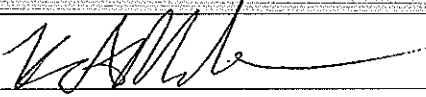
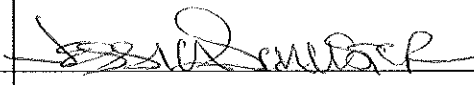
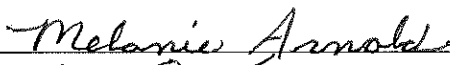
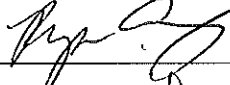

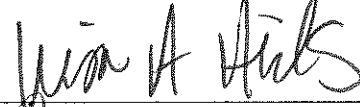

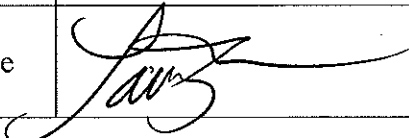


Laboratory Procedures for Macroinvertebrate Sample Processing and Identification

Commonwealth of Kentucky
Energy and Environment Cabinet
Department for Environmental Protection
Division of Water

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Action By	Signature	Date
Katie McKone Prepared, SOP Author		02/20/2015
Jessica Schuster Prepared, SOP Coauthor		02/20/2015
Melanie Arnold Reviewed		2/20/2015
Ryan Evans Reviewed		2/20/15
Andrea Keatley Approved, Branch Manager		2/20/15
Lisa Hicks Approved, DOW Quality Assurance Officer		03/13/15
Peter Goodmann Approved, Director Division of Water		5/8/2015
Larry Taylor Approved, DEP Quality Assurance Manager		5/13/15

**Commonwealth of Kentucky
Steven L. Beshear, Governor**

**Energy and Environment Cabinet
Len Peters, Secretary**

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2. Document Revision History

Date of Revision	Page(s) Revised	Revision Explanation
February, 2015	All	The macroinvertebrate processing procedures formerly found in “Laboratory Procedures for Macroinvertebrate Processing, Taxonomic Identification and Reporting” (KDOW 2011) have been updated and revised. This document only includes processing and identification procedures. A separate SOP is under development for data entry and analysis procedures.

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4. Scope and Applicability

Macroinvertebrates are utilized extensively as indicators of water quality and are integral in the decision making process regarding the aquatic life use support of individual stream segments throughout Kentucky. This document outlines laboratory methods used by KDOW for the processing of macroinvertebrate samples.

5. Executive Summary

This document includes the procedures that should be followed by Kentucky Division of Water (KDOW) staff for the uniform and accurate processing and identification of benthic macroinvertebrate samples collected from the surface waters of the Commonwealth. The recommended quality assurance/quality control (QA/QC) measures that should be taken when conducting these activities are also outlined.

Macroinvertebrate data are used by KDOW to:

1. Assess aquatic life use support as defined by Kentucky Water Quality Standards 401 KAR 10:026 and 10:031
2. Fulfill the requirements of sections 303(d) and 305(b) of the Clean Water Act
3. Determine of the effects of point or nonpoint source pollution on aquatic biota
4. Determine background conditions within particular watershed drainages or ecological regions
5. Maintain the accuracy and precision of Kentucky's Macroinvertebrate Biological Index (MBI)

This manual is considered a dynamic document that is reviewed and updated as new procedures and methods are adopted.

6. Acronyms

CMC- Slide mounting media

COC- Chain of Custody

EtOH- Ethyl Alcohol

KDOW- Kentucky Division of Water

KN- Kicknet

MBI- Macroinvertebrate Bioassessment Index

MH- Multi-habitat

QA- Quality Assurance

QC- Quality Control

RBP – Rapid Bioassessment Protocols

SDS- Safety Data Sheet

WQB – Water Quality Branch

7. Health & Safety Issues

Safety procedures outlined in the Kentucky Safety Manual (KDEP 2013) should be followed by staff employees while working in the laboratories.

When working with chemicals that cause harmful fumes, personnel should use a fume hood to reduce the threat of inhalation exposure to them and their fellow coworkers. When any chemical spill occurs, the first line supervisor will be notified. The first line supervisor will notify the second line supervisor and the division safety officer. The division safety officer will then notify the department safety officer. Do not attempt to clean-up a chemical spill if inhalation exposure or skin, throat, or eye irritation is a threat.

