STANDARD OPERATING PROCEDURE
for the
BENTHIC MACROINVERTEBRATE LABORATORY

Central Plains Center for BioAssessment
Kansas Biological Survey
University of Kansas

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Final Draft

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This benthic macroinvertebrate laboratory protocol closely follows that of the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP) (USEPA 1995, USEPA 2004).

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I. Introduction

Benthic macroinvertebrate communities are reliable and sensitive biological indicators of water quality. Measurements of community taxonomic composition and abundance quantifiably report biological responses of benthic macroinvertebrate communities to their respective aquatic environments.

The use of valid and widely accepted standard benthic macroinvertebrate laboratory procedures for collecting, processing, and storing data promotes a greater understanding of aquatic environments, both spatially and temporally, by ensuring the comparability of data. Benthic macroinvertebrate laboratories that adopt and adhere to these laboratory protocols are better able to maintain high standards and attain compatibility with other benthic macroinvertebrate laboratories, both regionally and nationally.

The Environmental Monitoring and Assessment Program (EMAP) of the U.S. Environmental Protection Agency (USEPA) has established such standard laboratory practices. Therefore, the Central Plains Center for BioAssessment (CPCB) of the Kansas Biological Survey (KBS) has developed a standard operating procedure (SOP) based on USEPA EMAP methods to process benthic macroinvertebrate samples in the laboratory for the purpose of measuring the taxonomic composition and abundance of benthic macroinvertebrate faunas found in freshwaters.

In developing standard, accepted methods of measuring the effects of water quality on aquatic biotic communities, the USEPA has produced a number of rapid bioassessment protocols for use in collecting and processing benthic macroinvertebrate samples from streams and rivers in an effort to promote consistent bioassessments of high quality (Plafkin, et al. 1989; Klemm, et al. 1990; USEPA 1995; Lazorchak, et al. 1998; Barbour, et al. 1999; Lazorchak, et al. 2000; USEPA 2004; Angradi, et al. 2006; Peck, et al. In Press). However, only a few of these manuals (Klemm, et al. 1990; USEPA 1995; Barbour, et al. 1999; USEPA 2004) include protocols for laboratory processing of the collected benthic macroinvertebrate samples. The CPCB benthic macroinvertebrate laboratory SOP borrows heavily from the most recent of these EMAP manuals (USEPA 1995; Barbour, et al. 1999; USEPA 2004), but incorporates limited modifications based on CPCB's experience using these protocols (i.e., working the *bugs* out).

This standard operating procedure describes only the 500-count subsampling method used by the benthic macroinvertebrate laboratory of the CPCB to process benthic macroinvertebrate samples. The subsample count can be modified as projects dictate.

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II. Sample Processing

Samples will undergo four stages of laboratory processing:

A. Receipt of samples from the field
B. Initial rinse of field fixative
C. Sample set-up, subsampling, and sorting of benthic macroinvertebrates, and sample cleanup
D. Taxonomic identification and enumeration of benthic macroinvertebrates

Recommended EMAP quality control (QC) measures will accompany each of these four stages in order to meet or exceed the EMAP quality assurance (QA) standards.

Laboratory equipment and supplies needed for the receipt of samples from the field; initial rinse of field fixative; and sample set-up, subsampling, sorting, and cleanup are listed in Attachment 1.

A. Receipt of samples from the field

Benthic macroinvertebrate samples will be delivered by field crews or will arrive by a mail delivery service. When samples arrive via non-CPCB personnel, a Chain-of-Custody form (Attachment 2) must be completed and signed by CPCB laboratory personnel, returned to the party of origin with a copy, signed by the party of origin, and returned to the CPCB laboratory. Completed, signed, and returned Chain-of-Custody forms will be stored in an "all-projects" Chain-of-Custody binder in the benthic macroinvertebrate laboratory (Room 38).

All samples must be recorded in a Sample Log upon arrival from the field to the laboratory to verify arrival, condition, and number of containers (Attachment 3). Missing or damaged samples must be reported to the laboratory supervisor immediately. Samples will be carefully tracked within the lab throughout the project using the "sample ID" number recorded on the Sample Log. Laboratory personnel must keep accurate records of the location and status of all samples in their custody. The Sample Log will be stored in a project-specific binder with other project forms.

Samples should arrive at the laboratory in pre-labeled, plastic screw-top jars, preserved in a Rose Bengal-stained, Sodium Borate (Borax) buffered, 10% formalin solution. These samples will be stored at room temperature in closed, plastic containers (ice chests) in a designated room (Tile Room/Room 20B) until samples can be rinsed of the Rose Bengal/formalin fixative. This room is well ventilated and is used primarily for storage of sample and field gear.

B. Initial rinse of field fixative

The Rose Bengal/formalin fixative is replaced with 80%ethanol after repeated water rinses within seven to ten days after collection. This procedure renders the samples formalin free and safer for laboratory personnel who will subsample and sort the samples in the benthic macroinvertebrate laboratory.

A Sample Pre-Sort Rinse form (Attachment 4) must be completed in the Tile Room/Room 20B and later placed in the project-specific binder with the Sample Log in the benthic macroinvertebrate laboratory (Room 38). The rinsed samples will also be moved to Room 38 where sample setup, subsampling, sorting will occur.

Use the following procedure to rinse the samples:

1. Obtain a sample from the ice chest.
2. Record the sample label information, number of jars per sample, initials of the person rinsing the sample, and the date of sample rinse on the Sample Pre-Sort Rinse form.
3. Pour and spread the sample evenly onto a U.S. standard soil sieve #35 (500µm) held above a funnel in the opening of a formalin waste container in the sink. Use large forceps if necessary to empty a sample container and spread the sample over the sieve, allowing the fixative solution to drain into the waste container. Remove the waste container and gently rinse the sample with water, draining the rinse water into the sink until the water runs clear.
4. Return the rinsed sample from the sieve to the original sample jar. Rinse the sieve with a water wash bottle to collect all sample residue for return to the sample jar.
5. Inspect the sieve for remaining sample material using a 3X magnifying ring light or magnifying glass.
6. Add 80% denatured ethanol (EtOH) to the rinsed sample and close the sample jar tightly.
7. Backwash the sieve to prevent cross-contamination of samples.
8. Complete the previous seven steps until all samples are rinsed and recorded.
9. Transport rinsed samples and the Sample Pre-Sort Rinse form to the benthic macroinvertebrate laboratory (Room 38). Store samples in an appropriate cabinet to await the subsampling procedure. Place the Sample Pre-Sort Rinse form in the project specific binder with the Sample Log form.
10. Contact the University of Kansas office of Environmental Health and Safety to pickup and discard the used Rose Bengal/formalin fixative (see section IV. Laboratory Safety).

C. Sample set-up, subsampling, sorting of benthic macroinvertebrates, and cleanup

Quantitative samples are subsampled to a randomized fixed count of 500 +20% benthic macroinvertebrate specimens per sample using standard EPA EMAP laboratory sorting methods or sorted whole if the target number of 500 is not reached. All subsampling is done using the Caton standardized subsampling apparatus, which consists of a standardized gridded screen (370µm opening) and a white tray (Caton 1991, USEPA 2004).

1. Sample setup

1a. Remove the lid from the sample jar and pull out the internal sample label using forceps. Rinse the label over the U.S. standard soil sieve #35 (500µm) in the sink using a water wash bottle and set the label aside. Record the sample collection information from the sample label to a Benthic Macroinvertebrate Sample Sort sheet (Attachment 5). Required header information includes: project name, site ID, sample ID, waterbody name, collection date, sorter name, and sort dates (start and finish dates). Set the Sample Sort sheet and label aside. The label will later be returned to the sample container with the unsorted portion of the sample to be archived.

