

Exhibit A - Statement of Work Mosquito Taxonomic Identification

1.0 Background

The National Ecological Observatory Network (NEON) project is funded by the National Science Foundation (NSF) and managed by Battelle Ecology, Inc. (BEI). The mission of the NEON project is to design, implement, and operate continental scale research infrastructure to open new horizons in ecological science and education, and to enable ecological analyses and forecasts for the benefit of society. A major part of the NEON project's measurement system is the organism sampling to measure the impacts of ecological changes. Additional Information on the project mission can be found at <http://www.neonscience.org/>.

This statement of work (SOW) addresses BEI's need for contracting services for mosquito taxonomic identification for species collected as part of mosquito abundance and diversity sampling for a subset of NEON sites. Mosquitoes are collected from up to 47 sites from 20 NEON domains across the continental United States, Alaska, Puerto Rico, and Hawaii. Collections occur in 'bouts' of sampling (approx. 40 hours of continuous sampling) conducted biweekly or monthly, depending on the location, throughout the season in which mosquitoes are flying. Accurate and consistent identification of specimens from NEON sites is critical to NEON's mission of measuring changes in abundance, diversity, and phenology of species across time.

Acronyms and Definitions

| Acronym or Term | Definition |
|-----------------|---|
| BEI | Battelle Ecology, Inc. |
| g | grams |
| NEON | National Ecological Observatory Network |
| NSF | National Science Foundation |
| PDE | percent difference in enumeration |
| PTD | percent taxonomic disagreement |
| QA | quality assurance |
| SOW | statement of work |

2.0 Required Services

This section describes the services to be performed by Contractor. Contractor's price for the proposed effort must include the cost of instruments, equipment (e.g., balances, chill tables, -80°C freezers), space, storage units, supplies or consumable materials (e.g., all vials, labels¹, cardstock, point punches, etc. - including any materials non-standard to Contractor and needed for NEON samples) and fees required to sort, store, pack, and ship samples as described in this SOW. Contractor should also include in their price the cost of participating in

¹ Labels suited for -80°C storage such as Cryo-babies (<http://www.e-lspi.com/cryo-babies-and-cryo-tags-on-rolls>), Tough Tags (<http://www.e-lspi.com/tough-tags-for-thermal-transfer-printer-ther-1128>), or equivalent are required.

quality assurance audits as described in Attachment 1, preparing and returning data in NEON datasheets (Attachment 2), and attending any needed meetings or teleconferences as noted in Section 3.

All work is to be conducted at Contractor’s facilities. Any work to be subcontracted must be approved by BEI, and subcontractor is subject to the same quality requirements and schedule as Contractor.

The range in expected total number of samples for each required service is listed in Exhibit B. The general workflow for identification and pooling services is indicated in Figure 1 and described in Sections 2.1 and 2.3. Preparation of voucher specimens is described in Section 2.2.

2.1 Mosquito Taxonomic Identification

Contractor is required to identify and group specimens from bouts at NEON sites to species and sex. Specifically:

- Mosquito specimens collected by BEI have been transferred from each trap into one or more vials (typically 15mL or 50mL tubes, BD Falcon or similar). Each sample (vial or Ziploc of multiple vials) is labeled with a sampleID in the following format: plotID.collectDate.collectTime (e.g., OSBS_001.20160601.0725; see Figure 2) and/or a sample barcode. Samples have been frozen and stored in a -80°C freezer prior to shipment to Contractor.
- Vials of mosquitoes will be shipped to Contractor on dry ice with a hard-copy shipping manifest. Prior to delivery, Contractor will also receive an electronic copy of the shipping manifest with tracking information, which will include sampleID and/or sample barcode information for each sample, and a receipt form. Upon receipt of each package, Contractor will:
 - Open the container and ensure all samples listed on the manifest have been received and are in good condition (e.g., not broken or missing; labels are legible);
 - Record date received and sample condition on the electronic receipt form provided by BEI. Contractor will return this electronic receipt form provided by BEI to confirm receipt of the package and all samples (see Figure 3) to a website specified by BEI. Contractor must notify the BEI Technical Representative of any problems with the shipment within 48 hours of receipt;
 - While in Contractor’s custody, samples must be stored according to the conditions in Table 1.

Table 1. Storage Conditions and Hold Times for Mosquito Taxonomic Identification

| Grouping | Analysis | Hold Time | Storage Conditions |
|-----------------|--------------------------|---|---------------------------|
| Mosquitoes | Taxonomic identification | No known hold time applies. Identifications to be conducted within the timeframe listed in Exhibit B. | -80°C |