If injury or exposure occurs within the laboratory facilities, then proper first aid attention will be administered by other lab personnel as soon as possible. If the condition is serious, the victim should be transported to a medical facility as soon as possible. For chemical exposures refer to the appropriate Safety Data Sheet (SDS) for first aid treatment. SDS sheets shall be maintained in a readily accessible location in the lab for each chemical stored or used in the lab. If any exposure occurs while in the laboratory, a 1A1 exposure or injury form needs to be submitted to the Division of Workman's Compensation within 24 hours of exposure or injury. Accidents which occur in the laboratory facility will be immediately reported to the acting supervisor or manager.

8. Cautions and Interferences

8A. Cautions

- Samples should be processed according to the method specified in the project study plan for which the sample was collected.
- Care and maintenance of microscopes should be performed on a regular basis by a professional service contractor.

8B. Interferences

- Samples should be stored in a secure location to ensure that they cannot be tampered with.
- All samples should be entered in an appropriate logbook.
- All samples collected by KDOW staff not located in the Frankfort office must be accompanied by Chain of Custody (COC) documentation.
- Samples stored in containers using ethyl alcohol (EtOH) are not always airtight. As a consequence, EtOH may evaporate leading to desiccation of the sample. EtOH levels should be checked periodically to ensure that they are adequate.
- Any equipment/supplies should be free and clear of debris and organisms. Always wash sorting pans, mesh sieves and storage containers prior to processing.

9. Personnel Qualifications/Responsibilities

Processing procedures are performed by personnel trained in this SOP. Macroinvertebrate identifications are performed by personnel with special expertise. Personnel have a basic understanding of laboratory practices and safety. Personnel performing these procedures are responsible for reading and fully understanding the methods and quality assurance procedures presented in this document.

10. Equipment and Supplies

An itemized list of common equipment and supplies typically used to process macroinvertebrates can be found below (Table 1).

Table 1. Recommended KDOW Macroinvertebrate Equipment and Supplies

<u>Microscopes</u> <ul style="list-style-type: none">• Stereomicroscope (dissecting scope) with 10X ocular and appropriate zoom magnification• Standard light source• Compound microscope with 40, 100, and 1000x total magnification and phased contrast	<u>Tools</u> <ul style="list-style-type: none">• Fine-tip forceps• Probes• Counter• Spoons
<u>Sorting and Identifying</u> <ul style="list-style-type: none">• Approved Subsampling Pans<ul style="list-style-type: none">○ 10" x 15" Pyrex pan○ Caton Grid○ 5" x 8" 24 Grid sorting pan○ 10 x 10 cm gridded Plexiglas pan○ Glass quadrant Petri dish• Paper layout with 24 grids• 5" x 8" Sorting Pan• Glass Petri dishes of various sizes• Watch glasses of various sizes• Random numbers table	<u>Preservation & Storage</u> <ul style="list-style-type: none">• 70% EtOH• Glass specimen jars• Glass shell vials• CMC mounting media• Glass slides and cover slips• Cotton
<u>Record Keeping</u> <ul style="list-style-type: none">• Sample log book (Appendix A)• Macroinvertebrate bench sheet (Appendix B)• Chironomid slide bench sheet (Appendix C)	<u>Taxonomic Literature</u> <ul style="list-style-type: none">• See Section 8.0 (Appendix D)

11. Sample Processing Procedures

KDOW utilizes separate field collection methods for low- and high-gradient streams, which are outlined in "Methods for Sampling Benthic Macroinvertebrate Communities in Wadeable Waters" (KDOW 2011). The criteria used for the selection of macroinvertebrate field sampling procedures can be found in Figure 1. To briefly reiterate, in high-gradient streams, collections produce two types of samples; a semi-quantitative (1m² kicknet or KN) sample composed of four 0.25 m² quadrat kicknet samples and a qualitative (multihabitat or MH) sample consisting of macroinvertebrates collected from various habitats present within the stream reach. Low-gradient streams are sampled using a proportional sampling technique which follows the Mid-Atlantic Coastal Plain Streams Workgroup (MACS) protocol (EPA 1997) also detailed in Barbour et al. (1999). Using this methodology, a semi-quantitative collection consists of 20 D- or A- frame net jabs composited into a single container. Laboratory processing methods approved for these field collection procedures were developed from guidance provided by Barbour et al. (1999) and are discussed in detail in the following sections.