1b. Carefully decant the ethanol from the sample jar by pouring the fluid through a 500µm sieve held over a bucket then set the sieve aside. Save the ethanol to later preserve any unsorted sample portion. Repeat this step for remaining jars of the same sample. Rinse the sieve over the sample jar using a wash bottle of water to collect any organisms or debris that may have been poured onto the sieve. Inspect the sieve with a 3X magnifying ring light for organisms or debris. Backwash the sieve to clean it and prevent cross contamination of other samples processed later.

1c. If the sample contains a low amount (one jar) of inorganic substrate such as sand or gravel, transfer the sample material to the Caton gridded screen that is placed on the dish drain board. If there is more than one jar for any particular sample, empty and wash each jar onto the screen one at a time, making sure to spread each jar's contents evenly across the entire screen. If the amount of leaf litter or other detrital material exceeds that which fills the screen nearly to the level of the screen walls, divide the sample among two or more Caton tray assemblies. Carefully rinse any remaining fine sediment from the sample by pouring a gentle wash of water over the sample until the water rinses clear. Proceed to step 1e.

1d. If the sample contains a large amount (two or more jars) of inorganic substrate such as sand or gravel, carefully empty all or a portion of the sample (depending on the amount of inorganic material) into a large (four-to-six quart) plastic container and fill it nearly to the top with water. Gently circulate (elutriate) the sample using a large, long-handled metal spoon to suspend organisms and fine silt. Gently pour the water and suspended solids evenly across the surface of the Caton gridded screen. Repeat elutriation, pouring, and sieving until the elutriated water is clear of macroinvertebrates, plant debris, and sediment.

Use a white plastic paint spatula to inspect the elutriated sand/gravel remaining in the large plastic container under a 3X magnifying ring light searching for any remaining organisms that did not suspend during elutriation. These heavier organisms may include: clams, snails, caddisfly cases, crayfish, and/or larger worms and insects. Ask a lab supervisor to check the elutriated sand/gravel for missed organisms. Place any found organisms onto the Caton gridded screen with the main portion of the sample. Drain the remaining elutriated sand/gravel and dispose the inorganic material into a trash container. Rinse the plastic elutriation container. Proceed to step 1e.

1e. Place the Caton gridded screen in the white Caton tray and add water to nearly full or to a level well above that of the sample material. Remove large objects (sticks, stones, empty clam shells) and carefully search them under the 6x stereoscope or 3X magnifying ring light for organisms. Return found organisms to the sample on the gridded screen and place the large objects in the original sample jar that is now labeled as sorted residue. Use a spoon, spatula, or long forceps to gently spread the sample material over the bottom of the gridded screen as evenly as possible. Move the sample material into the corners and along the edges of the screen. Gently vibrate or shake the screen to help spread the sample.

1f. Lift the gridded screen out of the white tray to drain on the dish drain board. Pour off most of the excess water from the tray, leaving just enough water to nearly touch the screen and allow the sample to remain moist. Return the screen to the tray.

1g. Make a note on the backside of the sort sheet of any macroinvertebrates $\geq$0.5 inch ($\geq$12.7mm) long and occurring in four or fewer grids per Caton tray assembly. Those that remain after subsampling is completed will be placed in a sample vial and labeled "large/rare".

2. Subsampling (picking) of the subsample

2a. Use a random number generator (e.g. 6- and10-sided dice) to select the subsample grids to be sorted from the Caton gridded screen. The goal is to randomly select at least 10% (three) of the grids from the 30 grids on the screen in an effort to ensure that the subsample material is representative of the overall sample. The gridded screen was previously marked with a permanent black felt-tip marker into 30 equal 6cm square grids, by way of the letters "A" through "E" on the short side and the numbers 1 through 6 on the long side. A roll of the 10-sided die corresponds to the short side of the screen as follows: 1 or 2 equals A; 3 or 4 equals B; 5 or 6 equals C; 7 or 8 equals D; and 9 or 10 equals E. A roll of the 6-sided die corresponds to the same number along the long side of the gridded screen. Record the randomly chosen grid coordinates chronologically on the Benthic Macroinvertebrate Sample Sort sheet (Attachment 5) in the "Random Number Grid ID" column. Mark the grids chosen by an "X" in the coordinates box at the bottom of the page.

2b. Place the metal dividing frame (6cm square cookie cutter) over the sample at the approximate location of the grid selected for processing. Use a framing square to facilitate lining up the cookie cutter on the grid lines of the screen if necessary. If two or more Caton tray assemblies are being used to hold a large sample, remove the same grid on each screen and treat all grids as one. Write in the "General Comments" section of the Benthic Macroinvertebrate Sample Sort sheet the number of Caton tray assemblies that were used in sorting the sample.

2c. Remove or extract the sample material within the cookie cutter (grid or quarter-grid (step 2f)) using a white plastic teaspoon, 5/8-inch artist water brush, other small artist brush, paint spatula, and/or forceps. Depending on the consistency of the sample material, it might be necessary to cut the material along the inside of the cookie cutter with scissors or an Exacto knife or separate it with forceps so that only the sample material in the chosen grid is extracted. A wash bottle with a small nozzle may be used to clear small sand grains from most of the grid. Place the extracted sample material in a 5x7 inch white plastic photo tray with a pour spout and add water to cover the sample material. Rinse the extraction tools and the inside surface of the cookie cutter over the plastic tray and inspect these tools and the grid for any remaining organisms using the 3X magnifying ring light.

Use the following rules when dealing with organisms that lie on the line between two grids:

- An organism belongs to the grid containing its head.
- If it is not possible to determine the location of the head (i.e., for worms), the organism is considered to be in the grid containing most of its body
- If the head of an organism lies on the line between two grids, all organisms on the top border of a grid and those on the right border of a grid belong in that grid, and are picked with that grid.

2d. Set the Caton tray and screen aside until the macroinvertebrates have been sorted and counted from the previously extracted grid material (Section 3). Cover the gridded screen and tray with aluminum foil to prevent desiccation of the sample and damage to the specimens. Label the foil-covered sample using a self-stick note. Periodically moisten the sample with water from a spray bottle if the top layer begins to dry and/or add water to the tray under the screen. Spray the sample with ethanol if the sample contains large, fleshy invertebrates that may decompose more quickly than smaller invertebrates. Between each subsampling operation, be careful not to disturb the subsampling device to prevent redistribution of specimens, which could possibly change the probability of selection.

2e. If the number of organisms within any three grids appears to be lower than the sample target count (500 +20%), proceed in the following manner, otherwise go to 2f.

- If, after sorting and counting all the organisms from the first grid as explained in Section 3 and the organism count is greater than 350, put aside the organisms for that count and randomly choose a new first grid following the quarter-grid method

(step 2f); or at the lab manager's discretion, retain the organisms for that count and randomly choose a second grid following the quarter-grid method (step 2f).

- If, after sorting and counting all the organisms from the first grid, the organism count is fewer than 350 but greater than 150, then choose the second and third grids at random following the quarter-grid method (step 2f).

- If, after sorting and counting all the organisms from the first grid, the organism count is fewer than 150, then randomly choose a second full grid. If, after sorting and counting all the organisms from the first and second grids, the count does not exceed 300, then randomly choose a third full grid. Continue until the target level is reached.

