Updated: 8 October 2020

Vector Disease Control International Denver Surveillance Laboratory
Standard Operating Procedures
[NEON Sample Processing Version, 2020]

Introduction:

The Vector Disease Control International (VDCI) Denver Surveillance Laboratory is currently located at 7000 Broadway, Suite 108, Denver, Colorado 80221 USA (N39°49'30.50", W104°59'6.75"). Formerly known as Colorado Mosquito Control, we have been at this location since June 2014. The main laboratory is approximately 325 sq. ft. and includes eight microscope stations (see below under "Equipment"). The staff is supervised by Michael "Doc" Weissmann, Ph.D., Chief Entomologist for VDCI (see below under "VDCI Surveillance Laboratory Personnel"). The VDCI Surveillance Laboratory has the capability to do taxonomic identification for many groups of insects but specializes in mosquitoes (Culicidae) from North America north of Mexico. The VDCI Denver Laboratory works cooperatively with other mosquito surveillance laboratories around the country associated with VDCI, as well as with museum collections in Colorado and elsewhere in the USA.

All identification records are maintained a minimum of 7 years, primarily electronically but in some cases in hard copy printed format as well. All NEON electronic data files are backed up bi-monthly on a hard drive that is kept off-site, and all data files are uploaded on a monthly basis into the NEON data system. Access to all VDCI computers and the NEON files and databases contained within are password protected.

Vector Disease Control International, initially as Colorado Mosquito Control, began working with the National Ecological Observatory Network (NEON) during the 2009 summer season, assisting with trapping and mosquito identifications at sixteen trap sites in Colorado (four each at the CU Mountain Research Station, Fraser Experimental Forest, Central Plains Experimental Range, and Sterling Agricultural Experiment Station). VDCI is currently involved with NEON performing identification services for adult mosquito samples collected in 2020 from various sites throughout the United States.

This Standard Operating Procedures (SOP) document is revisited annually, with the date of the current revision listed on the upper left corner of the first page. See Appendix A for images/figures listed in the SOP text.

Equipment:

The Vector Disease Control International Denver Surveillance Laboratory is located at the Denver VDCI office. The following equipment is available for use by technicians in the laboratory. Those items marked with * are used exclusively in processing and storing samples for NEON:

1) Microscopes: Eight microscope stations are dedicated to mosquito identification, two of which are regularly used for processing NEON samples. These two are Omano zoom scopes (7.5x – 35x, with capability to double the magnification to 15x – 70x with higher-powered eyepieces). Each is illuminated with internal 25wt. lighting, and supplemental external lighting if necessary. Additionally, a National Digital Microscope is available

with a camera that can be used for taking photographs of specimens, or projecting onto a computer screen for identification training purposes. The microscopes are serviced and cleaned every other year by Spectrum Microscope Services in Denver, Colorado, and are certified by their technician with a sticker stating the date of last servicing.

2) * Chill Tables: Three chill tables are available for use when identifying NEON samples to keep specimens from decomposing while being processed (see below under "Sample Processing Procedure"). These are manufactured by BioQuip Products, Rancho Dominguez, CA (http://www.bioquip.com/search/DispProduct.asp?pid=1431). These were purchased in March 2014 and will be serviced (or replaced) by the manufacturer as needed.
3) * Digital Scale: A U.S. Solid USS-DBS3-2 Model Electric Balance 200/0.001g (https://ussolid.com/) is used for determining total sample, subsample, and bycatch weights (see below under "Sample Processing Procedure: Sample Identification"). The scale is calibrated (sensitivity calibration) at least once every month and/or every time it is moved, using a NIST Certified 100g calibration weight certified by Rice Lake (www.grainger.com catalog #15F141). The calibration weight will be sent to Rice Lake Weighing Systems for re-certification if it is dropped, or every 5 years, whichever comes first (last certification dated 13 November 2018).
4) * CMOS optical imaging 2D barcode scanner (Model MJ-4000)
5) * 2 Fisher Scientific Isotemp ULT Chest Freezers (Catalog No. 1913CA; Model No. 5153) [see Figure 1]: These freezers include 12.7 cubic feet of storage space each and are always set at -80ºC when samples are stored within. The freezers include a battery backup device that comes on automatically during a loss of regular power in the building. They also include an alarm system that activates if the temperature falls out of range or the power goes out. Regular preventative maintenance (air filter cleaning, condenser cleaning, lid gasket cleaning) is conducted by our staff according to directions in the Instruction Manual in Section 5, page 5-5, and recorded on the maintenance log [see Figure 2 form maintenance log example]. The freezers are defrosted completely whenever it is completely emptied (projected to occur in between each trapping season) according to directions in the Instruction Manual. One freezer was purchased in February 2014, and the second freezer was purchased in August 2016.
6) * BradyPrinter i5100 300dpi Industrial Label Printer: The printer is used annually at the end of each season to produce vial cryolabels for archive specimens deposited at the liquid nitrogen bioarchives at Arizona State University. The printer undergoes cleaning and maintenance each year prior to use according to the instructions on page 28 of the printer User Manual.
7) * BioQuip 6-drawer Cornell-System Insect Specimen Cabinet for temporary storage of labelled voucher specimens prior to sending them to the Arizona State University museum collections for permanent storage.
8) Library: The VDCI Surveillance Laboratory has an extensive library that includes several mosquito identification references for use as needed. Mosquito identification references include the following:
    - Darsie, R. F., and R. A. Ward. 2005. Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico. University Press of Florida. 383 pp. [This is the primary reference for all regions of North America]