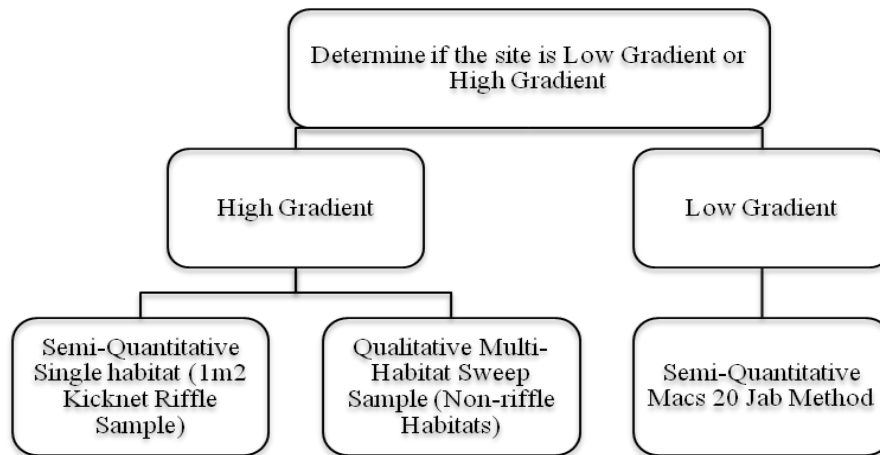


Figure 1. Criteria for Selection of Macroinvertebrate Field Sampling Methods

11A. Sample Log

All samples are logged in the appropriate sample logbook (electronic or hard copy) by the collector upon returning from the field (Appendix A). Information required in the logbook includes DOW Station identification, stream name, county, latitude and longitude, date collected, collectors, type of sample (i.e., quantitative or qualitative), number of samples, and date-to-lab.

11B. Labeling

If a label was not placed in the sample container while in the field, a label is placed on or in the container when the sample is returned to the laboratory. The label should include the station ID, stream name, location, county, date sampled, collection method, and the collectors' initials. (KDOW 2009a)

11C. Preservation

Upon returning to the laboratory, the 90% EtOH added to the sample for preservation should be switched out with 70% EtOH for storage until processing occurs. Precautionary methods must be implemented to reduce the possible loss of organisms during preservative exchange by draining the contents over a U.S. Standard No. 30 sieve (600 µm wire mesh). Organisms captured in the sieve must be returned to the sample container.

11D. Bench Sheet

Prior to processing, a bench sheet must be filled out for the macroinvertebrate sample (Appendix A or located at: \\eas\dfs\EEC-DEP-

DOW\J_Drive\sharedworkingfolders\DOWWORKS\DOWWQB\Data Sheets). The following information should be filled out by staff performing macroinvertebrate processing:

- Sample location information (stream name, location, station id, county, program, latitude, longitude, river basin, drainage area, river mile)
- Collector(s) name and date collected
- Sorters name ("Picked by") and date sorted

- Field Collection and Lab Processing information (field collection methodology, lab processing methodology)
- QA/QC related boxes

Any questions relating to the information required on the bench sheet should be directed to the sample collector(s).

11E. Sample Preparation

Remove the sample from the container using the following steps:

1. Remove the lid and place a #30 (600 μ m) mesh sieve over the container opening
2. Holding firmly, invert and drain any remaining liquid from the container into the sink, keeping the sample in the container.
3. Flip the sieve and container right side up after draining and examine the sieve for any organisms that may have escaped during draining. Return any organisms left in the sieve to the container.
4. Alternatively, the entire sample may be gently rinsed with water in a #30 sieve in order to remove preservative, fine sediment, large organic material, rocks (twigs, whole leaves, etc.). Rinse any material removed from the sample, visually inspect for organisms, and discard.
5. Transfer the sample into an appropriate pan.
6. Use a little bit of water at a time to rinse the container of any remaining sample, dumping it into the pan. Ensure that the entire sample and all organisms are removed from the container and added to the pan.
7. If the sample was stored in more than one container, the contents of all containers for a given sample should be combined at this time.
8. Evenly distribute the sample in the pan. If large clumps of filamentous algae (*Cladophora* sp.) are present in the sample, cut them into small pieces with scissors to allow for easier spreading in the pan.

If the sample is dehydrated, it may be rehydrated using the following method:

1. Place a #30 sieve over the container opening
2. Holding firmly, invert and drain any remaining liquid from the container into the sink, keeping the sample in the container
3. Flip the sieve and container right side up after draining and examine the sieve for any organisms that may have escaped during draining. Return any organisms left in the sieve to the container
4. Refill the container with water and allow the sample to sit for several minutes to rehydrate the organisms.
5. Dump the sample into an appropriate picking pan and ensure all organisms and sample are removed from the container, as in steps 4 and 5 above.

