Standard Operating Procedures:
Laboratory Bioassessment Sample Processing and Identifications

Fill out log sheet records of samples processed (including initials of sorter/IDer and checker). Each individual should process and ID all replicates from a single sample reach site.

Minimum count of 250 organisms. Complete counts of any subsamples taken. [average counts will be in the 300-500 range]

Sample Splitting – Split sample to get into target range of organism count (250) but make no more than 3 splits ($1/8^{th}$ the sample) on the first 2 samples, other samples may use more splits. Use Folsom plankton splitter (rotating drum) and record number of splits on post-it labels in pencil for sorted samples (post-it placed on sorting tray lids), and on invertebrate ID record sheets when doing identifications and counts. This is essential to making density calculations from samples. Use a random number chart to select the sample split to be sorted, and save unprocessed splits until sorting is complete. One of the unprocessed fractions from the 5 samples taken at a site is saved as a sample voucher (this may be stored in the field sample container). Adjust sample splits after initial sample to be in the range of 300-500 counted. Be sure splitter is centered/balanced and inspect splits to verify that splits were equally divided.

Sample Sorting:
Each subsample may be sorted initially by eye or with the aid of an Optivisor or swing-arm lens. Search may then continue or even begin with microscope scan of samples (10-15X). Small portions of subsamples will be placed into gridded trays for picking out invertebrates. Only nematodes should not be picked - all others including mites and ostracods should be removed. Look-alike taxa or broad taxonomic groupings should be placed into separate cells of sorting trays for subsequent identification. One cell of each tray should be reserved for midges and mites (placed together for later IDs by Herbst). The cells of this tray should be about half-full with 80% ethanol/ 5% glycerol mix. Very small or early instars of abundant insects may not be removed completely but as many as practical should be sorted. Following sorting of the subsample, the unprocessed portion of each full sample should be scanned for large and rare taxa not encountered in the sorted subsample. A representative specimen of each large/rare (if present) should be put into the sample sorting tray. For one of five sample replicates sorted (20%), a verification check by another lab worker is required (w/inititals) to ensure that a sample has been completely picked. This should be done by pooling of the contents of a sample as it is picked, then having a co-worker examine this with an optivisor or under a 10-15X microscope. Records of incomplete sorts (number missed) should be kept in the log records. This cross-check should be done on the first of each of the five replicates taken for a stream site (this will establish a search image for the remainder of the set of samples). One of the 5 unprocessed sample fractions is to be saved as a sample voucher (record the fraction remaining).

If the sample is to be stored in the refrigerator, place a zip-lock plastic bag over the tray and tray lid (w/field bottle label and # of splits on post-it note) placed over the bag. All 5 samples from a site should be stacked so they can be IDed together without having to search for each. All placed inside a storage tub.

Sample Identification:
Sorted specimens will be IDed to the lowest taxonomic level possible given the availability of keys. Each identification will have a taxonomic certainty rating attached to it for use in evaluating problems with taxonomy (see taxa record sheets). Also record life stage(s) and any notes on ID traits or specimen condition. Uncertain identifications should be checked with other technicians and the reference collection. **Each** IDed replicate is stored in a 20 mL vial, then reviewed for QC verification of identifications with Herbst (technician checking off each ID sheet). The conservative guidance for IDs is not to push rare organisms beyond a verifiable identity (or exclude/pool). Uncertain taxa and 10% of all samples will be sent to taxonomic authorities and external labs for final verification and used as part of an archived reference collection. Representative identified specimens are archived in a reference collection. After data compilation and verifications, all replicates from a single site will eventually be pooled and permanently archived (80% EtOH-5% glycerol). All midges/mites from each replicate should be placed in a separate small glass vials, labeled with stream/site/date/ #splits, and stored in a rack for further ID. Ostracods and oligochaetes are not IDed further; copepods are recorded when present but not counted.