- Wood, D.M., P.T. Dang, and R.A. Ellis. 1979. The Mosquitoes of Canada (Diptera: Culicidae). Part 6 of The Insects and Arachnids of Canada. Agriculture Canada Publication 1686. 390 pp. [A helpful reference, especially for the northern states and Alaska, and for determining high-elevation black-legged *Aedes* spp.]
- Burkett-Cadena, N.D. 2013. Mosquitoes of the Southeastern United States. The University of Alabama Press, Tuscaloosa. 188pp. [one of the newest and best illustrated guides, very helpful for all domains in the SE USA, especially those from Florida (D03)]
- Nielsen, L.T., R.J. Brand, and G.C. Collett. 2002. An Identification Guide to the Mosquitoes of Utah (revision). The Utah Mosquito Abatement Association. 97pp. [A useful guide for species in the Rocky Mountain regions]
- Rose, D.A., B.C. Kondratieff, and M.J. Weissmann. 2017. Insects of Western North America. 9. Colorado Mosquitoes (Diptera: Culicidae). Contributions of the C.P. Gillette Museum of Arthropod Diversity, Colorado State University. 194pp. [A useful guide for species in the Rocky Mountain regions]
- Harrison, B.A., B.D. Byrd, C.B. Sither, and P.B. Whitt. 2016. The Mosquitoes of the Mid-Atlantic Region: An Identification Guide. Western Carolina University, Cullowhee, NC. 201pp. [A guide to the species found in the Eastern USA]
- In addition to these, there are several other books and pdf references specific to various regions and states that are consulted as needed for certain domains and/or species groups. Many additional literature references, original species identifications, and illustrations are available through the Walter Reed Biosystematics Unit/Smithsonian Institution website for Vector Identification Resources (http://wrbu.si.edu/) and the Mosquito Taxonomic Inventory website (http://mosquito-taxonomic-inventory.info/).

9) Museum Collections: VDCI Surveillance Laboratory staff has access to the major museum insect collections in Colorado (CP Gillette Museum at Colorado State University in Fort Collins, the University of Colorado Museum of Natural History collection in Boulder, and the Denver Museum of Nature and Science collection in Denver). VDCI Chief Entomologist Dr. Michael Weissmann is affiliated with each collection in an associate capacity (see below under "VDCI Surveillance Laboratory Personnel"). The CSU collection includes extensive samples from across the country and various CDC research sites around the world. A small voucher collection is maintained at the VDCI office as well for staff use as needed.

<u>VDCI Surveillance Laboratory Personnel</u>:

1) Michael J. Weissmann, Ph.D., Chief Entomologist – Dr. Weissmann has been with Colorado Mosquito Control from April 2003 until January 2017 (seasonally in 2003-2013, year-round beginning in January 2014), after which time CMC rebranded and Dr. Weissmann became an employee of VDCI. He has been involved with mosquito identification since 1986 while serving as Assistant Curator at the University of Colorado Museum, assisting with identifications for the Boulder County Health Department. He has a B.A and M.A. in Biology from the University of Colorado in Boulder, and a Ph.D. in Entomology from Colorado State University in Ft. Collins. Complete Curriculum Vitae available upon request.