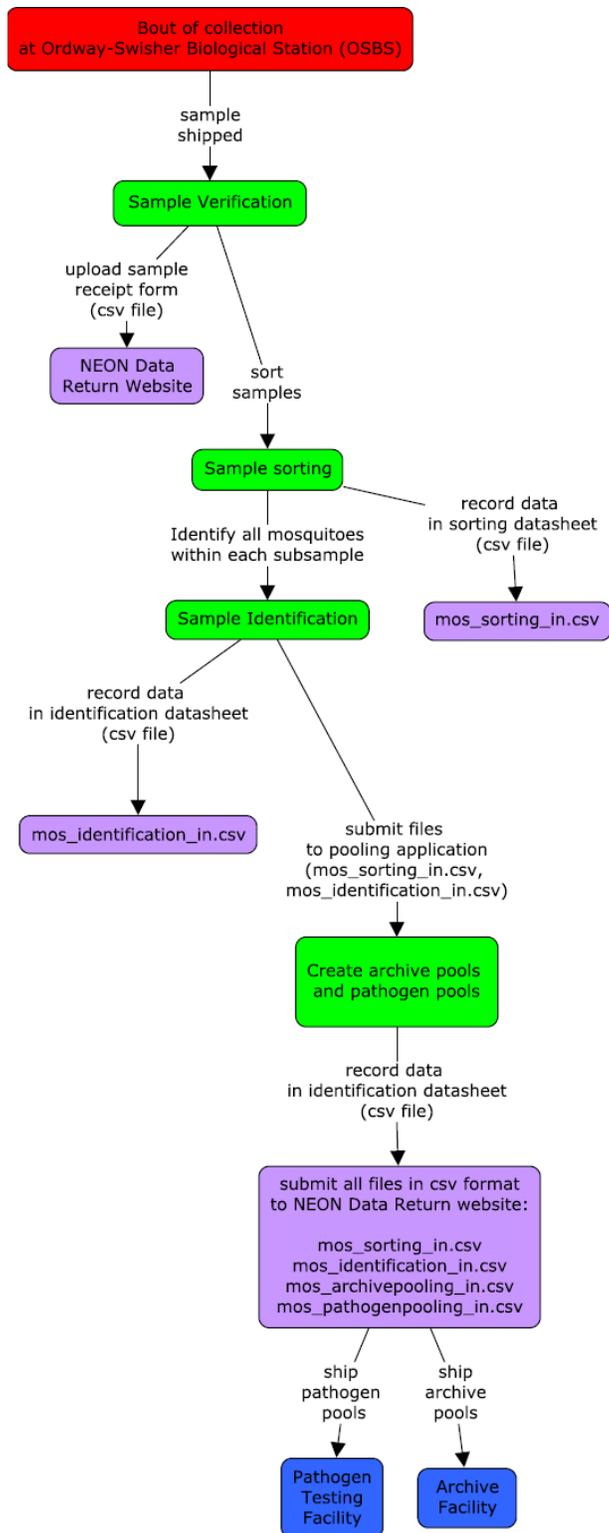


Figure 1. Summary of the mosquito processing workflow. Red boxes are performed by BEI and green boxes are performed by Contractor. Purple boxes highlight instances of data return.