- If three complete grids have yielded fewer than 60 organisms, then group the next three randomly chosen grids and sort and count the target organisms. If the organism count from the first six grids is lower than 120, then extract the entire remaining sample from the gridded screen; combine the extracted material in a 5x7 inch white tray; and sort and count the benthic macroinvertebrates from it. Write in the "General Comments" section of the Benthic Macroinvertebrate Sample Sort sheet that the remaining grids were combined for sorting. If the organism count from the first six grids is higher than 120, then resume random selection of individual grids until the target count (500 +20%) is reached. The laboratory supervisor is responsible for the determining whether a sample can be processed whole or by individual grids.

2f. If the number of organisms in any three grids <u>appears</u> to exceed the target count (500 +20%), then randomly choose grids and quarter them in the following manner.

- Assign numbers one through four to each quarter section of the grid, then roll a 4-sided dice and extract the sample material from the numbered quarter section that corresponds to the roll of the dice.
- After sorting and counting the first quarter section from the first grid, randomly choose a second grid. Randomly quarter the second grid as before.
- If, after sorting and counting the first quarter section from the second grid, the organism count is lower than the target count, then randomly choose a third grid. Randomly quarter the third grid as before and sort and count the organisms.
- If, after sorting and counting the first quarter section from the third grid, the organism count is below the target count, then return to first grid that was quartered and randomly choose a second quarter section from it. Repeat this procedure throughout the three grids until the target count is reached or until all quarter sections are completed for the first three grids.
- Note in the "General Comments" section of the Benthic Macroinvertebrate Sample Sort sheet which grids were quartered and the number of quarter sections that were chosen from each grid.

2g. If the total of all grids (or quarter-grids) processed consists of more than 600 organisms (i.e., >20% above the target number of 500), combine and evenly distribute all of the sorted, counted organisms evenly in a quarter-gridded petri dish or in a 5x7 inch gridded plastic tray. Choose grids at random, picking and counting organisms from each until the target count (500+20%) is reached. The sorter must pick an entire grid once started. Combine the remaining organisms in the dish in excess of the target count with the unsorted residue of the sample. Write in the "General Comments" section of the Benthic Macroinvertebrate Sample Sort sheet that the sort count exceeded 600 and was subsampled to the target count (500 +20%).

3. <u>Sorting of benthic macroinvertebrates</u>

All samples will be sorted under a minimum of 6x and a maximum of 10x magnification using a dissecting microscope (stereomicroscope).

3a. Pour a small amount of an extracted subsample from the 5x7 inch tray into a gridded 100x15mm glass petri dish that is marked underneath with a permanent felt tip marker into grids slightly smaller than the 6x field of vision of the microscope. Fill the petri dish to no more than half full with water and sample material. Too much sample material and/or water in the petri dish will slow sorting time and hinder search image success. Slowly search all grids in a systematic pattern to locate and identify all benthic macroinvertebrates specified in the Standard Taxonomic Level of Effort for Sorting list (Attachment 6). Use the recommended general taxonomic literature (Attachment 7) to identify organisms to order-level. First search over a black background, then search again over a white background, and search a third time focusing on the surface of the water, looking for surface floating organisms. Remove organisms and place in appropriately labeled 20ml scintillation vials. Each vial will contain a complete sample label, a phylum, class, subclass, or order label (Attachment 6), and 10ml of 80% ethanol. Write the "sample ID" number on the vial lids using a permanent felt tip marker.

3b. Do not remove or count: empty snail or bivalve shells; empty caddisfly cases; fragments such as legs, antennae, gills, wings, or headless bodies; round worms (Nematoda); microcrustacea (copepods, ostracods, branchiopods); eggs; or winged adult aquatic insects (except Hemiptera and Coleoptera). Search inside empty snail and bivalve shells and caddisfly cases for the presence of smaller target organisms. Also, search inside aquatic plant stems and leaves for small invertebrates such as dipteran or lepidopteran larvae and pupae that mine such tissues. Insects thought to be terrestrial should be verified as such by a taxonomist at sorting time or placed in a labeled vial for later verification but should not be counted. For segmented worms (Oligochaeta) remove and count only whole bodies and fragments that include a rounded end that could be a head or tail end. Count Oligochaeta end fragments as 1/2 counts (two ends equals one count). Count a whole worm as one count. If unsure as to whether any specimen should be counted, place the organism in a 20ml vial without counting it (the final identity and count will be made by a taxonomist).

3c. Use a tally counter to keep a running count of the total number of target macroinvertebrates removed from a grid or quarter section and record it on the Benthic Macroinvertebrate Sample Sort sheet. Enter the sorter's initials in the appropriate column on the Benthic Macroinvertebrate Sample Sort sheet for each completed grid or quarter section.

3d. When the randomly chosen grids are completely sorted, search the remaining sample on the gridded screen for "large and rare" organisms (Vinson and Hawkins 1996). These are organisms $\geq$0.5 inch in length which occurred in four or fewer grids per Caton tray assembly before subsampling. Place "large and rare" macroinvertebrates in a labeled sample vial (20ml or larger) and write L/R on the lid of the vial.

3e. Record the sorting completion date next to the sorting start date on the front side of the Benthic Macroinvertebrate Sample Sort sheet. Record on the backside of the Benthic Macroinvertebrate Sample Sort sheet record: the number of grids picked, the hours spent sorting the sample, the number of organisms picked, and the types and numbers of "large and rare" organisms found in the sample.

3f. Place the completed Benthic Macroinvertebrate Sample Sort sheet in the project-specific binder with the Benthic Macroinvertebrate Sample Log and the Benthic Macroinvertebrate Pre-sort Rinse form. Add the organisms found in the quality control check of sorted material to the appropriate 20ml vials. Place labeled sample vials in designated taxonomic groups in the wooden project vial trays to await further taxonomic identification.

3g. Record the completion of the sorting portion of each sample on the Benthic Macroinvertebrate Sample Progress form (Attachment 8) that is kept in the project specific binder.

4. Sample clean up

4a. Return all the sample material not subsampled from the gridded screen to the original sample container with the original ethanol preservative that was reserved earlier. Search the gridded screen for remaining sample residue using the 3X ring light. Put the original internal sample label and an"unsorted" sample label in the jar and attach another "unsorted" sample label to the outside surface of the jar using clear packing tape. Sieve all subsampled, sorted sample residue to drain the water, and put the drained, sorted sample residue and 80% ethanol preservative into a sample jar. Add a "sorted" sample label inside and outside the jar. Clean the sieve, gridded screen, and white Caton tray in a sink using antibacterial dish soap or cleaner and a brush and then backwash the sieve and screen.

4b. Store the sorted residue and unsorted residue sample portions separately in designated storage cabinets.

D. Taxonomic identification and enumeration

This procedure is to be used to facilitate identification and enumeration of benthic macroinvertebrate organisms collected from freshwater systems. Recommended EMAP quality control (QC) methods will be used in order to meet or exceed the EMAP quality assurance (QA) standards. The taxonomists must have training and experience in the identification of freshwater benthic macroinvertebrates or must undergo a rigorous QC of each sample by an experienced taxonomist in the lab.

It is important that the CPCB taxonomists maintain contact with other taxonomists through professional societies and other interactions, and keep up with the pertinent literature, since systematics and species identifications change over time.

1. Laboratory equipment and supplies

Laboratory equipment and supplies needed for the identification and enumeration of benthic macroinvertebrate samples are listed in Attachment 9. These supplies and equipment are quite numerous and some will require occasional repair or replacement. Therefore, laboratory personnel will maintain a running list of items that need service, replacement, or acquisition in order to continue and improve our laboratory standards.

