

Preparation and Submission of Mosquito and Tick Samples to PHEL 2024

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DANA WOELL, PROGRAM MANAGER
MOLECULAR VIROLOGY UNIT
NEW JERSEY PUBLIC HEALTH AND
ENVIRONMENTAL LABORATORIES



Preparing samples for submission

PHEL will provide RNA/DNA free safe lock tubes containing one copper BB for pool submission

- **Green vials** are for tick submissions, **clear vials** for mosquitos!!

Keep samples in the refrigerator (2-8°C) or freezer (-20°C) prior to submission

Maintain samples on ice or at 2-8°C as often as possible while preparing samples

Wear powder free latex or nitrile gloves

Do not touch the inside of the cap or insides of tubes to prevent DNA/RNA contamination

Make sure the lids on all tubes are snapped **completely** shut

Submit no more than 20 samples per week- additional samples should request permission from state mosquito program



Tube Labels

All tubes must be labeled with county code + pool number on **the top AND side**

Paper labels must be used on all tubes

- Labels should be wrapped around horizontally
- Use a laser printer (no ink-jet)
- If you are unable to print labels hand-write **ON the provided labels**
- **Handwrite on the top of the tubes** (ok to use single-letter county code) with sharpie

Label sheets are distributed along with testing supplies

Labels are automatically generated by JerseySurv

- Print as many as you like in advance by selecting 'pool labels' on the right hand side of the 'pools' tab
- Can also select 'print labels' when entering/managing existing pools

This guidance is for all tubes, whether they contain mosquitos or ticks!

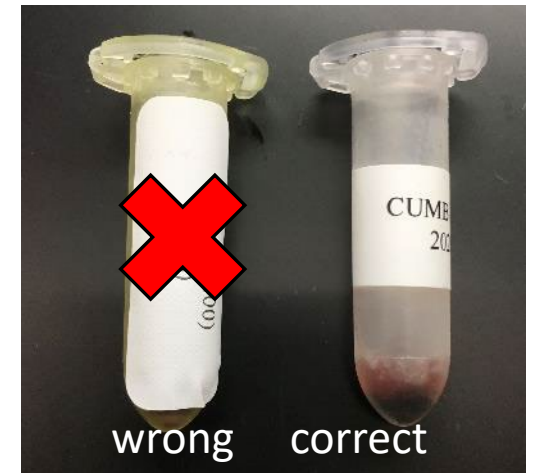
Labels for 2020 Pools

Label Type: TTLW2016 (33 mm x 13 mm x 85)

Label Print Direction: Top -> Bottom, Left -> Right

Pools to Include: 1 - 100

Next Step... Close



Ordering Tests in JerseySurv-Mosquito Collections

Testing panels

- **WESJ:** WNV, EEE, SLE and JCV
- **ZCD:** Exotic Aedes panel (upon request): Zika, Chikungunya and Dengue
 - NOTE: the ZDC Panel does NOT automatically test for West Nile Virus- **Select BOTH WESJ and ZDC if you want both**
- **LAC** (upon request): Lacrosse and WNV

Select one or multiple test panels for each sample

- **Create separate submissions for samples that have different test requests**
- All samples in a given submission should be requesting the same test(s)
- Example: 20 samples- 15 for WESJ only, 3 for ZDC and WESJ, and 2 for LAC- these should be submitted as THREE different submissions

Put all samples from a single submission form ID# in biohazard Ziploc bags, provided by PHEL

- Print the submission form from JerseySurv, and place in the outer pocket of each biohazard bag
- **Label bags** with county name, date and submission **form ID#** (labels provided on request, ok to handwrite)

Keep all samples cold at until shipment, transport on ice packs

EEE Reporting

Identification and Reporting of EEE- Form 4

EEE is a select agent. Select agents are pathogens which have been determined to be weaponizable and of high consequence to public health. Possession, use and transfer of select agents is regulated by the Select Agent Regulation 42 CFR part 73, through the Division of Select Agents and Toxins (DSAT) of the CDC and APHIS (Animal, and Plant Health Inspection Services) of the USDA