2) Kelsey L. Renfro, B.S., Laboratory Manager – Ms. Renfro has been with CMC/VDCI since May 2015, originally as a Field Technician but switching over to the Surveillance department part way through that season. She stayed on part-time after the regular season to assist with NEON identifications in the "off-season." After receiving her B.S. in Biology from Metropolitan State University of Denver in December 2016, Ms. Renfro began working full time in the VDCI Surveillance Laboratory, focusing primarily on NEON identifications and data management. During the hurricane emergency recovery efforts in September 2017, she was deployed to Texas and Florida to assist with various VDCI post-hurricane mosquito surveillance and control contract as well as in 2018 to North Carolina. Ms. Renfro is a Certified Mosquito Identification Specialist, receiving extensive training in mosquito identification through the Advanced Mosquito Identification and Certification Workshop at the University of Florida's Medical Entomological Laboratory, March 2019.
3) Anna E. Wanek, B.S., VDCI Denver Surveillance Manager – Ms. Wanek started at VDCI in summer 2018 as a seasonal Surveillance Technician, setting traps and identifying mosquitoes for regional control contracts. In Spring 2020, she was promoted to the year-round position of Surveillance Manager, supervising the seasonal surveillance trapping team in the VDCI Denver Surveillance Laboratory. During the "off-season" she is being trained to assist with NEON identifications and data management, as well as assisting with pathogen test vialing and archive vialing for NEON samples. Ms. Wanek received her B.S. in Biology from Metropolitan State University of Denver in May 2018.
4) Seasonal Surveillance Technicians – VDCI employs several seasonal technicians to work in the Surveillance Laboratory, setting traps and identifying mosquitoes. Most work only with Colorado specimens, but if needed some are trained to identify other species from outside of Colorado and assist with pathogen test and archive vialing of VDCI samples. All seasonal technicians are fully trained in mosquito taxonomic identification by Dr. Weissmann and Ms. Renfro, and regularly checked for accuracy (see below under "Technician Training Protocol").
5) Vector Disease Control International (VDCI) has laboratories nationwide that can, when necessary, assist with all areas of mosquito control and surveillance services. Scientific services at VDCI are directly supervised by Dr. Dan Markowski, Ph.D., Entomologist. Dr. Markowski is available to assist VDCI personnel with mosquito identification questions when needed and makes approximately quarterly visits to Colorado to visit the VDCI Denver office and laboratory.

Technician Training Protocol:

Although most NEON samples are identified by the primary VDCI Denver Surveillance Laboratory full-time staff members (see above under "VDCI Surveillance Laboratory Personnel"), occasionally seasonal Surveillance Technicians employed at VDCI will be used for processing some of the NEON samples as well. All technicians must have a minimum of at least one month prior experience identifying routine VDCI trap samples prior to being trained to process NEON samples.

1) When a technician first starts working on NEON material, they are already familiar with use of dichotomous keys and with the basic mosquito anatomy that is used in those keys, usually from previous work in the VDCI lab with routine Colorado surveillance trapping.
2) All new technicians must spend a minimum 20 hours per NEON domain location under direct supervision by either Dr. Weissmann or Ms. Renfro. If the technician is to be trained to identify species from a different domain, they must undergo an additional 20 hours minimum of supervised identification training. Dates/hours of training and name of supervisor are recorded in the training log, maintained on file at VDCI.
3) All new technicians are required to read this SOP, as well as associated referenced NEON protocol instructions on file, prior to identifying any samples.
4) Prior to processing samples, new technicians are shown literature images, descriptions, and when available actual specimens of the most commonly encountered species from the domain to be identified, and key features used to distinguish each species are reviewed. The technicians are instructed as to which VDCI library references are most appropriate to the domain samples being processed.
5) During the first day of training, the technician is shown how to subsample using the sampling protocol (described below under "Sample Processing Procedure: Sample Identification").
6) Initially all specimens identified by a new technician are verified by the supervisor/trainer prior to being pooled with the other identified specimens from that bout, or to being processed as voucher specimens (as described below under "Sample Processing Procedure: Sample Identification").
7) After the initial 20-hour training period, competency is evaluated and proficiency determined by random verification by the supervisor, the dates of which are added to the training log.
8) At all times, technicians are required to have verified by a supervisor any specimen that is of uncertain identification or that is unfamiliar to the technician (for example, rare/uncommon species).