2. Taxonomic level of effort

The objective of species identification and enumeration is to accurately identify all organisms found in a sample to the lowest possible taxonomic category consistent with study objectives and to accurately count the number of organisms in each taxon. Except as noted in this SOP or dictated by a project, specimens will be identified to the genus level whenever possible (mature and well preserved specimens). Due to various taxonomic difficulties, certain groups will not be identified to genus (Attachment 10). These groups and the associated level of expected taxonomic classification include the following:

Phylum Porifera	Family
Phylum Cnidaria	Order
Phylum Platyheminthes	Order
Phylum Nematomorpha	Family
Phylum Annelida	Order
Subclass Acarina	Subclass
Order Hymenoptera	Order

Identification of the Oligochaeta to family or genus level can be accomplished but at greater costs in time and resources, and therefore must be negotiated on a project-by-project basis.

All remaining groups of macroinvertebrates will be identified to genus except the gastropod family (Hydrobiidae) and five dipteran families (Dolichopodidae, Muscidae, Phoridae, Scathophagidae, Syrphidae).

3. Taxonomic literature

Identifications will be based on current published taxonomic references (Attachment 11) and more recently published primary literature. Each taxonomist will maintain a list of primary and secondary technical literature used in completing the identifications for each macroinvertebrate group and regularly update CPCB's list of taxonomic literature (Attachment 11). Questions of nomenclatural validity and classification will defer to the Integrated Taxonomic Information System (ITIS) that is available on the Internet at http://www.itis.gov/taxmatch_ftp.html.

4. Reference collection

Each taxonomist will prepare a reference collection of the organisms identified by him for each project. A designated taxonomist will organize a complete reference collection for each project by combining the reference specimens prepared by all the taxonomists involved. The reference collection will consist of the best available specimen(s) in various life stages of each species or morphospecies in each genus (or lowest taxonomic level identified). When a reference specimen(s) is removed from a sample and placed in the reference collection, a label will be placed in the original sample vial that indicates a specimen(s) was removed for the reference collection. Labels will be placed in sample slide boxes to denote the removal of reference-specimen slides to the reference collection. Slide-mounted specimens will be denoted using a fine-tipped permanent marker to indicate those specimens that belong to the reference collection. The reference collection is discussed further in the Quality Assurance and Quality Control Section III.

5. Taxonomic identifications

Specimens from most of the taxonomic groups will be identified and enumerated from visual inspection in 80% ethanol using a high quality dissecting microscope (stereomicroscope) and will be stored in alcohol vials. The larvae and pupae of the dipteran family Chironomidae and the Collembola, and the Oligochaeta (when necessary) will be mounted on microscope slides for identification using a compound microscope and stored in numbered slide boxes. These two procedures are described separately.

5a. Alcohol specimens

Sample processing for identification and enumeration begins by retrieving all the sample vials for a particular taxonomic group (Gastropoda, Ephemeroptera, etc.) for the entire project. Obtain an Original Data Sheet for Alcohol Specimens (Attachment 12) for each major taxonomic group to record identifications and counts. Record the sample label information on the original data sheet. Use a colored highlighter marker to note the sample label information. Next remove all labels from the vial; search them under the dissecting scope for organisms; and set the labels aside to reuse. Next pour the

specimens from the vial into a small glass petri dish. Rinse the vial with 80% ethanol into the petri dish. Examine the vial and its lid under the stereomicroscope for specimens.

Examine the macroinvertebrates using a stereomicroscope and taxonomic keys and other supportive taxonomic literature to identify the specimens. Record on the original taxonomic data sheet: the Family and Genus identifications; counts of larvae, pupae, and adults as appropriate for the taxonomic group; distinct-taxon status; and comments if any. Store the completed Original Data Sheets for all alcohol specimens in the project-specific binder.

After identification and enumeration, each taxon will be stored separately in 0.25-dram or 0.5-dram microvials within the original 20ml sample vial. Each microvial must contain a taxonomic determination label that includes the family name, genus name, taxonomist's identity, and the year of the taxonomic determination on the front side; and the life stage count on the backside. Use computer-generated labels or hand print the labels using archival ink pens or pencil. Use the cotton rag label paper described in Attachment 9 for all labels. Fill the microvials with 80% ethanol and plug with a cotton ball. Five 0.25-dram microvials can be stored in the original sample vial. Larger sized or more numerous specimens may be stored in 0.5-dram vials or directly in the original 20ml sample vial if the specimens are too large or too numerous to fit into a microvial. Each 20ml sample vial must contain: a sample collection label, an order label, and a hand-printed label that accounts for all the taxa, the respective counts, and respective life stages contained within that sample. When multiple 20ml vials are used to store identified specimens for an order within a sample, note on the vial lid with permanent felt-tip marker the vial number and total number of vials i.e. 1 of 3, 2 of 3, etc.

The identification of early-instar specimens can sometimes be determined if they can be associated with one or more mature specimens that have a more developed morphology.

5b. Slide-mounted specimens

Use a semi-permanent, slide-mounting medium (CMC-9) for most or all specimens from ecological projects. Use a permanent slide-mounting medium such as Euparol or Canada Balsam for reference specimens when possible. Prepare slide mounts of specimens using the methods explained by Epler (2001).

Attach an adhesive label printed with the sample label information on the left side of each slide. Mount five chironomid larvae or oligochaetes per slide, one chironomid pupa per slide, and one to five collembolans per slide. Dry and ring the slides as needed per mountant (Epler, 2001). Store the mounted, labeled slides in numbered slide boxes.

Number the slides consecutively within each project, beginning with the first sample to be identified. Record the identifications and counts of slide-mounted invertebrates for each sample on the Original Data Sheets for Slide-mounted Specimens (Attachment 13). Keep these original data sheets in a project-specific, three-ring binder.

5c. Enumeration

Identify and count only the specimen heads when organism fragments are encountered; do not identify, count, or store posterior body fragments. Do not count empty snail or clamshells. Larval and pupal exuviae (shed skins) will not be identified or counted, nor will fully emerged, aerial adults of Ephemeroptera, Odonata, Plecoptera, Megaloptera, Neuroptera, Hymenoptera, Diptera, Trichoptera, or Lepidoptera; or any terrestrial organisms. Adult aquatic Hemiptera and Coleoptera will be identified and counted.

The organism's life stage will be recorded on the original data sheet (Attachments 12 or 13) as larva, pupa, or adult when discernible. It is often difficult to identify the life stage in the non-insect groups.

If the taxonomic target level cannot be achieved due to immature, damaged, or pupal specimens this should be noted in the comments section of the original data sheet (Attachments 12 or 13) as early-instar, damaged, or pupal specimen(s).

5d. Distinct taxon

Each taxon will be recorded as "distinct" or as "not distinct" within a sample to avoid double counts of any taxa in later determination of diversity counts. When all macroinvertebrates within a sample are identified to the taxonomic target level (usually genus), then all taxa at the target level within that sample are considered to be "distinct taxa" and are recorded as "yes" in the "distinct taxon" column for each genus-level identification on the original data sheet.

However, when one or more macroinvertebrates within a sample are identified to the target level (usually genus), and one or more individuals are identifiable only to some taxonomic level above the target level (usually family), the designation of "distinct-taxon" status becomes important in forming correct taxa counts within a sample. Identification of macroinvertebrates at a taxonomic level higher than the target level (such as family) often occurs because the individuals are early instars or are damaged beyond recognition at the target level (genus).

If the taxonomist believes the specimen(s) identified to the higher level may belong to a lower target taxon that is represented in the same sample, then "no" is recorded in the "distinct-taxon" column for that higher-level identification. Example: Ten caddisfly larvae are identifiable to the target level of genus Hydropsyche, and three caddisfly larvae are such early instars (or damaged late instars) that identification is possible only to the family Hydropsychidae. The taxonomist sees nothing to indicate that the three early-instar (or damaged) specimens definitely represent a genus other than Hydropsyche. Therefore, although the genus Hydropsyche is considered a "distinct taxon", the family Hydropsychidae is not considered a "distinct taxon" in this sample. In cases of uncertainty, the conservative choice of "no" is recorded in the "distinct-taxon" column.

