

Figure 1: Venoset displaying modifications that need to be made

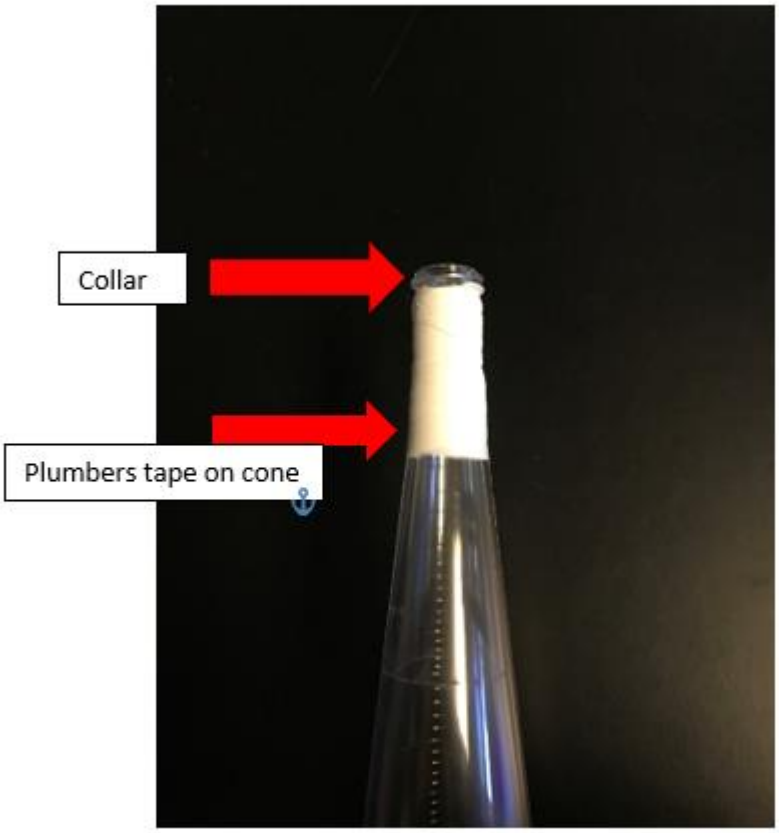

Figure 2: Plumber's tape added to base of cone (step 7)