<https://www.selectagents.gov/index.htm>

PHEL is a registered entity with DSAT to possess, use and transfer select agents

Notification of Positive EEE

PHEL will notify you by email that one or more of your mosquito pools tested positive for EEE and indicate that you are required to submit Form 4A, parts C and D **within 7 calendar days**

The email will be sent to the POC on your mosquito submission, and the public contact(s) for your CMCA

The email will contain


- A link to the downloadable form 4A [APHIS/CDC Form 4A-CD Fillable PDF](#)
- The sample ID of the positive pool(s), the date it was identified
- The due date to submit the form 4 back to CDC
 - The seven day count down begins on the day you receive the email from PHEL, the email will have a due date

Follow the instructions on the top of Form 4A to submit via email or fax

Form 4A guidance is available here: [Guidance Document for the Completion of APHIS/CDC Form 4A-CD](#)

Include PHEL.EEEform4@doh.nj.gov cc'd as a recipient on all Form 4 submissions to CDC

Form 4 Section C- Provider Information



REPORTING THE IDENTIFICATION OF A SELECT AGENT OR TOXIN FROM A CLINICAL/DIAGNOSTIC SPECIMEN (APHIS/CDC FORM 4A)

FORM APPROVED
OMB NO. 0579-0213
OMB NO. 0920-0576
EXP DATE 11/30/2018

INSTRUCTIONS
Detailed instructions are available at <http://www.selectagents.gov/form4.html>. Answer all items completely and type or print in black ink. This report must be signed and submitted to either APHIS or CDC:

Animal and Plant Health Inspection Service
Agriculture Select Agent Services
4700 River Road Unit 2, Mailstop 22, Cubicle 1A07
Riverdale, MD 20737
FAX: (301) 734-3652
E-mail: AqSAS@aphis.usda.gov

Centers for Disease Control and Prevention
Division of Select Agents and Toxins
1600 Clifton Road NE, Mailstop A-46
Atlanta, GA 30329
FAX: (404) 471-8469
E-mail: CDCForm4@cdc.gov

Reference ID Number:

Submit completed form only once by either e-mail, fax, or mail

SECTION C – SAMPLE PROVIDER INFORMATION					
1. Name of individual completing Sections C and D: First: MI: Last:		2. E-mail address:		3. Telephone #:	
4. <input type="checkbox"/> Registered Entity (APHIS or CDC Registration #: _____) <input type="checkbox"/> Clinical or Diagnostic Laboratory (non-registered entity (NRE)) (NRE # (provided by APHIS or CDC): _____)		9. Entity name:			
5. Responsible Official or Laboratory Supervisor name (if same as field 1 then skip to field 9): First: MI: Last:		10. Address (NOT a post office address):			
6. E-mail address:	7. Telephone #:	8. Fax #:	11. City:	12. State:	13. Zip Code:

SECTION D – SPECIMEN(S) CONTAINING SELECT AGENT OR TOXIN PROVIDED TO REFERENCE LABORATORY

Form should be filled out by “the personnel at your entity that is most familiar with the case(s) being reported on the APHIS/CDC Form 4 report”

4 – Registration Information

- **You are all non-registered entities**
- NRE# will be provided to your entity after the first APHIS/CDC Form 4 report is submitted
- If your county has submitted CDC Form 4A in the past, you should have an NRE#
 - If you do not know your entity’s NRE number please contact APHIS or CDC to obtain the NRE number.
- If you have not previously submitted a CDC Form 4A, check the box for non-registered entity and leave the NRE# blank- one will be provided to you

Form 4 Section D- Sample Information

SECTION D – SPECIMEN(S) CONTAINING SELECT AGENT OR TOXIN PROVIDED TO REFERENCE LABORATORY		
1. Select Agent or Toxin Identified: {Select}		2. Date notified by reference laboratory of select agent or toxin identification:
3. # of select agent/toxin samples shipped:	4. Sample type provided: {Select}	5. Zip code for case/patient/sample origin:
6. Date sample(s) shipped to Reference Laboratory:		7. Name of Reference Laboratory:
8. Disposition of any remaining select agent or toxin listed by entity: <input type="checkbox"/> Destroyed (Provide destruction method and date. Must be onsite. Method: _____ Date: _____) <input type="checkbox"/> Retained (Provide name of Principal Investigator retaining sample. Name: _____) <input type="checkbox"/> Not applicable, the entire specimen was transferred to the Reference Laboratory.		
9. Were any of the samples containing a select agent or toxin handled outside of primary containment which may have led to an unintentional release and/or exposure to the		