If the taxonomist believes the specimen(s) identified to family level may belong to a genus that is not otherwise represented in the same sample, then "yes" is recorded in the "distinct-taxon" column for that family-level identification. Example: Thirty riffle beetle

larvae occur in a sample. Twenty larvae are identifiable to the target level Dubiraphia, six larvae are identifiable to the target level Stenelmis, and four larvae are identifiable only to the family level as Elmidae. Although the four elmid larvae cannot be identified to genus, it is clear to the taxonomist that they do not belong to either genus already represented in the sample (Dubiraphia, Stenelmis). Therefore, the four larvae identified to the family Elmidae are considered to represent a "distinct taxon" in this sample in addition to Dubiraphia and Stenelmis.

5e. Tally Data

After the taxonomic identifications and enumerations are completed for all samples in the project, the original data sheets will be used to tally identifications and counts for all taxa within a sample and for the project (Attachment 14). The taxonomist supervising the project is responsible for completing the tally data sheets and passing them to the data manager for data entry.

E. Data entry and management

Bench sheet (tally data sheet) data will be entered into an MS-Access database developed by CPCB. To reduce entry errors, the database is set up with a drop down list for the taxonomic names, and contains queries to compare specimen counts reported on the bench sheet (tally data sheet) with counts totaled by the database. One person enters the data from the bench sheets, and a Quality Control (QC) officer checks all records for accurate entry. The data enterer and QC officer initial each sheet. The QC officer will report gross mistakes or consistent errors to both the data enterer and also the database manager who will take corrective action. The database manager will additionally QC at least three samples from each project.

The data will be transferred to a project-specific database and archived. From a copy of the data table the database manager will randomly remove specimen counts in samples that exceed 500+20% (specimen counts greater than 600) to bring the count down to 600. This count excludes "large and rare" taxa. A random-number generator in MS Excel or MS Access will determine specimens removed. This new table of adjusted counts will also be archived in the project database.

A final table will be produced that excludes all nondistinct taxa and counts. This final table will be used for analyses.

III. Quality Assurance and Quality Control

A. Responsibility and personnel qualifications

Quality control (QC) procedures are used to ensure that the data consists of ≤10% total error for the extraction of benthic macroinvertebrates from samples and ≤10% total error for the identification and the enumeration of the extracted organisms.

All laboratory personnel will receive basic instruction and evaluation in the sample processing procedure from experienced laboratory staff (preferably QC personnel). The qualifications of QC personnel include consistent achievement of ≥90% sorting efficiency and taxonomic knowledge of benthic macroinvertebrates as well as experience in sorting benthic macroinvertebrate samples.

The roles and responsibilities of the QC personnel in regards to sample processing are described below.

- Provide oversight of daily operations and sample processing; monitor QC activities to determine conformance; and conduct performance and systems audits of the procedures.
- Verify the completeness of every Benthic Macroinvertebrate Sample Sort sheet to ensure header information is correctly entered.
- Check the sorted material of all inexperienced laboratory personnel (those who have not achieved a ≥90% sorting efficiency) for missed organisms; and record the number of missed organisms in the appropriate blank on the backside of the Benthic Macroinvertebrate Sample Sort sheet.
- Check 10% of the sorted material of all experienced laboratory personnel (those who have achieved a ≥90% sorting efficiency) for missed organisms.
- Determine the sorting efficiency for each sample and sorter. Record the sorter's sorting efficiency on the bench sheet.
- Perform evaluations to ensure that QC is maintained throughout the laboratory sorting and subsampling procedure. Evaluations include double-checking work as it is completed and providing written documentation of these reviews to ensure that the standards set forth in the QAPP are met or exceeded.

B. QC of sample set-up, subsampling, and sorting of benthic macroinvertebrates

The Quality Control procedure for monitoring taxonomic sample set-up, subsampling, and sorting of benthic macroinvertebrates uses a re-sort method to identify unacceptable (>10%) levels of error in the data, and implements corrective actions that decrease the data error to acceptable levels ($\leq$10%).

An experienced QC person will use a 6-10x stereomicroscope to check all sorted grids from each sample. The QC person will calculate percent sorting efficiency (*PSE*) for each sample as follows:

$$PSE = [A / A + B] \times 100$$

where A = the number of organisms found by the primary sorter and B = the number of organisms missed by the primary sorter and found during the QC check.

If the sorting efficiency for each of five consecutive samples is $\geq$90% for a particular individual, this individual is considered "experienced" and can serve as QC personnel. In the event that an individual fails to achieve $\geq$90% sorting efficiency, he will be required to sort up to an additional five samples to continue to monitor sorting efficiency. If he shows marked improvement in sorting efficiency prior to completion of the additional five samples, whereby he acquires the $\geq$90% sorting efficiency, the QC person may, at his discretion, consider this individual to be "experienced."

After an individual passes QC requirements, 10% of his sorted samples will be randomly chosen and checked for each project.

If an "experienced" individual fails to maintain a $\geq$90% sorting efficiency as determined by QC checks, QC checks will be performed on every grid of five consecutive samples until a $\geq$90% sorting efficiency is achieved on all five. During this time, that individual will not be able to perform QC checks.

Organisms missed by the primary sorter and recovered during the QC check will be given to the sorter to put (do not count) into the appropriate vials for that sample. The QC officer will record and add the counts of recovered organisms to the sort sheet.

C. QC of taxonomic identification and enumeration

The Quality Control procedure for monitoring taxonomic identification and enumeration uses a re-identification and recount method to identify unacceptable (>10%) levels of error in the data in the form of misidentifications and/or specimen counts, and implements corrective actions that decrease the data error to acceptable levels (≤10%).

In addition, a taxonomic reference collection will be established for each project to assist in achieving consistently correct identifications.

1. QC re-identification and recount

All samples processed by a less experienced taxonomic technician will be checked by an experienced taxonomist to verify the accuracy of species identifications and counts and to correct the mistakes. After consistent achievement of ≥90% taxonomic efficiency by a taxonomic technician, an experienced taxonomist will check at random a minimum of 10% of all samples within each project processed by that taxonomic technician. If >10% error occurs in the checked samples, all the specimens in question will be re-identified and recounted by an experienced taxonomist. Re-identifications and recounts will be compared to the original data sheet, and corrections to data sheets will be made, initialed, and dated by the person conducting the QC check. The taxonomic technician will receive further training and Quality Control checks until taxonomic efficiency equals or exceeds 90%.

Experienced taxonomists will check at random 10% of the samples identified by each other. Corrections will be made to the original data sheet as described above.

2. Taxonomic reference collection as QA/QC

A reference collection will be established for each project. This collection will be stored in designated cabinets in the benthic macroinvertebrate laboratory (Room 36) for a time period designated per project agreement. The collection should consist of representative specimens of each species or representative morphotypes of each lowest identifiable taxon (i.e., genus, family, order, subclass).

In addition, taxa or morphotypes new to the laboratory's comprehensive reference collection will be sent to recognized experts for taxonomic verification. The verified specimens should then be added to the comprehensive reference collection.

All specimens in each alcohol reference collection will be preserved in 80% ethanol in vials with labels made of waterproof, 100% rag paper and archival ink or pencil. More than one specimen from a sample may be stored in a vial for each project. Reference specimens will be organized alphabetically within major taxonomic groups.