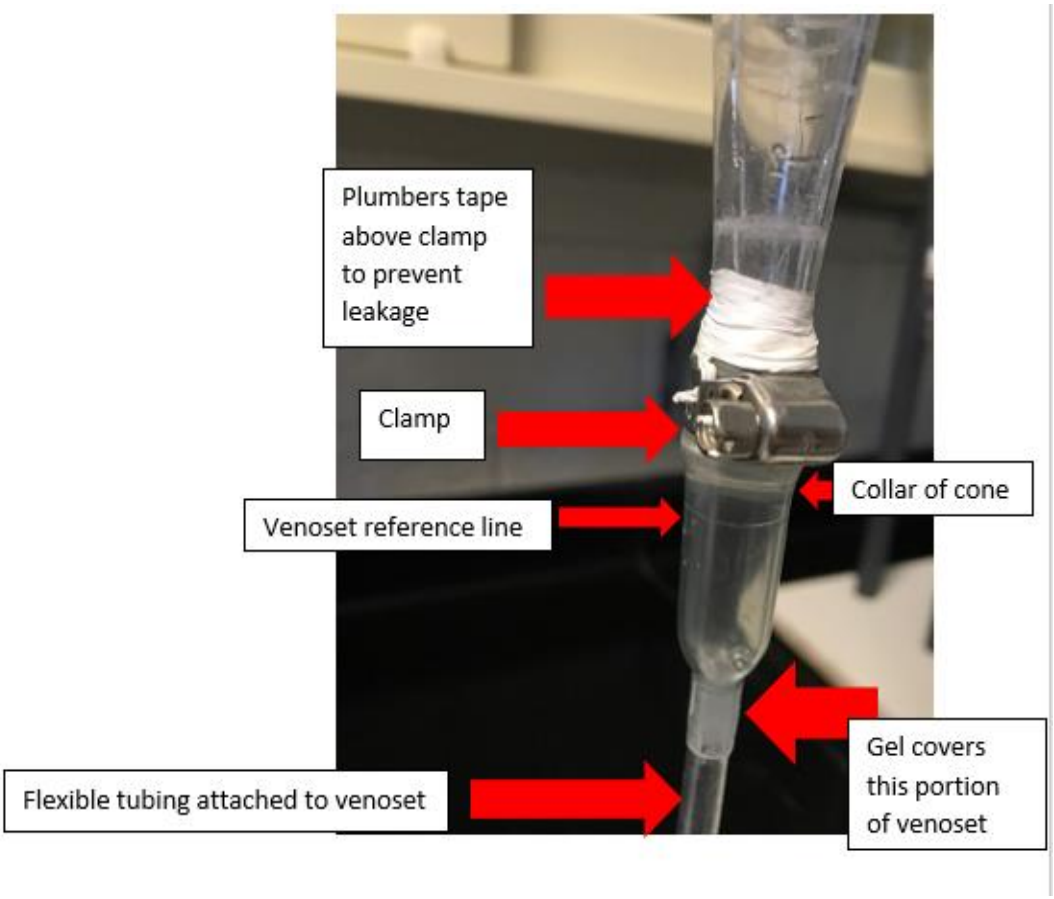

Figure 3: Assembled bottom portion of cone

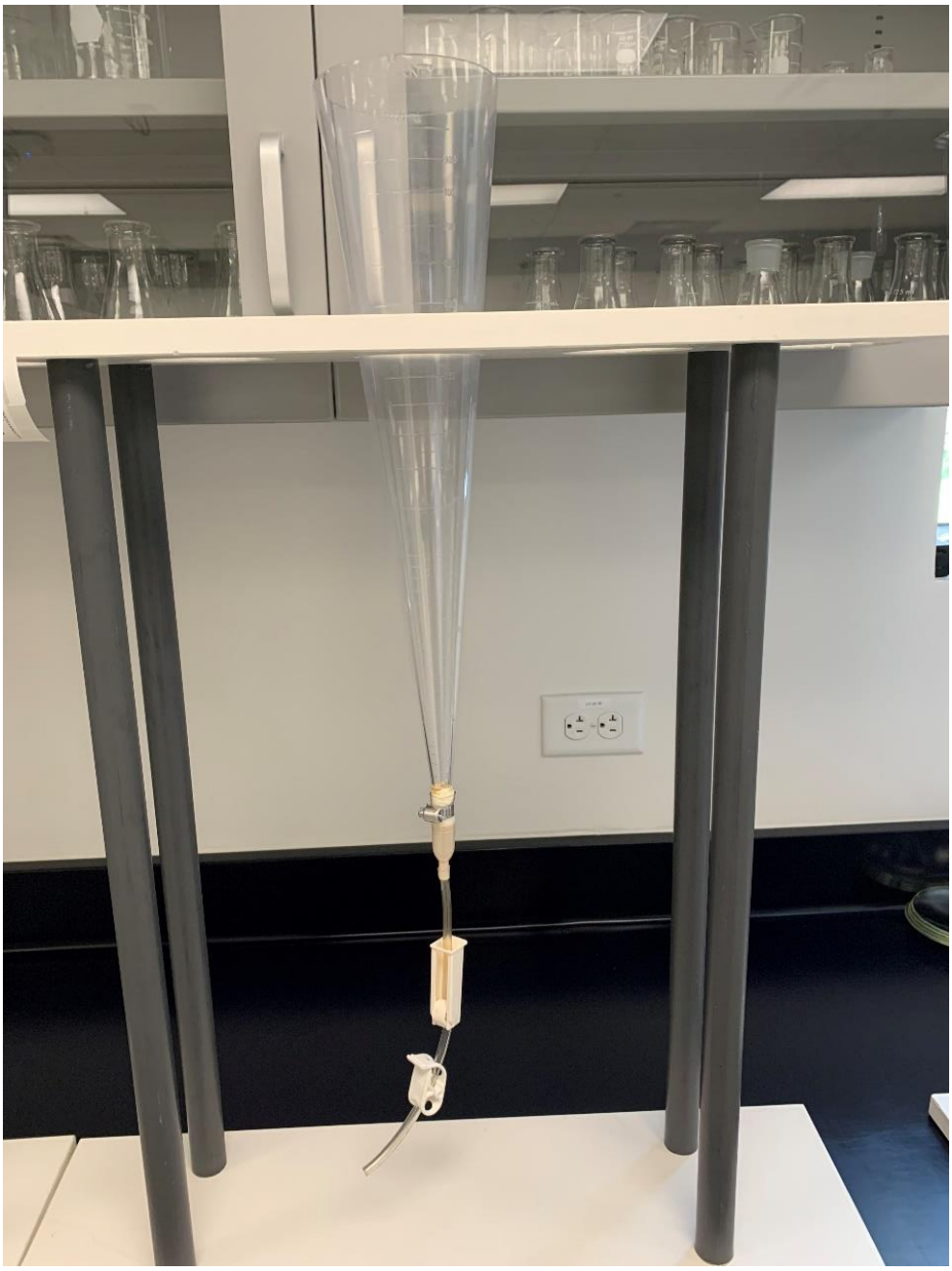

Figure 4: Fully assembled cone on stand

Appendix C – Cone Washing and Decontamination

After all samples have been taken down, follow the steps below to clean and disinfect cones.

1. Take empty cones and stands to sink area. Do not block fire cabinet
2. Remove pinch clamp. Do not remove roller clamp, but ensure it is fully open.
3. Using HOT tap water and the sprayer, thoroughly rinse the inside of any cone that has stained due to sample contents, or any cone with significant debris remaining. Start at the bottom and work up the sides as the cone fills. Allow water to run freely through the tube.
4. Once cone has filled, dump rinse water and return cone to stand.
5. Apply pinch clamp to the bottom of the tube and slide roller clamp down, ensuring the roller is fully closed.
6. Fill cones with at least 300 mL 5% acetic acid and allow to sit overnight. Do not move cones/stands from the sink area, and do not cover with watch glasses (this is a high traffic area and breakage is likely).
7. After sitting overnight, take each cone to the sink. Remove pinch clamp and fully open roller clamp – do not remove roller clamp.
8. Allow acetic acid to flow freely through the tube while using the appropriate dish brush (blue/black=negative, green=positive) to scrub the inside of the cone, inserting brush as far down the cone as possible and twisting gently. Scrub inside of cone from top to bottom, paying particular attention to places where debris collects. It is not necessary to scrub the outside of the cone.
9. Dump out any acetic acid remaining in cone.
10. Using HOT tap water and sprayer, rinse the inside of the cone starting at the bottom and working up the sides as the cone fills. Allow water to run freely through the tube. When cone is full, dump water and repeat, thoroughly rinsing cone three times. Quickly rinse outside of cone.