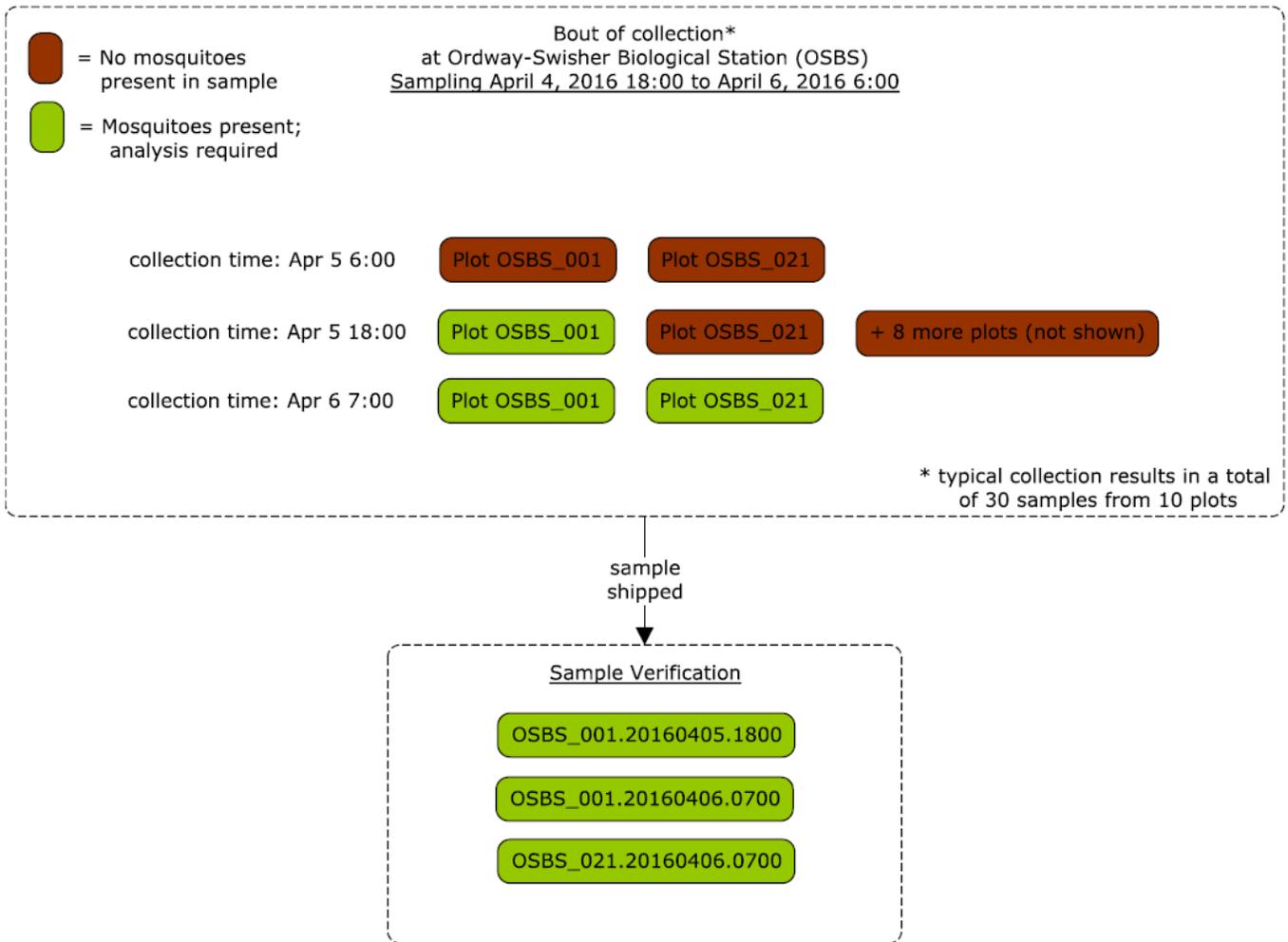


Figure 2. Mosquito collection and labeling. Each sampleID label contains a plotID, the collection date (YYYYMMDD) and the collection time (HHMM).

- During processing, mosquitoes shall be handled on a chill table and not allowed to thaw in order to preserve samples for subsequent pathogen testing. If a chill table is not available, Contractor’s proposal must describe alternatives. BEI must approve any alternatives to a chill table.
- Consolidate all the mosquitoes from one trap into a single batch of mosquitoes and weigh and record the weight of the total contents of this trap in grams (g) prior to sorting mosquitoes from the trap (enter as ‘totalWeight’ on the sorting datasheet; see Figure 3). BEI requires that scales have a precision of one milligram.
- Visually assess if a sample likely contains either 1) 200 or fewer mosquitoes or 2) greater than 200 mosquitoes.
 - If the complete sample is estimated to contain 200 or fewer mosquitoes, then the subsample will comprise the entire contents of the trap. Record the weight of this

subsample in grams (enter as 'subsampleWeight' on the sorting datasheet; see Figure 3). All Culicidae will be identified from the sample and the weight of any unidentified non-Culidae bycatch will be recorded as the 'bycatchWeight'.

- If the complete sample is estimated to contain greater than 200 mosquitoes, a randomly selected proportion of 200 specimens and any bycatch (non-mosquitoes) associated with those 200 will be removed and weighed on the digital scale to determine subsample weight (a typical subsample of ~200 specimens will weigh approximately 500 mg, but this weight may vary with species composition and sample condition). This value will be recorded in the field 'subsampleWeight'. All Culicidae will be identified from the subsample. The all unidentified non-mosquito bycatch from the subsample will be removed and weighed on the digital scale to determine sample bycatch weight (recorded in the field called 'bycatchWeight'). Any remaining sample which is not part of the subsample shall be returned to their original vial(s) and stored at -80°C until BEI has received and approved identification data and provided information on disposition of samples.
- Subsample Identification: Sort the subsample into mosquitoes and non-mosquito (by-catch) organisms. All mosquitoes will be counted and the number of mosquitoes of each sex and species will be recorded in the identification datasheet (described below). Weigh and record the weight of the by-catch organisms for this subsample (enter as 'bycatchWeight' on the sorting datasheet; see Figure 3). The bycatch associated with the subsample may then be discarded.
- Contractor will record the number of individual mosquitoes (enter as 'individualCount' on the identification datasheet; see Table 2) of each species (enter as 'scientificName' on the identification datasheet; see Table 2) and sex (enter as 'sex' on the identification datasheet; see Table 2) present in each subsample. Afterward, Contractor will sort mosquitoes from each subsample into separate containers or vials, grouping mosquitos by species and sex (the subsample from one trap may generate multiple vials). These temporary vials or containers may be any size or shape, but mosquitoes are to be maintained in groups of the same species, sex and bout (collection window). Each grouped vial shall be labeled with the scientific name, sex and bout to which the mosquitoes pertain and stored in -80C freezer until pooling instructions are available.

Table 2. Selected Columns from the Identification Datasheet

| subsampleID | individualCount | scientificName | sex |
|-----------------------------|-----------------|------------------------|-----|
| OSBS_001.20160405.1800.S.01 | 81 | Aedes aegypti | F |
| OSBS_001.20160405.1800.S.01 | 5 | Aedes vexans | F |
| OSBS_001.20160406.0700.S.01 | 10 | Aedes aegypti | F |
| OSBS_001.20160406.0700.S.01 | 102 | Aedes vexans | F |
| OSBS_021.20160406.0700.S.01 | 1 | Aedes aegypti | F |
| OSBS_021.20160406.0700.S.01 | 18 | Uranotaenia sapphirina | F |
| OSBS_021.20160406.0700.S.01 | 4 | Aedes vexans | M |