Sample Processing Procedure:

The following sample processing procedures are specific to dead mosquito samples received from NEON domain centers, and do not necessarily apply to other mosquito samples processed at the VDCI Surveillance Laboratory for other clients or for internal surveillance projects.

Sample Receipt:

1) Regular samples are received at the VDCI main office in Denver via overnight courier on an occasional basis, either directly from various NEON domain offices around the country, from NEON contractors, or from the NEON national office in Boulder. Each sample received is accompanied either physically or electronically by a sample inventory spreadsheet.
2) Immediately upon receipt, the sample box is opened and checked for the presence of residual dry ice and cold to verify that the specimens were kept frozen during transit. When requested, an acknowledgement email is sent to the NEON field office to confirm receipt of the package and condition of the samples.

3) The vials are checked against the inventory manifest to make sure that all samples were received and that the sample numbers match the manifest. If it does not match, the NEON office contact is immediately notified, and any discrepancies are resolved prior to sample identification. A receipt spreadsheet is uploaded to the NEON portal certifying that the samples were received.
4) When samples from multiple bouts and locations are received at the same time, each bout is processed separately, and all other bouts are kept frozen until inventoried.
5) When a trap sample contains multiple vials, they are placed together and numbered (ex: 1 of 5, 2 of 5, 3 of 5, etc.) to ensure that all vials for a specific trap are processed together.
6) Vials are stored by bout and domain in the freezer at -80ºC until processed for identification.

Sample Identification:

1) Samples are removed from the -80ºC freezer one bout at a time and kept in the surveillance laboratory freezer (-20ºC) during identification and processing. Samples stored in multiple vials are removed together and checked to make sure all vials are included for that trap/night sample.
2) If the sample is estimated to contain less than 200 mosquitoes, all are placed in a weigh boat on the digital scale to obtain the sample weight [see Figure 3]. The bycatch is removed and weighed on the digital scale to determine sample bycatch weight, and all weights are recorded on the hard-copy internal data sheet [see Appendix B for a sample internal data sheet]. If there are too few mosquitoes to register a weight, the weight is listed in the data sheet as "< 0.02g." The scale is cleared of debris and tared between each use. The sample is then placed into a petri dish for identification under the microscope.
3) If the sample is estimated to contain more than 200 mosquitoes, all specimens from each vial in the sample are placed together in a weigh boat on the digital scale to obtain the total sample weight [see Figure 3]. A randomly selected portion of 200 specimens and any bycatch (non-mosquitoes) associated with those 200 is removed and weighed on the digital scale to determine subsample weight, then placed in a petri dish for identification. The bycatch is removed and weighed on the digital scale to determine sample bycatch weight, and all weights are recorded on the hard-copy internal data sheet [see Appendix B for a sample internal data sheet]. The scale is cleared of debris and tared between each use.
4) Samples up to 200 are identified under the microscope and placed in petri dishes on the chill table as they are identified, in separate places for each species.
5) Identification data is recorded on the hard-copy internal data sheets. These data are retained on file at the VDCI office for at least 7 years.
6) Voucher specimens (up to 10 specimens in good condition per species/sex for each location) are removed from the sample during identification. These are pointed on pins and labeled using current NEON mosquito pointing and labeling protocols (a hard copy of which in printed form for the most recent version is kept on file in the in-house "NEON Instructions – Voucher" file folder). The voucher specimens are stored at room temperature in Schmidt boxes provided by NEON or temporarily in insect drawers and housed temporarily in the VDCI Insect Specimen Cabinet. Specimens removed for vouchering are recorded on the NEON database spreadsheet.