11. It is not necessary to do any special rinse of the tubing (such as turning the cone upside down and spraying into the tube) – acetic acid and water flowing through the tube is sufficient.
12. DO NOT USE DI WATER!
13. Return cone to stand, return to stands to storage areas, and allow to air dry.

Prior to using again, inspect each cone for debris and/or film. If present, repeat cleaning with the appropriate brush and HOT tap water, until cone is clean.

Appendix D - Subsampling Instructions

1. Prepare a glass petri dish with a grid pattern using a Sharpie.
2. Set a micropipette to 75 µL and add a yellow pipette tip.
3. Cut approximately 5mm off the pipette tip with a clean razor.
4. With cap still on, mix the 15 mL sample by inverting multiple times to get an even distribution of material throughout sample.
5. Remove the cap and quickly take two pulls of the sample with the pipette (before settling occurs). Dilute with DI water in the petri dish.
6. Count and record the number of veligers.
    a. If less than 20 veligers are observed in 150 µL, the full 15 mL sample must be counted using standard methods.
    b. If 20 or more veligers are counted, replace cap on the sample and repeat steps 4-6 for a total of six counts.
    c. If 500 or more veligers are counted at any point in one subsample, the pipette can be set to a lower µL – don't forget to adjust calculations!
    d. If pipette is set to 10 µL and there are still 500 veligers in one subsample STOP! This sample will be recorded as "Too Many to Count".
7. Use the standard deviation calculator at https://www.calculator.net to obtain the **mean** and **sample standard deviation** for the counts.
8. Standard deviation should be within 10-15% of the mean (standard deviation/mean). If it is not, perform additional counts and recalculate.
9. Once an acceptable standard deviation has been obtained, multiply the mean by 100 to obtain the average number of veligers in the 15mL sample.
   (150µL = 15,000µL = 15mL).
10. Record this number as the total count for the sample in the notebook and include the note "sub-sampled".