11F. Processing Procedures for Semi-quantitative Samples

KDOW utilizes two processing methodologies: full pick and fixed-count subsampling. Both methodologies may be applied to the 1m² kicknet (KN) and 20 Jab collection methods. In order to determine which processing methodology is appropriate, best professional judgment should be used to estimate how many organisms are in the sample. If there obviously appears to be 300 or

fewer organisms, apply the full pick method. If there appears to be greater than 300 organisms, apply the 300 pick method.

11F.1. Full Pick Processing Method

1. Remove a small amount of sample from the container using a spoon or forceps and place in a sorting pan or Petri dish (Table 1).
2. Add enough water to disperse and thin out the sample.
3. Using a dissecting microscope, carefully scan the sample for organisms.
4. As organisms are found, pick them from the sample and place them in glass shell vials filled with 70% EtOH. Continue picking until all organisms have been removed from the sample.
5. Debris from the sample can be discarded as the sample is picked.
6. When processing is complete, store organisms in a sample container filled with 70% EtOH. Ensure that the container is labeled with all pertinent sample information.

11F.2. Fixed-count Subsampling (300 pick) Method

The fixed-count subsampling processing method was adapted from “Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers” (Barbour et al. 1999). The sample (KN or 20 Jab) should be randomly subsampled to a fixed-count of 300 individual organisms $\pm 20\%$, with an effort made to count a *minimum* of 300 organisms.

Subsampling Pan Selection

Prior to preparing the sample, obtain an appropriate subsampling pan (Table 2). Examples include a 10” X 15” Pyrex baking dish that is placed over a paper grid containing 24 evenly sized grids or a Caton tray (Caton 1991). If a sample does not contain much material, and evenly distributing the sample over a larger dish would be difficult due to lack of material, a 5” x 8” 24 grid sorting pan can be used initially.

Subsampling Procedures

There are special considerations to be aware of before subsampling begins. An organism lying on the line between two grids is considered to be in the grid in which its head lies. In those instances where it may not be possible to determine the location of the head (i.e. worms), the organism is considered to be in the grid containing most of its body. Once picking of a grid begins, all organisms in that grid must be picked (i.e. picking doesn’t stop once the target number is achieved, but until the current grid has no remaining macroinvertebrates). Once a sample has been picked, organisms should be stored in an appropriately labeled container in 70% EtOH. Samples should be picked using a dissecting microscope as a visual aid.

Level 1 Subsampling

A minimum of four randomly chosen grids must be picked from the sample, but it may take >4 grids to achieve the desired number ($300 \pm 20\%$), with an effort made to count a *minimum* of 300 organisms (Barbour and Gerritsen 1996, Barbour et al. 1999).

1. Evenly distribute the sample within a 10” x 15” Pyrex pan or Caton tray. If a 10” x 15” Pyrex pan is being used, place the pan on top of the paper layout with 24 grids, ensuring that the pan is evenly centered on the grid.

2. Based on the number of grids in the pan, generate a random number and pick the corresponding grid in the pan. Place the contents of the grid in a 5" x 8" sorting pan or glass Petri dish.
3. Pick all organisms from this grid and place in glass shell vials filled with 70%EtOH.
4. Using a counter, track the number of organisms as they are picked.
5. IF greater than 70 organisms are picked from the first square, stop picking and follow the procedures outlined in Level 2 Subsampling.
6. IF fewer than 70 organisms are picked from the first square, continue using randomly generated numbers to pick grids until 300 to 360 individuals are picked.

Some samples may contain a very large number of organisms that may result in >360 individuals being picked from four squares. Therefore, the following guidelines can be applied to these samples. If, after a thorough visual inspection, it is apparent that >70 organisms are in one square, randomly choose 4 grids from the pan and place them in a Level 2 5"x8" gridded sorting pan and proceed with Level 2 subsampling.

Level 2 Subsampling (subsampling a subsample)

No minimum number of grids is required when subsampling a subsample, so it is recommended to pick one grid at a time until the desired number ($300 \pm 20\%$) is achieved, with an effort made to count a *minimum* of 300 organisms.