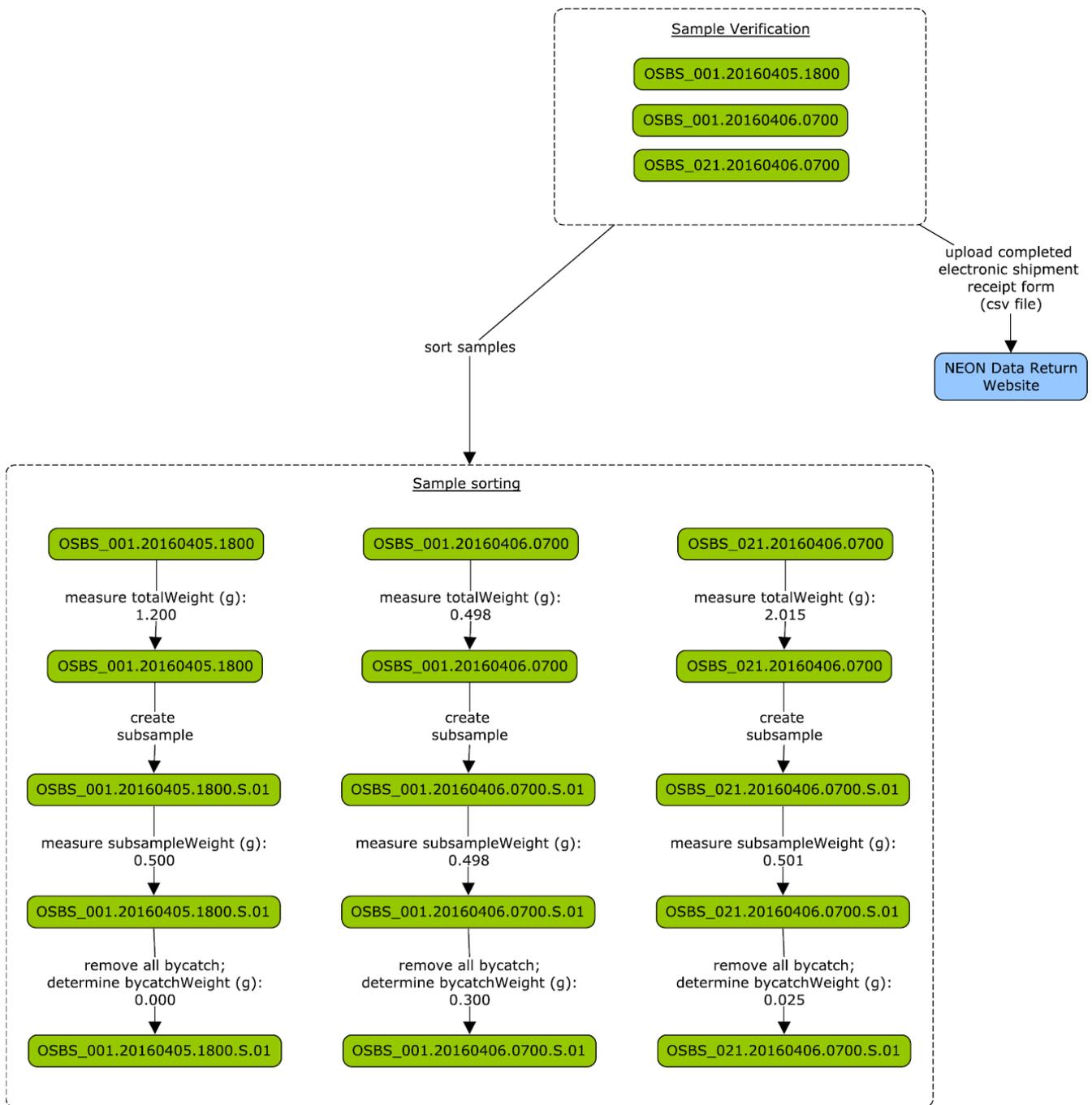


Figure 3. Upon sample receipt, Contractor must first verify the condition and presence of samples, then process samples as shown in this diagram.

In the example illustrated in Figure 4, Contractor sorts 3 subsamples containing mosquitoes of the composition noted in Table 2. Contractor would be required to place these mosquitoes in 4 separate containers or vials, segregated by species and sex. Vials would then be labeled with all relevant sample information as noted above. Other mosquitoes from different subsamples from the same bout, species and sex could be consolidated into those same 4 vials (i.e., other female *Aedes aegypti* from OSBS within the

same bout from different subsamples could be consolidated.)