Fill out sections 1-7 with the sample information

D3- specifically refers to the number of **pools** that tested positive

- Do NOT put the number of mosquitos in the pools

D5- The zip code for the specimen collection **site**

D7- Reference Laboratory: New Jersey Public Health and Environmental Laboratories

D8- This specifically is asking for you to destroy any remaining sample retained by your facility

- You can select not applicable since the entire positive pool was sent to PHEL**

Questions on Form 4? Email us at
PHEL.EEEform4@doh.nj.gov

Collection and Submission of Tick Samples to PHEL



Tick Collection

Follow the NJ Tick Surveillance guide for site selection, number of ticks to collect, and documentation

NJ DOH is putting on tick training next week that will go over this in detail

Ticks collected in the field should be sorted and stored by collection site, date, species, and life stage

Ticks **MUST** be surface-sterilized by submersion in 70-95% ethanol before submission- either in the field or before/during sorting.

- It is recommended to collect ticks directly into vials containing 70-95% ethanol
- PHEL or DEP can provide collection vials on request, or counties can provide their own

Submission to the Lab



Select one panel per sample pool based on species

Tick Species	Panel to request	Targets
I. scapularis	T-IS	Borrelia burgdorferi Borrelia miyamotoi Anaplasma phagocytophilum Babesia microti Powassan virus
A. americanum and	T-AA	Ehrlichia chafeensis Ehrlichia ewingii Rickettsia rickettsii Heartland virus Bourbon virus
D. variabilis	T-RR	Rickettsia rickettsii
A. maculatum	T-AM	Rickettsia parkeri
H. longicornis	T-HL	Rickettsia rickettsii Heartland virus Bourbon virus

Pool ticks according to species and life stage

Species	# of adults/pool	# of nymphs/pool
I. scapularis	1	1
A. maculatum		
A. americanum	1	up to 5
H. longicornis	up to 5	up to 10
D. variabilis		



Submitting Ticks in JerseySurv

Send to PHEL tick tests once monthly at minimum

- If you're collecting a lot of ticks, we would encourage you to submit more frequently instead of batching and sending once a month

One Bag, One Species, One Form

- Submissions should be broken up based on **species**
 - Do not create separate submission forms based on different collections or life stages
 - Select only one test for all samples in one submission form
- No more than 94 samples per submission
 - If you have a large submission with more than 94 samples, create two different submissions (e.g. sample # 1- 94, sample # 95 – 110)
- All samples in one submission should go in one biohazard bag
- If you are submitting multiple species for testing, create multiple submission forms

Print out submission form from JerseySurv to include with shipment- place in outer pocket of biohazard bag

Label the bag with county name, date and submission ID#

Keep all samples cold until shipment, transport on ice packs

Supply Requests

Requesting Additional Supplies

Today PHEL is only distributing 300 tubes instead of the usual 500

When we have more tubes in and ready to distribute, we will notify Scott, and he will notify you

After that, you can submit a request for more tubes

Request Supplies by:

- Email Usha.Yadav@doh.nj.gov and James.Occi@doh.nj.gov
- We will send 200 additional tubes at that point unless a different number is specified.
- If you're requesting any other supplies such as labels, biohazard bags etc. in addition to tubes, please specify that
- You can either pick them up in person at the labs, or we will mail to you

Thank you!

Please reach out to the molecular virology laboratory with any questions or comments about submissions, supplies or and testing concerns

- **Lab phone number: 609-718-8336**
- EEE Notifications: PHEL.EEEform4@doh.nj.gov
- Dana Woell: Dana.Woell@doh.nj.gov
- Usha Yadav: Usha.Yadav@doh.nj.gov,
- Jim Occi: James.Occi@doh.nj.gov, ph. 609-718-8352



Jim Occi