1. Take all organisms picked from the four grids selected above and dump them into the 5" x 8" gridded sorting pan.
2. Randomly select a grid from the Level 2 pan and pick all organisms from this grid and place in glass shell vials filled with 70% EtOH.
3. Using a counter, track the number of organisms as they are picked.
4. Continue randomly picking grids until the desired number ($300 \pm 20\%$) is achieved, with an effort made to count a *minimum* of 300 organisms. No minimum number of grids is required at Level 2 subsampling.

Level 3 Subsampling (subsampling a subsample of a subsample)

In rare circumstances, ≥ 120 organisms may be found in one grid of Level 2 subsampling. In this case, picking 2 grids may result in ~240 individuals. Picking a third grid is required to achieve $300 \pm 20\%$ individuals, but picking the entire grid may result in a subsample of >360 individuals. Follow these procedures if picking an entire next grid will likely result in a subsample of >360 individuals:

1. Remove all material from a Level 2 randomly selected grid and place into a 5" x 8" gridded sorting pan or a 10 x 10 cm Plexiglas gridded pan.
2. Randomly select a grid from the Level 3 pan and pick all organisms from this grid.
3. Using a counter, track the number of organisms as they are picked.
4. Continue randomly picking grids until the desired number ($300 \pm 20\%$) is achieved, with an effort made to count a *minimum* of 300 organisms. No minimum number of grids is required at Level 3 subsampling.

11G. Processing Procedures for Qualitative Samples

The qualitative (MH) sample collected in high gradient streams should be picked such that all unique taxa are removed from the sample. If excessively abundant organisms such as Chironomids are present, an effort should be made to subsample a representative number for identification. Organisms should be preserved in a properly labeled jar containing 70% EtOH until they are identified.

12. Taxonomic Identification Procedures

Identifications are made to the lowest practical level of identification (e.g., family/genus/species) using a dissecting scope for most organisms. Specimen condition (damaged, early instar, poor slide mount) may result in the situation of having to leave identification at more coarse levels (e.g., family instead of genus). Individuals in the Family Chironomidae are mounted on slides in CMC mounting medium and identified using a compound microscope. A list of the taxonomic references currently used by KDOW can be found in Appendix D.

12A. Recording Identifications

1. Record each taxon in the “Final ID” column of the bench sheet.
2. Enumerate individuals from the semi-quantitative sample (KN or 20 jab) in the “Quant” column. A running tally of the number of individuals can be kept in the “Tally” column. Upon completing the sample, the tally can be summed and recorded in the “Quant” column.
3. Mark the presence of taxa from the multihabitat (MH) sample by checking the “Qual” column. Taxa identified in the qualitative sample are not enumerated.
4. Difficulties encountered during identification (e.g., missing gills) are noted in the “Comments/Taxonomy QC” column.
5. If individuals are removed for QA/QC purposes, make note in the “Comments/Taxonomy QC” column.
6. Information on samples completed through the id process will be recorded in the sample log book to track the progress of each sample.
7. Archive samples according to the methods in Section 12D.

12B. Identifying Chironomids

1. Mount larvae on slides using techniques found in Epler (2001).
2. Every slide must be labeled with the following information:
 - Site number
 - Stream name
 - County
 - Sample date
 - Sample method (MH, KN or 20 jab)
 - Collector(s) initials
 - Slide number

3. Record each taxon in the “Final ID” column of the slide bench sheet (Appendix C or located at: \\eas\dfs\EEC-DEP-DOW\J_Drive\sharedworkingfolders\DOWWORKS\DOWWQB\Data Sheets).
4. Record the slide number, cover slip ID and specimen number for each individual identified. See Figure 2 for the schematic to be used to assign specimen numbers to Chironomids.