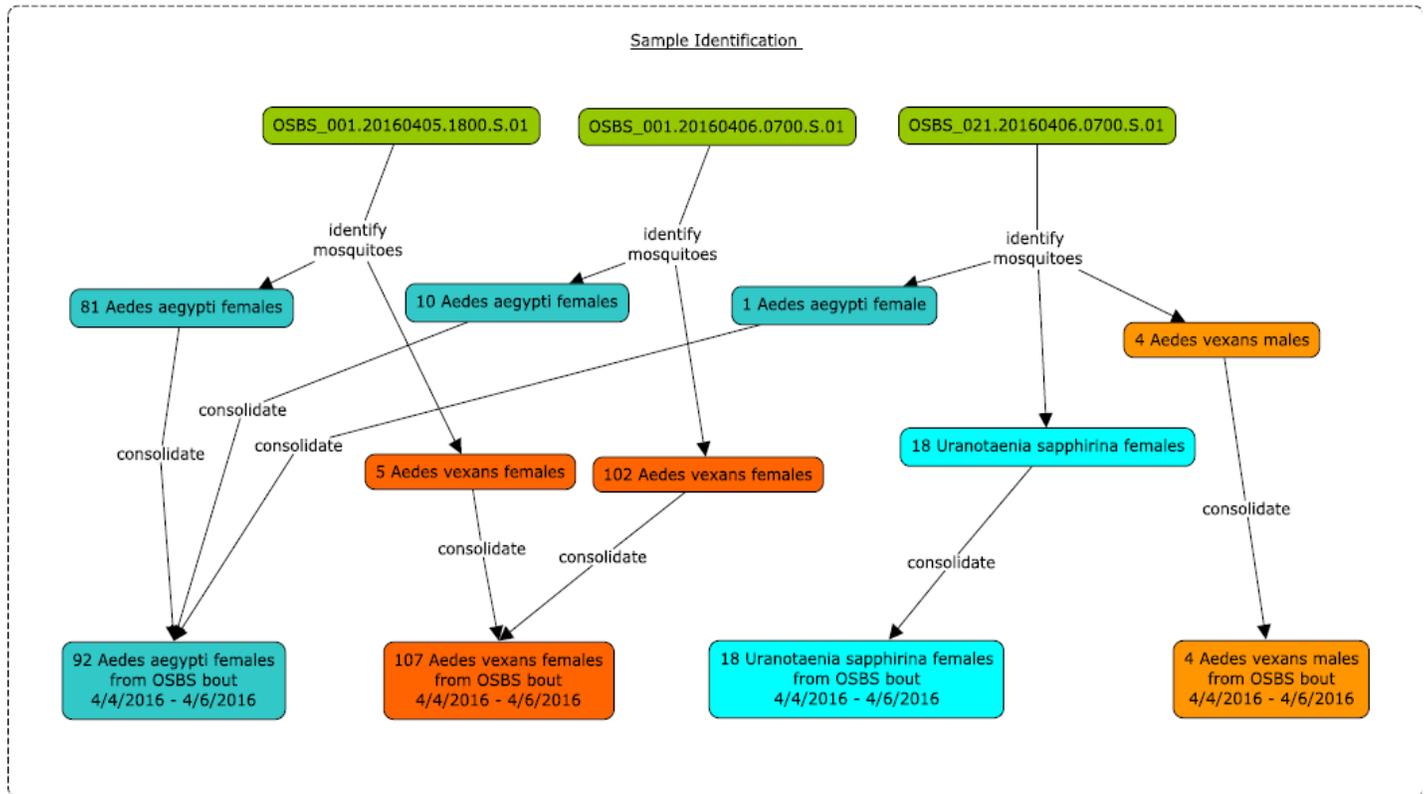


Figure 4. Contractor will identify all mosquitoes from each subsample, recording the number of each species and sex present in each subsample. Afterward, Contractor may store individuals of the same species and sex from the same bout together.

- Provide quality assurance (QA) checks as described in Table 3. QA data shall be returned to BEI along with standard datasheets.

Equation 1, Percent Difference in Enumeration (PDE):

$$PDE = \left(\frac{|Count_1 - Count_2|}{|Count_1 + Count_2|} \right) \times 100$$

Equation 2, Percent Taxonomic Disagreement (PTD):

$$PTD = \left(1 - \frac{agreements}{N} \right) \times 100$$

Where:

agreements is the number of consistent identifications (species and sex match; if species and sex are identical, but identification qualifier differs, that is still considered an agreement)

N is the total number of individuals in the larger of the two counts

Table 3. QA Requirements for Mosquito Taxonomic Identification

| QA Check | Frequency | Acceptance Criteria | Corrective Action | Reporting Requirements |
|--|--|--|--|--|
| Verify that samples are accurately enumerated. | Rolling, at a minimum 3% of subsamples (approx. 1 subsample per bout). | Contractor will count and identify all mosquitoes within a subsample, reporting the data on the identification spreadsheet. That subsample will be reprocessed (enumerated and identified a second time). The percent difference in enumeration (PDE; see Equation 1) will be calculated. PDE must not exceed 5% (0.05). | If PDE>0.05, use the results of the second count in the identification datasheet and explain the discrepancy in the “remarks” column in addition to the requisite language described in the reporting requirements. | Reconcile count data in final identification datasheet. Report QC results as a numeric value (no percent sign) in the column ‘PDE’ in the sorting datasheet. In the sorting datasheet “remarks” indicate that QC was performed. Use format “QC check; PDE XX; Genus level PTD YY; Species level PTD YY” where XX is the calculated PDE value for the subsample (should match the numerical value given in the ‘PDE’ column). |
| Verify that subsamples have accurate identifications | Rolling, at a minimum 3% of subsamples (approx. 1 subsample per bout). | Using the same sample for the PDE analysis, Contractor will calculate the percent taxonomic disagreement (PTD; see Equation 2). Genus level disagreement must not exceed 2% (0.02), while species level disagreement must not exceed 5% (0.05). | If PTD values are > 0.02 or 0.05, as applicable, use the second identification to update the scientificName and sex in the identification datasheet and explain the discrepancy in the “remarks” column in addition to the requisite language described in the reporting requirements. Acceptable explanations may include any information that affected the analysis (i.e., specimen integrity, etc.) | Reconcile taxonomic data in final identification datasheet. Report QC results as a numeric value (no percent sign) in the columns ‘genusPTD’ (genus-level PTD value) and ‘speciesPTD’ (species-level PTD value) in the sorting datasheet. In the sorting datasheet “remarks” indicate that QC was performed. Use format “QC check; PDE XX; Genus level PTD YY; Species level PTD YY” where YY is the calculated PTD value at each resolution (should match the numerical values given in the ‘genusPTD’ and ‘speciesPTD’ columns). |