All slides containing reference specimens should be mounted with Euparol or Canada Balsam or must be ringed when using CMC-9 mountant. A reference specimen's

location on a slide must be marked with a fine-point permanent felt-tip marker. All slides containing reference specimens will be stored alphabetically within major taxonomic groups in labeled slide-boxes. A corresponding list of reference taxa will be included in each box.

An experienced taxonomist will curate the laboratory's comprehensive reference collection and maintain a collection log. The collection log will include the taxon name, the name of the taxonomist who originated the reference specimen, the location of the reference specimen, the status of the specimen if it has been loaned to outside experts, information about the species' confirmation by outside experts, and references to pertinent literature describing the species (USEPA 1995).

IV. Laboratory Safety

Safe laboratory practices must be followed at all times.

Materials Safety Data Sheets for all chemicals must be available in the rooms in which the chemicals are stored and used.

The chemical used in the macroinvertebrate laboratory that is the greatest safety concern is formalin. Formalin is a known carcinogen; therefore samples containing formalin and formalin waste containers should be stored in a room where personnel are not usually working (Tile Room/Room 20B). Samples in formalin must be stored in closed, plastic containers such as ice chests until drained, rinsed, and transferred to ethanol. Laboratory personnel must wear protective eyewear, a respirator, nitrile gloves, and a lab coat or apron when working with samples containing formalin. The laboratory or room must be ventilated by a fume hood and/or by the use of a fan and open door when personnel work with this chemical.

Formalin and ethanol waste must each be stored in appropriately labeled, plastic waste containers. Waste containers can be requested from the Environment, Health, and Safety (EHS) office of the University of Kansas (KU): http://www.ehs.ku.edu/ehs form/.

EHS approved labels are available online:
http://www.ehs.ku.edu/document/ehs_forms/hazardous_materials_waste_labels.aspx.

The waste containers when filled must be picked up for disposal by KU's EHS personnel:
http://www.ehs.ku.edu/document/ehs_forms/hazardous_materials_pickup_request.aspx.

Keep work areas clean and neat.

Report possible safety hazards to the CPCB director.

Refer to the safety manuals provided online by the EHS of KU for more detailed laboratory guidelines: http://www.ehs.ku.edu/documents/ehs.

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V. Literature Cited

Angradi, T.R. (editor). 2006. Environmental Monitoring and Assessment Program: Great River Ecosystems, Field Operations Manual. EPA/620/R-06/002. U.S. Environmental Protection Agency, Washington, D.C.

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Caton, L.W. 1991. Improved Subsampling Methods for the EPA "Rapid Bioassessment" Benthic Protocols. Bulletin of the North American Benthological Society of America 8(3):317-319.

Epler, J.H. 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. Special Publication SJ2001-SP13. North Carolina Department of Environment and Natural Resources, Raleigh, NC, and St. Johns River Water Management District, Palatka, FL. 526 pp.

Klemm, D.J., P.A. Lewis, F. Fulk, and J.M. Lazorchak. 1990. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/4-90/030. U.S. Environmental Protection Agency, Washington, D.C.

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Lazorchak, J.M., B.H. Hill, D.K. Averill, D.V. Peck, and D.J. Klemm (editors). 2000. Environmental Monitoring and Assessment Program – Surface Waters: Field Operations and Methods for Measuring the Ecological Condition of Non-wadeable Rivers and Streams. U.S. Environmental Protection Agency, Cincinnati, OH.

Peck, D.V., J.M. Lazorchak, and D.J. Klemm. In Press. EMAP – Surface Waters. Western Pilot Study Field Operations Manual for Wadeable Streams. U.S. Environmental Protection Agency, Office of Research and Development, Washington, D.C.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish. EPA/444/4-89-001. U.S. Environmental Protection Agency, Washington, D.C.

USEPA. 1995. Environmental Monitoring and Assessment Program (EMAP): Laboratory Methods Manual – Estuaries, Volume 1: Biological and Physical Analyses. EPA/620/R-95/008. U.S. Environmental Protection Agency, Rarragansett, RI.

USEPA. 2004. Wadeable Stream Assessment: Benthic Laboratory Methods. EPA841-B-04-007. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, D.C.

Vinson, M.R. and C.P. Hawkins. 1996. Effects of sampling area and subsampling procedure on comparisons of taxa richness among streams. Journal of the North American Benthological Society 15(3):392-399.

ATTACHMENT 1

Materials List
for receipt, initial rinse, setup, subsampling, and sorting
of benthic macroinvertebrate samples

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Materials List for receipt, initial rinse, setup, subsampling, & sorting of samples

Attachment 1 – Materials List for receipt, initial rinse, setup, subsampling, and sorting of samples
Attachment 2 - Benthic Macroinvertebrate Chain-of-Custody form (one per project)
Attachment 3 - Benthic Macroinvertebrate Sample Log form (one per project)
Attachment 4 - Benthic Macroinvertebrate Sample Pre-sort Rinse form (one per project)
Attachment 5 – Benthic Macroinvertebrate Sample Sort Sheet (one per sample)
Attachment 6 – Standard Taxonomic Level-of-Effort for Sorting list
Attachment 7 – Taxonomic Literature for Sorting list
Attachment 8 - Benthic Macroinvertebrate Sample Progress form (one per project)

Taxonomic literature (see Attachment 7)

Storage cabinets for unsorted, sorted, and identified samples
Large capacity ice chests
5 to 10-gallon plastic storage/dispense container for denatured ethanol (EtOH)
Denatured ethanol (EtOH) 80% by volume
U.S. standard soil sieve # 35 (500 μm) or any smaller mesh size
2-gallon plastic round buckets with pour spout
Large jar funnels
Plastic dish drain board
4 - 6 quart, plastic tubs
Large, long-handled metal spoons
Large, long-handled forceps
Caton macroinvertebrate subsubsampling devices (370μm mesh, gridded screen and white plastic holding tray)
6cm square metal frames (cookie cutter)
8 - 12 inch L-shaped framing square
Random number generators (dice: 10-sided die, 6-sided die, 4-sided die)
White plastic 5 x 7 inch photo trays with pour spout

Artist wash brushes (5/8 inch)
Artist angled brushes (3/8 inch)
Artist camelhair brushes (no. 2, medium)
Dropping pipettes
White plastic teaspoons
Artist white plastic paint spatulas
Fine point dissecting scissors
Exacto knives
Fine point jewelers forceps (#5 Rubis)
Butterfly forceps
Fine point dissecting probes (probe handles, #000 insect pins, minuten pins)
Diamond edge sharpener for forceps and micro tools.
Plastic wide-mouth pint jars
Plastic wash bottles (500ml)
Plastic spray bottles (500ml or smaller)
Aluminum foil

Glass petri dishes with lid (100x15mm)
Glass petri dishes with lid (60x10mm)
Glass stender dishes with lids (51x26mm)
Glass stender dishes with lids (37x25mm)
Glass scintillation vials (20ml) with cone-insert caps
Glass scintillation vials (40ml and larger) with cone-insert caps
Single, double, or multiple tally counters

Magnified ring lights (3X)
Stereomicroscopes (6X – 10X)
Fiber optic microscope lights
Lens paper
Kimwipes

Labeling paper (100% 13WCO acid free linen ledger paper, #36 short grain)
Preprinted sample-jar labels (sorted residue)
Preprinted sample-jar labels (unsorted residue)
Preprinted sample-vial labels
Preprinted phyla, class, subclass, and order labels