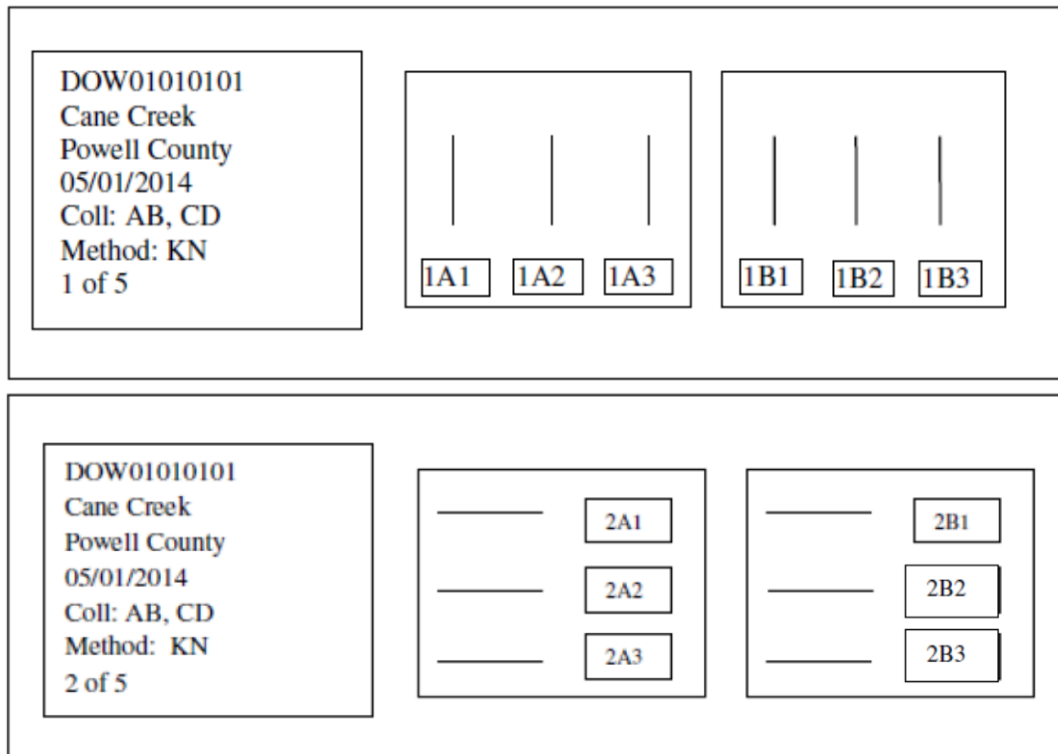


Figure 2. Schematic for Assigning Specimen Numbers to Chironomids

5. Enumerate individuals from the semi-quantitative sample (KN or 20 jab) in the “Quant” column. A running tally of the number of individuals can be kept in the “Tally” column. Upon completing the sample, the tally can be summed and recorded in the “Quant” column.
6. Mark the presence of taxa from the multi-habitat sample by checking the “Qual” column. Taxa identified in the qualitative sample are not enumerated.
7. Difficulties encountered during identification (e.g., poor mount) are noted in the “Comments/Taxonomy QC” column.
8. If slides are removed for QA/QC purposes, make note in the “Comments/Taxonomy QC” column.
9. Attach the slide bench sheet to the sample’s corresponding Macroinvertebrate Bench Sheet. Ensure that these two bench sheets accompany each other during data entry practices. Fill out the “Chironomid Slide Information” on the Macroinvertebrate Bench Sheet.
10. Archive samples according to the methods in Section 12D.

12C. Subsampling Excessively Abundant Individuals for Identification

Excessively abundant taxa (e.g., Chironomidae, Hydropsychidae and Baetidae) may occur in semi-quantitative samples. These taxa may be subsampled in order to reduce the effort and time it would require to identify all individuals. A 10 x 10 cm gridded Plexiglas pan or a glass quadrant Petri dish may be used to obtain a 10%, 25% or 50% subsample. A minimum of 25 individuals should be randomly chosen for identification.

1. Place all individuals from the excessively abundant family in an appropriate subsampling pan/Petri dish.
2. If using a 10X10 cm grid pan, evenly distribute the organisms. Randomly select a grid and pick all organisms from that grid and place in a vial with 70% EtOH. Continue randomly picking grids until a 10%, 25% or 50% subsample is achieved.
3. If using a quadrant Petri dish, evenly distribute the organisms into four quarters. Randomly select one (25%) or two (50%) quadrants and pick all organisms from the quadrant(s) and place in a vial with 70% EtOH.
4. Place remaining individuals, not to be identified, in a separate vial. This vial should be stored with the original sample and should be labeled as “residual”.
5. Fill out the fields in the “Excessively Abundant Taxa Subsampling Information” section of the Macroinvertebrate Bench Sheet.
8. As taxa are identified, record each taxon in the “Final ID” column of the slide bench sheet.
9. A running tally of the number of individuals in each taxon can be kept in the “Tally” column. Upon completing the sample, the tally should be summed, multiplied by the appropriate correction factor based on the percent subsampled, and the total recorded in the “Quant” column. For example, if a 25% subsampling method was used and 4 individuals were identified as Taxa X, a total of 16 would be recorded in the “Quant” column for Taxa X.