- Contractor is required to report the primary identification reference used to make the taxonomic determination (enter as ‘identificationReferences’ on the identification datasheet). The following taxonomic references are recommended to conduct identifications:
 - a. *Darsie Jr., R. F., and R. A. Ward. 2005. Identification and geographical distribution of the mosquitoes of North America, North of Mexico. University Press of Florida, Gainesville.*
 - b. *Darsie, R. F., and R. A. Ward. 1981. Identification and geographical distribution of the mosquitoes of North America, North of Mexico. Mosquito Sytematics Supplement 1: 1-313.*

- c. *Stanuszek, W.W. 2013. A dichotomous key to commonly encountered mosquitoes in Saginaw County, Michigan.*
- d. *Tulloch, G. S. 1937. The Mosquitoes of Puerto Rico. Journal of Agriculture of the University of Puerto Rico 21:137-167.*
- e. *Harrison, B. A., B.D. Byrd, C.B. Sither, and P.B. Witt. 2016. The mosquitoes of the mid-Atlantic region: an identification guide. Western Carolina University. Cullowhee, North Carolina, USA.*
- f. *Andreadis, T. G., M. C. Thomas, and J. J. Shepard. 2005. Identification guide to the mosquitoes of Connecticut. Connecticut Agricultural Experiment Station. 966.*

When referring to these references, shortened strings may be used for convenience and error reduction associated with long names. Each of these shortened references corresponds to the reference above with the same letter.

- a. *Darsie and Ward 2005*
- b. *Darsie and Ward 1981*
- c. *Stanuszek 2013*
- d. *Tulloch 1937*
- e. *Harrison et al 2016*
- f. *Andreadis et al 2005*

If any other reference is used, that reference must be approved by BEI and added to the list of accepted references. The NEON database will reject references that are not within the pre-approved list.

- In any case where species-level determination is not possible, identify to the lowest possible taxonomic level using visual examination of external morphology. Identification qualifiers may be also used to indicate uncertainty about the identification in the provided fields (i.e., an identification of *Aedes cf. dorsalis* would be entered as: scientificName = 'Aedes dorsalis', identificationQualifier = 'cf. species').
- Contractor must return sample data using the sampleID provided. Data are to be returned on the NEON datasheet (Attachment 2) according to the Required Turnaround Time in Exhibit B. The NEON database links data based on sampleID information and as a result it is imperative that this sampleID be correct. Contractor may not invoice for identifications performed on samples for which datasheets have been returned with inaccurate sampleID information.
- Verify certain results if BEI's post-analytical Quality Assurance Procedure, performed within 180 calendar days of BEI's receipt of data from Contractor, suggests the need for verification of certain specimens.
- For Domains that request shipping materials be returned, return all BEI supplied sample shipping materials (e.g. coolers) within 15 calendar days of receiving sample shipment. Domains will provide a return shipping label.

2.2 Mosquito Voucher Specimens and Preparation of Samples for DNA Barcoding

Contractor will select voucher specimens to point/pin. Contractor may receive instructions from BEI to remove leg from certain specimens after BEI reviews the identification data submitted by Contractor. For each specimen specified by BEI, Contractor will remove one leg from the voucher. This leg will be designated for barcoding. Contractor will send pinned/pointed specimens to a designated archive facility and the pulled legs to a barcoding facility as described below.

- Pull voucher specimens to pin/point and label appropriately during the sort of mosquitoes from a trap by species and sex. These specimens are not subject to cold chain requirements but can be kept cold during the process if it fits Contractor's workflow. Up to 10 specimens of a species/sex combination will be pinned/pointed from each domain over the course of a sampling season. Voucher specimens should be in very good condition and selected across sampling bouts and sites for each domain from which mosquitoes are obtained. Contractor shall attach locality, determination and individualID labels to each specimen.

| Locality label | Determination label | individualID |
|--|---|---------------------|
| USA, MASSACHUSETTS Worcester Co. Harvard Forest. 256m N42.456233 W72.22545 CO2 trap. 13Jun2014. CRitz. NEON HARV_077.20140613.0740 | <i>Culiseta melanura</i> det. M.J. Weissmann 2014 | MOS.D01.001010 |

Locality labels will include the country, state, county, locality name, spatial information about the sample (elevation, lat/lon), method of collection, collection data, collector name, 'NEON' and the sampleID. Determination labels will contain species information, identifier and year of identification. IndividualID is unique over the lifetime of the observatory and includes the information about the specimen type (i.e., 'MOS'), the domain (i.e., 'D01') and a unique six-digit number. BEI will provide a list of previously used individualIDs at the beginning of each field season.

BEI will provide an application that automatically creates locality, determination, and individualID labels from the identification data generated by Contractor. Contractor may then apply these labels to the specimen in lieu of generating them independently.