7) The remaining unidentified proportion in excess of the first 200 is discarded unless additional specimens are requested by NEON, or additional good quality specimens are retained for the in-house training collection.
8) For quality control purposes, every 20th sample is identified a second time by a different technician than the one that originally identified the sample. After the first technician completes the identifications for the sample, the mosquitoes are combined again, and re-identified. If there are any discrepancies between the first technician's results and the seconds, these are recorded and noted in the data. Samples that are double-processed in this manner for quality control have the sample number highlighted on the hard-copy internal data sheets. If there is a discrepancy in number of mosquitoes in the sample, the difference in quantity is divided by the total count by the first technician and reported as a decimal in the "PDE" column of the sorting datasheet (= Percent Difference in Enumeration). If there is a discrepancy in identification at the species level, the number of contested specimens is divided by the total count by the first technician and reported as a decimal in the "Species-level PTD" column of the sorting datasheet (= Percent Taxonomic Difference). In the unlikely event that the discrepancy in identification is at the genus level, this would be recorded as a decimal in the "Genus-level PTD" column of the sorting datasheet.
9) When identification is complete for the sample, each species is placed within labeled petri dishes, pooled into one dish per species/sex for each bout, and returned to the -80 freezer for storage [see Figure 4].
10) When the entire bout is completed, the petri dishes for each species are stored in plastic shoe boxes by domain and bout in the designated area of the -80 freezer to await instructions for pathogen testing and archive sample vialing.
11) Information from the hard-copy internal data sheets is recorded into the electronic NEON database, which creates sorting and identification reports in UTF-8 encoded comma-delimited csv files for uploading into the NEON data portal monthly. Data entry follows protocol supplied by NEON, including specifications for valid formats for each data category. A confirmation email is sent to NEON at the end of each month with a list of bouts processed during that month.

Sample Distribution:

1) Email instructions from NEON determine which samples are to be vialed for pathogen testing and which are to be vialed for archive storage, including the number of mosquitoes per vial and the destination of each pooled sample.
2) Samples for pathogen testing are removed from the -80 freezer and counted into 2ml snap-cap cryovials per emailed NEON instructions. Vials are labeled by hand using a permanent marker (cryopen) or with a label taped to the vial. These are placed into cryoboxes on the chill table, and as each box is filled it is returned to the -80 freezer until the shipment date. Pathogen testing vials are shipped to the testing lab(s) via FedEx Standard Overnight in insulated boxes and accompanied by at least 10 lbs. of dry ice to maintain cold during shipment. A spreadsheet of the inventory of samples shipped is sent electronically via email to NEON and to the pathogen testing laboratory, as well as a hard copy accompanying the actual shipment.
3) Samples for archive storage are removed from the -80 freezer and counted into various sized cryovials depending on the number of mosquitoes in the pooled sample and the size

of the specimens, per specific written or emailed instructions from NEON (which are specific to each particular archive shipment). Each vial has a cryolabel affixed to it with a distinct sample number provided in the NEON instructions. Vials are placed into cryoboxes on the chill table, and as each box is filled it is placed back into the -80 freezer until the shipment date. Archive storage vials are shipped to the storage NEON biorepository museum via FedEx Standard Overnight in insulated boxes and accompanied by at least 10 lbs. of dry ice to maintain cold during shipment. A spreadsheet of the inventory of samples shipped is sent electronically via email to NEON and to the archive storage facility, as well as a hard copy accompanying the actual shipment.

Contact Information:

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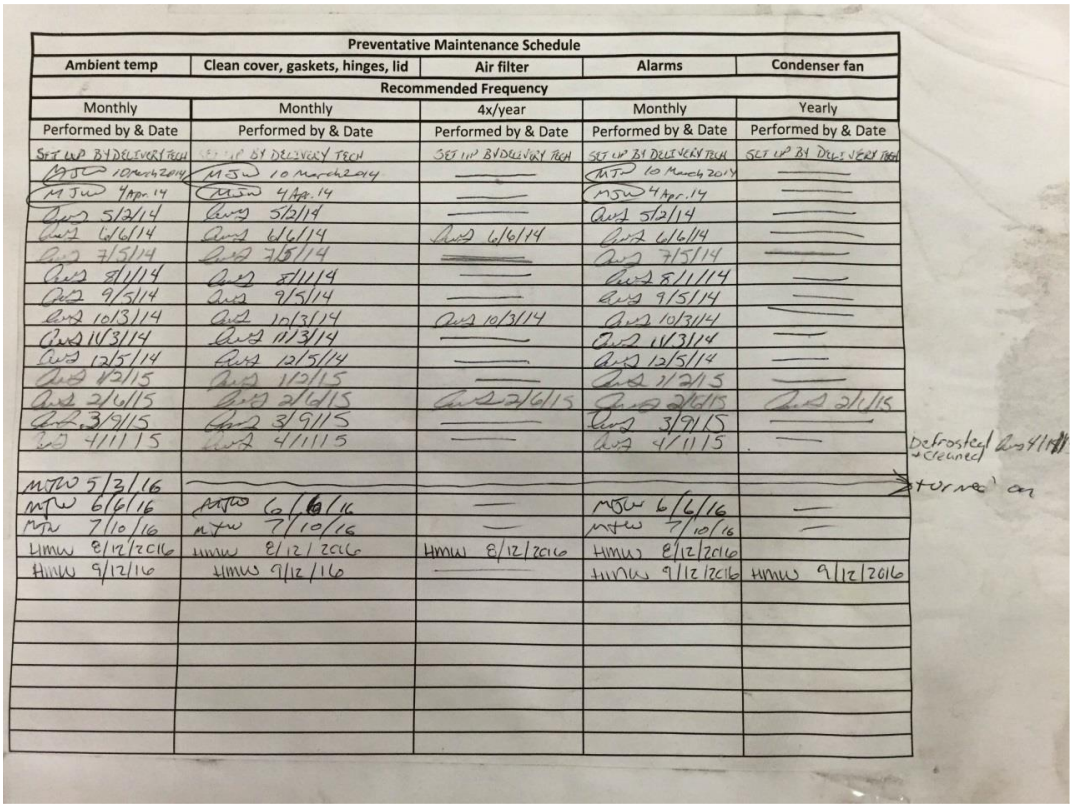
Website: www.vdci.net