12D. Archiving Specimens

All organisms identified from the same sample should be placed in a jar filled with 70% EtOH. The jar should be labeled with the following information: station ID, stream name, location, county, date sampled, sample type (KN, 20 jab, MH), collector(s) initials', and check mark (or some other marking) indicating the sample has been identified. The EtOH levels should be examined regularly and replenished as needed. Slides should be stored in appropriately labeled slide boxes. Samples should be stored in a secure location for a minimum of five years.

13. Data and Records Management

All documents, electronic data, and slides are retained in accordance to project specific Quality Assurance Project Plans (QAPP). Hardcopies of bench sheets, logbooks and COCs must be electronically scanned and stored in appropriate locations on WQB servers.

14. Quality Assurance and Quality Control

14A. Macroinvertebrate Sorting and Subsampling QA/QC

It is recommended that the first two samples processed by any sorter are subject to sorting and subsampling QA/QC to ensure that grids have been picked thoroughly. Sorters are categorized

as either inexperienced or experienced based best professional judgment and the following procedures are followed based on experience category:

Inexperienced Sorters

1. As the sample is picked, the sorter saves all sorted debris residue in a separate container labeled “sorted residue”. The number of organisms picked should be recorded in the “Sorting Pan QA” section of the bench sheet.
2. A second sorter scans the debris for remaining organisms, tallies the number of organisms found and records it on the bench sheet in the “Sorting Pan QA” section of the bench sheet.
3. Sorting efficiency (%) is calculated as follows and recorded on the bench sheet:

$$= \left(\frac{\text{\# organisms picked from sample}}{\text{\# organisms picked from sample} + \text{\# organisms found during check}} \right) \times 100$$

4. If >90% sorting efficiency is achieved for both samples, then every 10th sample will be examined for efficiency.
5. If <90% sorting efficiency occurs, subsequent samples will be checked for efficiency until a sorting efficiency of >90% is achieved.

Experienced Sorters

1. Follow steps 1-3 for inexperienced sorters.
2. If >90% sorting efficiency is achieved on those samples, then no more samples will be checked for efficiency for that year.
3. If <90% sorting efficiency occurs, subsequent samples will be checked for efficiency until a sorting efficiency of >90% is achieved.

All sorting pan QA/QC activities should be noted in the “Sorting Pan QA/QC Related” section of the macroinvertebrate bench sheet.

14B. Macroinvertebrate Taxonomy QA/QC

14B1. Reference Collections

It is recommended that every macroinvertebrate taxonomist maintain a verified reference collection. The concept for the reference collection is that if a taxonomist identifies macroinvertebrate ‘X’ correctly in the reference collection, then the same is true for that organism in all other samples. Specimens that are archived in taxonomists’ collections will be sent to an outside agency for confirmation. Specimens are not considered to be verified reference taxa until a third party taxonomist confirms that the specimen was correctly identified. Reference collections should be developed using the following guidelines:

- Reference specimens are archived in screw-top vials for every taxon identified by a taxonomist.
- The collection is built by cataloguing taxa as they are encountered.

- Reference specimens are labeled with a unique identifier that provides the taxonomist's initials, the year the specimen was collected, and a unique identifier for the taxon (i.e. klm14-001, klm14-002).
- No more than five individuals of the same taxon are placed in the same vial.
- Individuals of the same taxon should originate from the same sample.
- Individuals chosen for reference should be of high quality.

14B2. Sample Re-identification

It is recommended that five percent (5%) of all identified samples be re-identified by a second taxonomist. Samples selected for re-identification are chosen randomly using a random numbers table, or other random selection methodology, and are identified in house, by a second KDOW macroinvertebrate taxonomist. A second macroinvertebrate bench sheet is filled out with all pertinent station information, field collection information, and lab processing information. The "ID QA" section is filled out as well. This second bench sheet is attached and filed with the original bench sheet once data entry has been completed.

Percent Taxonomic Disagreement (PTD) is used to determine the taxonomic precision of KDOW biologists as described in Stribling et al. (2003) where:

$$PTD = \left[1 - \left(\frac{comp_{pos}}{N} \right) \right] \times 100$$

$comp_{pos}$ = number of agreements, and

N = total number of specimens in the larger of the two counts.

The below figure from Stribling et al. (2003) demonstrates how to determine counts of agreement, which is utilized in the PTD calculation.

TABLE 3. Example comparisons of re-identification results by 2 taxonomists showing counts of agreements. Target taxonomic level is based on program specifications.