- Voucher specimens should be stored as noted in Table 4.
- Based on the individualID information reported in the identification returned by Contractor, the BEI Technical Representative will notify Contractor at the end of the sampling season (typically November) which specimens require leg removal for DNA barcoding and Contractor will remove one leg for each specimen selected for DNA barcoding and place the leg into a designated container (i.e., a 96-well plate) to be shipped within 48 hours of removal from the vouchered specimens to a DNA barcoding facility chosen by BEI. Barcoding samples should be stored as noted in Table 4. Barcoding samples can be shipped at room temperature, but must be shipped to a barcoding facility within 48 hours of removal

from the vouchered specimens. Up to 95 legs per domain will be pulled for DNA. Each leg should be stored individually within one well of a 96-well plate. BEI will provide a datasheet for the contractor to indicate which specimens had a leg pulled and to which well it pertains.

- Once samples have been sent for DNA barcoding, the pinned/point specimens will be shipped at room temperature to an archive facility chosen by BEI, within the timeframe included in Exhibit B.

Table 4. Storage Conditions and Hold Times for Mosquito Pinned Voucher Specimens and DNA Barcoding Samples

| Grouping | Analysis | Hold Time | Storage Conditions |
|------------|--------------------------|--|---|
| Mosquitoes | Pinned Voucher Specimens | No known hold time applies. Samples are to be stored by Contractor until directed by BEI to ship to archive facility. | Room temperature in Schmidt boxes (or equivalent) with adequate preservation measures (e.g., moth crystals) |
| | DNA Barcoding Samples | No known hold time applies. Legs are not to be removed by Contractor until directed by BEI. Once legs are removed, samples should be shipped to designated barcoding facility within 48 hours. | Legs can be stored at room temperature, dry in 96-well plate until shipped to barcoding facility. |

- Contractor will create a shipping manifest that lists all samples included in the shipment. Contractor will provide the archive and/or DNA barcoding facility a hard-copy of the shipping manifest with the shipment and electronic copy of the shipping manifest when the specimens are sent. Contractor will also provide an electronic version of the shipping manifest to BEI in a .csv file.

2.3 Prepare Pooled Mosquitos for Shipment to Pathogen Testing or Permanent Archive Facility

Contractor shall prepare pooled samples of mosquitoes that have completed the taxonomic identification process as follows:

- Contractor will have recorded sorting information (i.e., weight) and taxonomic analysis (i.e., identifications and counts) as described in Section 2.1 above. All processed mosquitoes will have been stored in a -80°C freezer in groups segregated by species/sex/bout until pooling instructions are given. BEI will decide based on the data provided from Section 2.1 whether pooled mosquitoes will be pathogen tested or sent to an archive facility and provide that information to Contractor. Pooling instructions will be available once all samples have been identified.
- After all samples are identified, Contractor will upload the sorting datasheet and identification datasheet (as csv files) to a web-application² provided by BEI. Contractor must upload *all* data pertaining to each bout (do not upload partial bouts). Uploads of partial bouts (e.g., only half of bout 7 from OSBS

²This web-application is in development, but expected to be online for this service during the contract period of performance. Until the web application is online, samples will be uploaded to a BOX cloud-based data repository folder dedicated to the Contractor. More detailed instructions will be provided upon contract award.

is returned, with many records missing) will result in erroneous instructions. Contractor will upload data for all sites in one sorting data file and one identification data file.

- The web application will check the datasheet for common errors (other errors may be present); if errors are detected, Contractor must remedy those errors and re-upload the document. Once the web-application has an error-free copy of the sorting and identification datasheets, it will produce 4 files which Contractor will download. The data will be returned as 4 csv files with UTF-8 encoding; this is the format required by the NEON database. These files are: sorting, identification, pathogen pooling, and archive pooling datasheets.
- Based on the instructions in the pooling datasheets, Contractor will create pools of the size specified in the datasheet for each species/sex combination from all traps in a bout at a site (so by species/sex/bout/site) and place each pool in a new, labeled vial (or multiple vials depending on the number of mosquitoes in a pool; each vial will have a unique label) according to the instructions in the pooling datasheets (see Figure 5). If Contractor must deviate from the instructions for any reason, then Contractor will contact the BEI Technical Representative for further instruction. Pools shall be stored as indicated in Table 5. Final specimen vials and labels must be suited for cryo-storage (safe to -80°C). The following vial sizes should be used when storing various quantities of mosquitoes:
 - 2 mL snap cap centrifuge tubes for the pathogen testing and for archival of species with only one or two specimens per bout.
 - 2 mL cryovials for species with 3-50 specimens per bout
 - 5 mL cryovials for species with 51-200 specimens per bout
 - 15 mL cryovials for 201-500 specimens per bout

The pathogen testing facility will homogenize mosquitoes; therefore, it is unnecessary and not desirable for any homogenizing mechanism (i.e., ceramic beads) to be included in the vials. Archive pools must be stored in -80C freezer safe cryovials, but because the number of individuals to be included in an archive pool can vary (range anywhere from 1 – approx. 1500 individuals per vial) cryovials of size 2mL, 5mL or 15mL are appropriate for the storage of these samples.