APPENDIX A: Images

Figure 1: Freezer for NEON samples only (-80ºC).



Preventative Maintenance Schedule				
Ambient temp	Clean cover, gaskets, hinges, lid	Air filter	Alarms	Condenser fan
Recommended Frequency				
Monthly	Monthly	4x/year	Monthly	Yearly
Performed by & Date	Performed by & Date	Performed by & Date	Performed by & Date	Performed by & Date
SET UP BY DELIVERY TECH	SET UP BY DELIVERY TECH	SET UP BY DELIVERY TECH	SET UP BY DELIVERY TECH	SET UP BY DELIVERY TECH
MJW 10 March 2014	MJW 10 March 2014		MJW 10 March 2014	
MJW 4 Apr. 14	MJW 4 Apr. 14		MJW 4 Apr. 14	
Aws 5/2/14	Aws 5/2/14		Aws 5/2/14	
Aws 6/6/14	Aws 6/6/14	Aws 6/6/14	Aws 6/6/14	
Aws 7/5/14	Aws 7/5/14		Aws 7/5/14	
Aws 8/1/14	Aws 8/1/14		Aws 8/1/14	
Aws 9/5/14	Aws 9/5/14		Aws 9/5/14	
Aws 10/3/14	Aws 10/3/14	Aws 10/3/14	Aws 10/3/14	
Aws 11/3/14	Aws 11/3/14		Aws 11/3/14	
Aws 12/5/14	Aws 12/5/14		Aws 12/5/14	
Aws 1/2/15	Aws 1/2/15		Aws 1/2/15	
Aws 2/6/15	Aws 2/6/15	Aws 2/6/15	Aws 2/6/15	Aws 2/6/15
Aws 3/9/15	Aws 3/9/15		Aws 3/9/15	
Aws 4/1/15	Aws 4/1/15		Aws 4/1/15	Defrosted & cleaned Aws 4/1/15
MJW 5/3/16				turned on
MJW 6/6/16	MJW 6/6/16		MJW 6/6/16	
MJW 7/10/16	MJW 7/10/16		MJW 7/10/16	
HMW 8/12/2016	HMW 8/12/2016	HMW 8/12/2016	HMW 8/12/2016	
HMW 9/12/16	HMW 9/12/16		HMW 9/12/2016	HMW 9/12/2016

Figure 2: Freezer monthly preventative maintenance log.