Transparent plastic rulers (15mm)
Plastic rulers (30mm)
Calculators
Wooden or plastic open boxes or trays for temporary storage of sorted samples
Clear packing tape
Colored label tape (two colors)
Spray canned air
Mechanical pencils with 0.7mm lead refills
Paper scissors
Permanent felt-tip markers (fine tip, assorted colors)
Pigma micron archival pens (black, red) (#01, #005)
High lighters (assorted colors)
Self stick-it notepads (1.5 x 2 inch)
Self stick-it page markers
D-ring binders (1.5 inch, 2 inch, 3 inch)
Binder dividers
Note cards (3 x 5 inch)

Disposable nitrile gloves (M, L, XL)
Safety glasses
Respirator
Lab coats (M, L, XL, XXL) or aprons
Sharps box
Plastic container for formalin waste (properly labeled)
Plastic container for ethanol waste (properly labeled)
Materials Safety Data Sheets (MSDS) for all chemicals used in the lab

Paper towels
Dishtowels
Hand soap
Antibiotic dish soap
Antibiotic spray cleaner

ATTACHMENT 2

Benthic Macroinvertebrate Chain-of-Custody form

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Specimen Chain of Custody form

Shipped to:

Name

Agency

Address

Phone

Contents:	**Total number of:**
___ Macroinvertebrates	___ jars
___ Fish	___ vials
___ Other	___ slides
	___ other

From: **Page ___ of ___**

Central Plain Center for BioAssessment
Kansas Biological Survey
2101 Constant Ave.
Lawrence, KS 66047
Lab phone: 785-864-1549
Asst. Director phone: 785-864-1551
Fax: 785-864-1537

Site ID	Site name	Sample ID	Date collected	Slide (S), Jar (J), or Vial (V) #	Taxa	# of spec.	comments	h recipient recv'd	h CPCB recv'd

Comments:

CPCB Personnel
Packed by:
Date packed:
Shipped by:
Shipping method:

Recipient
Received by:
Date received:
PLEASE FAX THIS completed form back to CPCB at 785-864-1547
Or email Debbie Baker, dbaker@ku.edu that you received the package in full. (date emailed received:_____)

Specimens returned to CPCB on _____. Checked in by _____.

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ATTACHMENT 3

Benthic Macroinvertebrate Sample Log form

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Sample Log

Project:_____

Name:_____

#	Site ID	Sample ID	Waterbody Name	Collection Date	# of Jars	Sorted
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
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19						
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22						
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36						
37						
38						
39						
40						

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ATTACHMENT 4

Benthic Macroinvertebrate Sample Pre-sort Rinse form

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Sample Pre-Sort Rinse

Project name _____

#	Site ID	Sample ID	Waterbody Name	Collection Date	# of Jars	Rinser Initials	Date Rinsed
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25							
26							
27							
28							
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30							
31							
32							
33							
34							
35							
36							
37							
38							
39							
40							

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ATTACHMENT 5

Benthic Macroinvertebrate
Sample Sort sheet

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BENTHIC MACROINVERTEBRATE SAMPLE SORT SHEET (FRONT)

Project Name: _____
Site ID: _____ Sample ID: _____
Waterbody Name: _____ Collection Date: _____
Sorter Name: _____ Sort Date: _____

GRID ORDER	SORTER'S INITIALS	RANDOM NUMBER GRID ID	NUMBER OF INDIVIDUALS PER GRID	CUMULATIVE NUMBER OF ORGANISMS
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				

Check off grids as selected:

	1	2	3	4	5	6
A						
B						
C						
D						
E						

BENTHIC MACROINVERTEBRATE SAMPLE SORT SHEET (BACK)

SUBSAMPLING/SORTING INFORMATION Sorter: _____ Date: _____	Number of grids picked: _____ Time expenditure: _____ No. of organisms: _____ Indicate the presence of large or obviously abundant organisms: _____ **QC:** ☐ YES ☐ NO **QC Checker:** _____ ☐ # organisms originally sorted ☐ # organisms recovered by checker ☐ # organisms originally sorted ☐ % sorting efficiency ≥ 90%, sample passes: _____ < 90%, sample fails, action taken: _____ _____
TAXONOMY ID: _____ Date: _____	Explain TCR ratings of 3-5: Other Comments (e.g. condition of specimens): **QC:** ☐ YES ☐ NO **QC Checker:** _____ Organism recognition ☐ Pass ☐ Fail Verification complete ☐ YES ☐ NO

General Comments (use this space to add additional comments):

_____
_____
_____
_____
_____
_____

ATTACHMENT 6

Standard Taxonomic Level of Effort for Sorting

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Standard Taxonomic Effort List for Sorting
Central Plains Center for Bioassessment , KBS

Phylum Porifera	freshwater sponges
Phylum Cnidaria	hydroids, jellyfish
Phylum Platyhelminthes	
Class Turbellaria	free-living flatworms
Order Tricladida	macroturbellarians (planarians)
Phylum Nemertea	proboscis worms, ribbon worms
Phylum Nematomorpha	horsehair worms, gordian worms
Phylum Mollusca	
Class Gastropoda	snails, limpets
Class Bivalvia	clams, mussels
Phylum Annelida	
Subclass Oligochaeta	aquatic earthworms , branchiobdellid worms
Subclass Hirudinea	leeches
Phylum Arthropoda	
Class Arachnida	
Subclass Acarina	aquatic mites
Class Malacostraca	
Order Amphipoda	scuds, sideswimmers
Order Isopoda	slaters, aquatic sowbugs
Order Mysida	opossum shrimps
Order Decapoda	freshwater shrimps, crayfish
Class Entognatha	
Order Collembola	aquatic springtails
Class Insecta	
Order Ephemeroptera	mayflies
Order Odonata	damselfies, dragonflies
Order Orthoptera	semiaquatic grasshoppers & crickets
Order Plecoptera	stoneflies
Order Hemiptera	aquatic & semiaquatic bugs
Order Megaloptera	fishflies, alderflies, dobsonflies
Order Neuroptera	spongillaflies
Order Hymenoptera	aquatic parasitoid wasps
Order Coleoptera	aquatic beetles
Order Diptera	aquatic flies
Order Trichoptera	caddisflies
Order Lepidoptera	aquatic & semiaquatic moths

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ATTACHMENT 7

Taxonomic Literature for Sorting

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Taxonomic Literature for Sorting

Bouchard, R.W., Jr. 2004. "Guide to aquatic macroinvertebrates of the Upper Midwest". Water Resources Center, University of Minnesota, St. Paul, MN. 208 pp.

Huggins, D.G., P.M. Liechti, and L.C. Ferrington, Jr. (editors). 1985. "Guide to the freshwater invertebrates of the Midwest", 2nd ed. Technical Publications of the Kansas Biological Survey, University of Kansas, No. 11:1-221.

Merritt, R.W., K.W. Cummins, and M. B. Berg (editors). 2008. "An introduction to the aquatic insects of North America", 4th edition, revised printing. Kendall/Hunt Publishing Company, Dubuque, Iowa. 1158 pp.

Voshell, J.R., Jr. 2002. "A guide to common freshwater invertebrates of North America". The McDonald & Woodward Publishing Company, Blacksburg, VA. 442 pp.