Table 5. Storage Conditions and Hold Times for Mosquito Pools

| Grouping | Analysis | Hold Time | Storage Conditions |
|-----------------|------------------|---|---------------------------|
| Mosquitoes | Pooled Specimens | No known hold time applies. Samples are to be stored by Contractor until directed by BEI to ship to pathogen testing or archive facility. | -80°C |

- After the pooling datasheet instructions are implemented, Contractor will upload all 4 datasheets into the NEON database (see comment in footnote 2).
- Upon direction from the BEI Technical Representative, Contractor will pack pooled, identified mosquitoes with sufficient dry ice to keep samples frozen and ship them to a pathogen testing or archive facility as specified by the BEI Technical Representative according to the schedule in Exhibit B.

3.0 Other Requirements

3.1 Quality Assurance/Quality Control

In addition to QA/QC requirements described in each work package in Section 2.0. Contractor and any subcontractor(s) shall be subject to quality audits as described in Attachment 1.

3.2 Required Meetings, Travel, Telecon and other Events

Contractor shall participate in the following, if requested:

- An initial kick-off teleconference;
- Quarterly teleconferences until the work is completed and accepted by BEI. More frequent teleconferences (e.g., monthly) may be requested by BEI during the work period;
- Annual training teleconference on quality assurance requirements of the NEON program;
- Training on data return requirements for the NEON program by teleconference;
- A debrief teleconference at the conclusion of the Agreement.

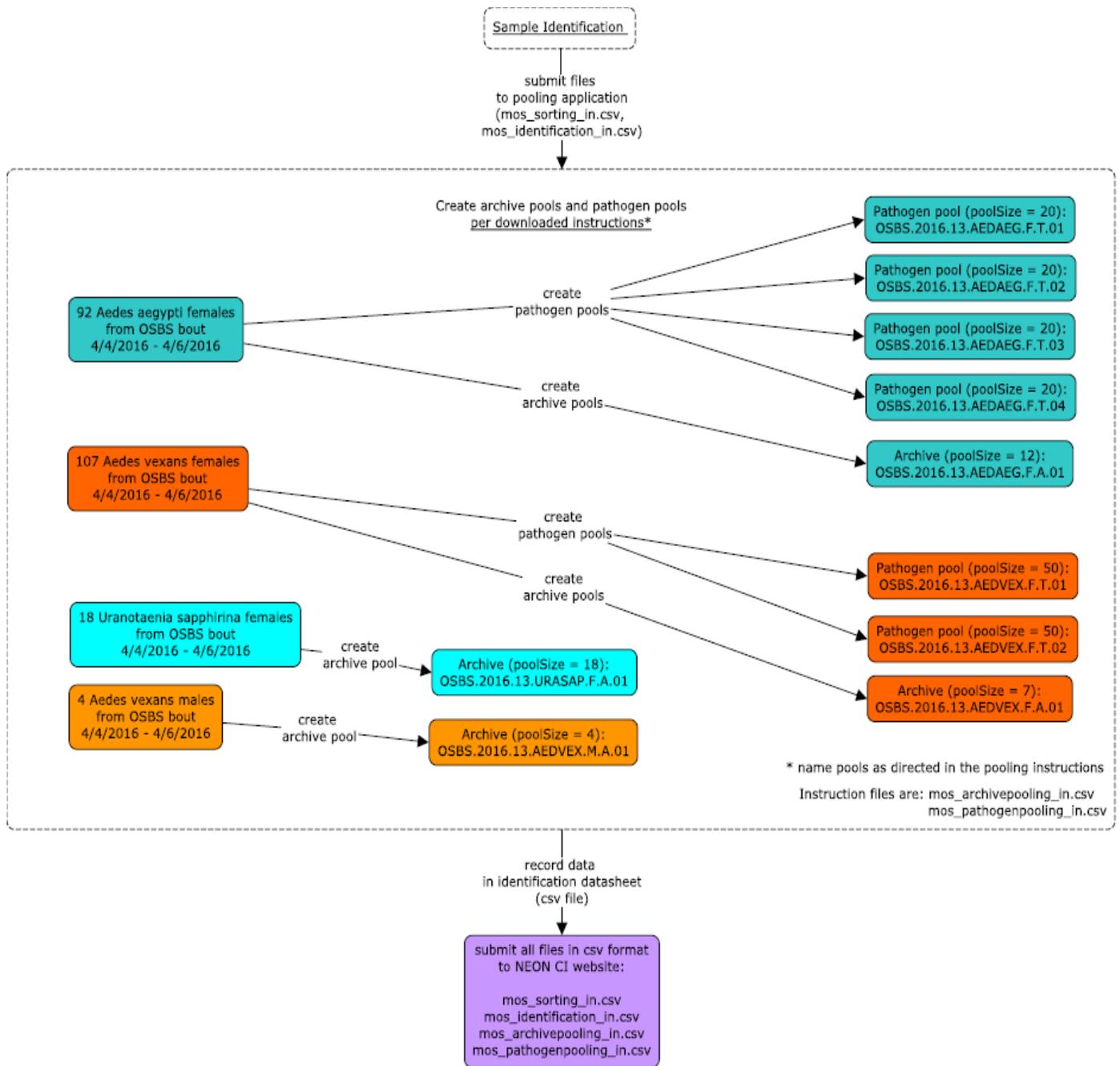


Figure 5. Contractor will submit sorting and identification data to receive pooling instructions. Contractor will implement the instructions and submit all 4 files to the NEON project web-based file upload site or to BOX while the web-based site is in development.