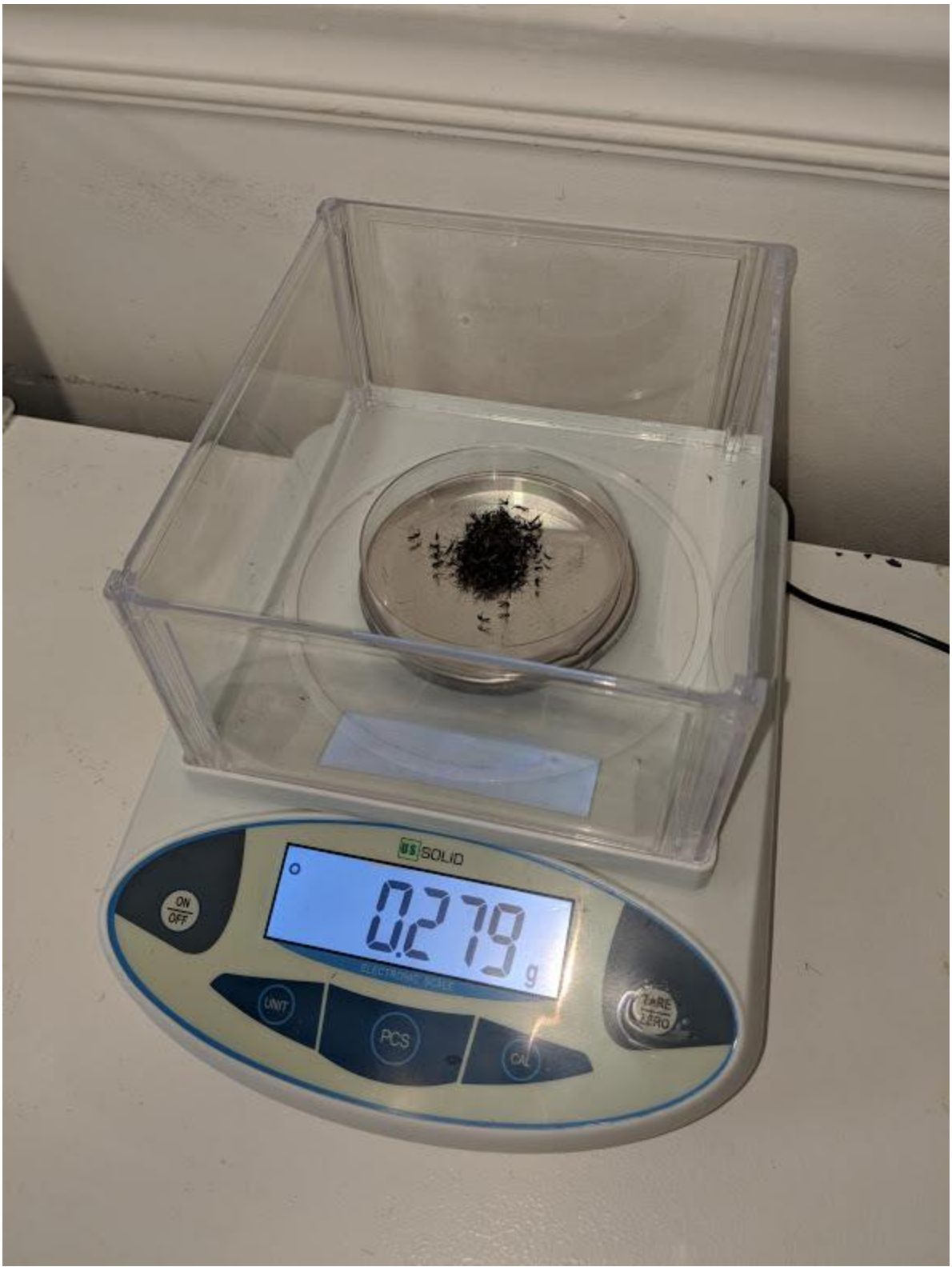
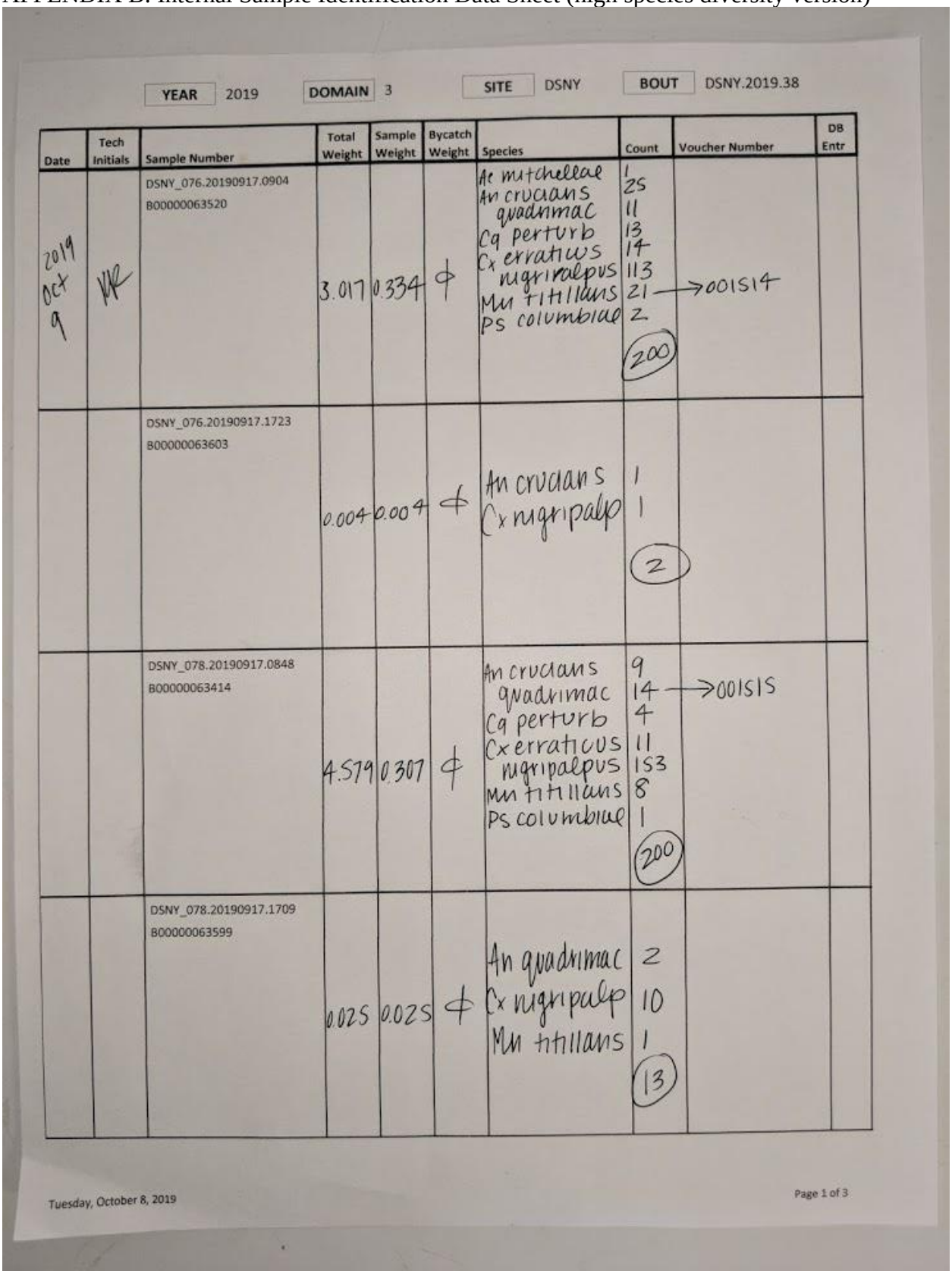