Target taxonomic level	Identification	Taxonomist		No. agreements
		1	2	
Genus	Baetidae		1	0
	<i>Procladius</i> / <i>Centroptilum</i>	1		
Genus	<i>Argia</i>	1	2	1
	Coenagrionidae	1		
Genus	<i>Bratislavia</i>		2	2
	<i>Bratislavia unidentata</i>	2		
Genus	<i>Ceratopsyche morosa</i>	12		12
	<i>Ceratopsyche bronta</i>		12	
Genus	<i>Phya</i>		4	0
	Physidae	4		
Genus	<i>Dugesia tigrina</i>	1	25	1
	<i>Cura foremanii</i>	25		
Genus	<i>Glyptotendipes</i>	58	32	32
	<i>Polypedilum halterale</i>		9	
Species	<i>Polypedilum obtusum</i>	9		0
Genus	<i>Hexatoma</i>	4	4	4

A PTD value $\leq 10\%$ is the target criterion. If a sample comparison fails to meet the target criterion, taxonomic discrepancies must be reconciled by the biologists, and if necessary, a third macroinvertebrate taxonomist is consulted to settle unresolved identifications. All ID QA/QC activities are noted on KDOW Benthic Macroinvertebrate Laboratory Bench Sheet.

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Appendix A. Macroinvertebrate Sample Logbook

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Appendix B. Benthic Macroinvertebrate Laboratory Bench Sheet

KDOW Benthic Macroinvertebrate Laboratory Bench Sheet										
Stream Name:				Location:						
Station ID:				County:			Program:			
Lat:		Long:		River Basin:			Drainage Area:		River Mile:	
Pan Scan? <input type="checkbox"/> Y <input type="checkbox"/> N		ID QA? <input type="checkbox"/> Y <input type="checkbox"/> N		Field Dup? <input type="checkbox"/> Y <input type="checkbox"/> N			Data Entry QA? <input type="checkbox"/> Y <input type="checkbox"/> N			
Collected by:		Date:		Picked by:		Date:		ID'd by:		
Field Collection, Lab Processing and MBI Template Information (check one option under each heading)										
Field Collection Methodology			Lab Processing Methodology			Excessively Abundant Taxa Subsampling Information				
<input type="checkbox"/> Macs 20 Jab <input type="checkbox"/> 1m ² Riffle Kick + Multihabitat <input type="checkbox"/> Other: _____			<input type="checkbox"/> Full Pick (%100) <input type="checkbox"/> Fixed Count Number of Organisms <input type="checkbox"/> 300 ± 20% (Actual #: _____) # of Squares Picked to achieve 300 _____ of 24 4 of 24, then _____ of 24 <input type="checkbox"/> Other: _____			Original # Picked: _____ Family: _____ % Subsample <input type="checkbox"/> 10% <input type="checkbox"/> 25% <input type="checkbox"/> 50% <input type="checkbox"/> Other: _____ Final # Identified: _____				
MBI Template To Be Used						Chronomid Slide Information				
<input type="checkbox"/> Headwater <input type="checkbox"/> Wadeable <input type="checkbox"/> Full Pick <input type="checkbox"/> 300 Pick <input type="checkbox"/> Other: _____						# Quant Slides: _____ #Qual Slides: _____				
Comments:										
Data Entry Information					MBI Scoring Information					
Data Entered into Database by:				Date:		Bioregion			MBI Score:	
MBI Score Entered into Database by:				Date:		<input type="checkbox"/> Mountain <input type="checkbox"/> Bluegrass <input type="checkbox"/> Pennyroyal <input type="checkbox"/> MVIR			Narrative Score:	
QA/QC Related										
Sorting Pan QA	Name:		Date:		% sorting efficiency:		> 90% efficiency passes: <input type="checkbox"/> Yes <input type="checkbox"/> No		< 90% efficiency fails, action taken:	
ID QA	Name:		Date:		PTD:		> 90% similarity passes: <input type="checkbox"/> Yes <input type="checkbox"/> No		< 90% similarity fails, action taken:	
Data Entry QA	Name:		Date:		Error Rate:		> 95% error rate passes: <input type="checkbox"/> Yes <input type="checkbox"/> No		< 95% error rate fails, action taken:	
Notes / Comments:										

[illegible]

Kentucky Division of Water Bench Sheet - Slides

[illegible]

Appendix D. Taxonomic References

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