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ATTACHMENT 8

Benthic Macroinvertebrate Sample Progress form

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Benthic Macroinvertebrate Sample Progress form

Site ID	Collection Date	Sample ID	Stream Name	Sort Count	Midges Slide Mounted	Chironomidae ID'ed	Diptera ID'ed (non-Chiro)	Ephemeroptera ID'ed	Plecoptera ID'ed	Trichoptera ID'ed	Odonata ID'ed	Coleoptera ID'ed	Hemiptera ID'ed	Megaloptera ID'ed	Lepidoptera ID'ed	Orthoptera ID'ed	Neuroptera ID'ed	Hymenoptera Id'ed	Collembola ID'ed	Amphipoda ID'ed	Decapoda ID'ed	Isopoda ID'ed	Mysida ID'ed	Gastropoda ID'ed	Bivalvia ID'ed	Oligochaeta ID'ed	Branchiobdellida ID'ed	Hirudinea ID'ed	Tricladida ID'ed	Acarina ID'ed	Nemertea ID'ed	Nematomorpha ID'ed	Porifera Id'ed	Cnidaria Id'ed	Large/Rare ID'ed

X = ID'ed, **0** = None exist for the sample, ***"Blank Space"*** = Not ID'ed yet

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ATTACHMENT 9

Materials List
for Taxonomic Identification and Enumeration of Samples

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Materials List for taxonomic identification and enumeration of samples:

Attachment 8 - Benthic Macroinvertebrate Sample Progress form (one per project)
Attachment 9 – Materials List for Taxonomic Identification and Enumeration of Samples
Attachment 10 - Standard Taxonomic Level-of-Effort for Taxonomy list
Attachment 11 – Taxonomic Literature for Taxonomy list
Attachment 12 – Original Data Sheet for Alcohol Specimens
Attachment 13 – Original Data Sheet for Slide-mounted Specimens
Attachment 14 – Tally Data Sheet (one per sample)

Taxonomic Literature (see Attachment 11)

Storage cabinets for sorted and identified samples
5 to 10-gallon plastic storage/dispense container for denatured ethanol (EtOH)
Denatured ethanol (EtOH) 80% by volume

Artist camelhair brushes (no. 2, medium)
Dropping pipettes
Fine point dissecting scissors
Iris scissors
Fine point jewelers forceps (#5 Rubis)
Butterfly forceps
Fine point dissecting probes (probe handles, #000 insect pins, minuten pins)
Diamond edge sharpener for forceps and micro tools.
Plastic wide-mouth half pint jars
Plastic wash bottles (500ml)

Divided (quartered) glass petri dishes (100 x 15mm)
Glass petri dishes with lid (100 x 15mm)
Glass petri dishes with lid (60 x 15mm)
Glass stender dishes with lids (51 x 26mm)
Glass stender dishes with lids (37 x 25mm)
Glass scintillation vials (20ml) with cone-insert caps
Glass scintillation vials (40ml and larger) with cone-insert caps
Single, double, or multiple tally counters

Stereomicroscopes (7.7X – 75X)
1.5 doubling lenses
Fiber optic microscope lights
Compound microscopes
Lens paper
Kimwipes

CMC–9 slide mounting medium (Masters Company brand)
Euparol slide mounting medium
Canada Balsam slide mounting medium
Clear nail polish
Glass dispensing bottles with fitted droppers for slide mounting medium
Microscope slides (17 x 25mm, 0.97 – 1.07mm thick, Goldseal brand)
Slide coverslips (18mm square, 0.17 – 0.25mm thick)
Slide coverslips (12mm circular)
Slide label paper (full sheet, self-stick labels; box of 100)
Slide storage boxes (wooden, Fischer brand)
Drying oven for slides

Curator's block (pin and tool holder)
Round wooden insect pin holder (holds shell microvials)
Shell microvials (0.25 dram, 9 x 30mm)
Shell microvials (0.50 dram, 12 x 35 mm)
Cotton roll or cotton balls

Labeling paper (100% 13WCO acid free linen ledger paper, #36 short grain)
Preprinted sample-vial labels
Preprinted phyla, class, subclass, and order labels (for scintillation vials)
Preprinted taxon labels (for microvials); includes: family name, genus name, taxonomist's name.

Transparent plastic rulers (15mm)
Calculators
Wooden or plastic open boxes or trays for temporary storage of sorted samples
Clear packing tape
Colored label tape (two colors)
Spray canned air
Mechanical pencils with 0.7mm lead refills
Paper scissors
Permanent felt-tip markers (fine tip, assorted colors)
Pigma micron archival pens (black, red) (#01, #005)
High lighters (assorted colors)
Self stick notepads (1.5 x 2 inch)
Self stick page markers
D-ring binders (1.5 inch, 2 inch, 3 inch)
Binder dividers
Note cards (3 x 5 inch)

Sharps box
Plastic container for ethanol waste (properly labeled)
Materials Safety Data Sheets (MSDS) for all chemicals used in the lab

Paper towels
Dishtowels
Hand soap
Dish soap

ATTACHMENT 10

Standard Taxonomic Level of Effort for Taxonomy

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Standard Taxonomic Effort List
Central Plains Center for Bioassessment , KBS

Phylum Porifera	freshwater sponges	family
Phylum Cnidaria	hydroids, jellyfish	order
Phylum Platyhelminthes		
Class Turbellaria	free-living flatworms	
Order Tricladida	macroturbellarians (planarians)	order
Phylum Nemertea	proboscis worms, ribbon worms	genus
Phylum Nematomorpha	horsehair worms, gordian worms	family
Phylum Mollusca		
Class Gastropoda	snails, limpets	genus (except 1 family)
Class Bivalvia	clams, mussels	genus
Phylum Annelida		
Subclass Oligochaeta	aquatic earthworms	order *
Order Haplotaxida	aquatic earthworms	
Order Lumbriculida	aquatic earthworms	
Order Branchiobdellida	branchiobdellid worms	
Subclass Hirudinea	leeches	genus
Phylum Arthropoda		
Class Arachnida		
Subclass Acarina	aquatic mites	subclass
Class Malacostraca		
Order Amphipoda	scuds, sideswimmers	genus
Order Isopoda	slaters, aquatic sowbugs	genus
Order Mysida	opossum shrimps	genus
Order Decapoda	freshwater shrimps, crayfish	genus
Class Entognatha		
Order Collembola	aquatic springtails	genus
Class Insecta		
Order Ephemeroptera	mayflies	genus
Order Odonata	damselfies, dragonflies	genus
Order Orthoptera	semiaquatic grasshoppers & crickets	genus
Order Plecoptera	stoneflies	genus
Order Hemiptera	aquatic & semiaquatic bugs	genus
Order Megaloptera	fishflies, alderflies, dobsonflies	genus
Order Neuroptera	spongillaflies	genus
Order Hymenoptera	aquatic parasitoid wasps	order
Order Coleoptera	aquatic beetles	genus
Order Diptera	aquatic flies	genus (except 5 families)
Order Trichoptera	caddisflies	genus
Order Lepidoptera	aquatic & semiaquatic moths	genus

* Oligochaeta can be identified to family or genus level for an additional cost

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ATTACHMENT 11

Taxonomic Literature for Taxonomy

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Taxonomic Literature for Taxonomy

General

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ATTACHMENT 12

Original Data Sheet for Alcohol Specimens

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Original Data Sheet for Alcohol Specimens **Taxonomic Group: ____________________**

Project: ______________________________ **Taxonomist/Date: _____________________**

Site ID Family	Stream Genus	# Larvae	Col.Date \# Pupa	# Adults	Sample ID Dist.Tax.	(highlight this line) Notes

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ATTACHMENT 13

Original Data Sheet for Slide-mounted Specimens

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Original Data Sheet for Slide-mounted Specimens

Project: ____________________ **Site ID:** ____________ **Sample ID:** ____________

Coll.Date: ________________ **Stream:** ______________________ **Det./Date:** ____________________

Slide#	Taxon	Stage	Notes

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ATTACHMENT 14

Tally Data Sheet

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Tally Data Sheet

Project Name: ______________________

Site ID: _________________________

Stream Name: ______________________________

Sample ID: _________________________

Collection Date: _____________________

Taxon Name	Total #	# of Larvae	# of Pupae	# of Adults	Distinct Taxon	Taxonomist	Comments