Figure 3: Trap sample being weighed to determine total sample weight.

Figure 4: Bout stored in petri dishes by species in plastic shoebox at -80ºC, awaiting pathogen testing and archive vialing.

APPENDIX B: Internal Sample Identification Data Sheet (high species diversity version)



YEAR 2019 DOMAIN 3 SITE DSNY BOUT DSNY.2019.38

Date	Tech Initials	Sample Number	Total Weight	Sample Weight	Bycatch Weight	Species	Count	Voucher Number	DB Entr
2019 Oct 9	KR	DSNY_076.20190917.0904 B00000063520	3.017	0.334	ϕ	Ae mitchellae An crucians quadrimac Cq perturb Cx erraticus nigripalpus Mu titillans Ps columbiae	1 25 11 13 14 113 21 2 200	→001514	
		DSNY_076.20190917.1723 B00000063603	0.004	0.004	ϕ	An crucians Cx nigripalp	1 1 2		
		DSNY_078.20190917.0848 B00000063414	4.579	0.307	ϕ	An crucians quadrimac Cq perturb Cx erraticus nigripalpus Mu titillans Ps columbiae	9 14 4 11 153 8 1 200	→001515	
		DSNY_078.20190917.1709 B00000063599	0.025	0.025	ϕ	An quadrimac Cx nigripalp Mu titillans	2 10 1 13		

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