July 2019

Washington State Department of Health
WNV Mosquito Surveillance
Protocol for Transporting, Packaging, and Shipping Mosquitoes

Section A. Live Mosquitoes

Follow instructions on how to transport, package, label, and ship **live** mosquitoes as Biological Substance-Category B, UN3373 in accordance with applicable government, International Air Transport Association (IATA) and International Civil Aviation Organization (ICAO) regulations and Department of Transportation (DOT).

TRAINING REQUIRED

Those who ship Biological Substance-Category B shipments must receive documented training on the government regulations that cover these substances and the proper method of packing. Free training can be obtained online at CDC Packing and Shipping Division 6.2 Materials Web-based Training. The length of this course is approximately two hours. Note accessing the CDC TRAIN course can be tricky. Under Resources, see *3 Steps to Successfully Access Your Online Course via TRAIN* and *Remove Compatibility Setting from MS Internet Explorer* to help you register and launch the training course. (**Tip**-If you experience problems with your IE browser, try Google Chrome.)

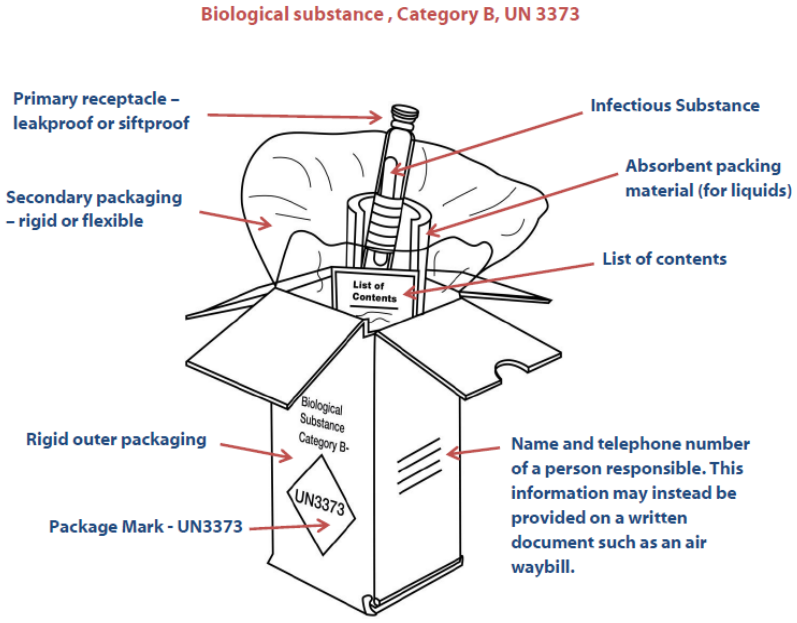
Another resource to keep handy is *FedEx Packaging UN3373 Shipments* instructions for packaging, marking, and labeling Biological Substance, Category B, UN3373 shipments for FedEx services.

HOW TO TRANSPORT

When in transport from the field, follow the tertiary containment shown in Figure 1. The primary receptacle is the mosquito catch bag. Place the catch bag in the secondary packaging—the filter patch plastic bag. The filter patch allows for air exchange. Clamp the bag close being careful not to block the filter patch. Place the bag into the tertiary, rigid outer packaging—the shipping cooler.

Before entering a building, open the cooler and ensure that both the primary and secondary containment are closed securely. Do your check outside of the building to avoid the escape of mosquitoes inside. If the secondary containment has been compromised, re-clamp the bag or seal with tape.

Figure 1



HOW TO PACK

Biological Substance-Category B requires tertiary packaging, but does not require UN specification packaging. Refer to Figure 1 CDC Packaging Biological Substance, Category B, UN3373 diagram.

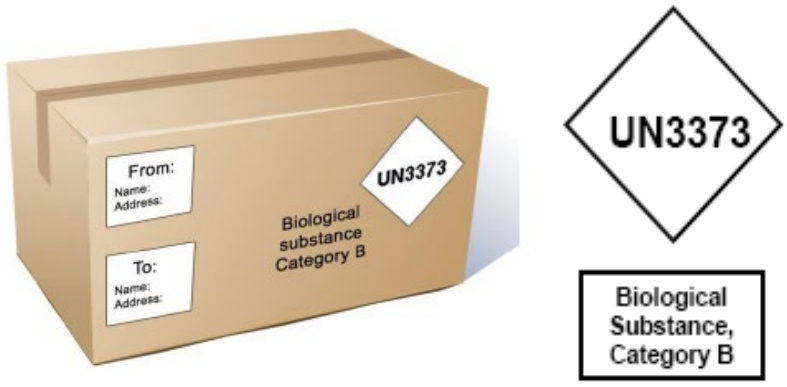
Pack the shipping cooler with two gel ice packs to keep mosquitoes cold. Place crumpled newspaper around the secondary packaging (filter patch plastic bag), or similar absorbent material in the cooler to fill unused space, provide insulation, and absorb any liquids. Seal the field data reporting forms in a Ziploc bag then place in the cooler. Tape cooler shut with strapping tape. Stick FedEx airbill and Biological Substance-Category B shipping labels onto the cooler.

HOW TO LABEL

The following labels must be included on Biological Substance-Category B shipment: Sender's name and address; Recipient's name and address; and the name, address and phone number of a designated person who will be responsible for the shipment during the time it's in transit. The designated "Responsible Person" should be the person who signs off on the airbill. This information may be provided on the airbill. Additionally, the following label must be included on the package: **BIOLOGICAL SUBSTANCE, CATEGORY B, UN3373** to cover federal shipping regulations.

Note: The mark "UN3373" must be in the form of a square set at an angle of 45° (diamond-shaped) with each side having a length of at least 2 inches square and the letters and numbers must be at least 1/4 inch high. The proper shipping name "Biological Substance, Category B" in letters at least ¼ inch high must be marked on the outer package adjacent to the diamond-shaped mark as shown in Figure 2.

Figure 2



HOW TO SHIP

Ship coolers Monday through Thursday by FedEx overnight service using Washington State Department of Health's account. Pre-printed FedEx airbill forms are available for your convenience. Do not ship on Friday; the laboratory is closed on Saturday.

Use our pre-printed FedEx airbill form or fill out a new airbill form:

Ship FedEx standard overnight to following address.

Field Surveillance
WDOH Environmental Public Health Science
234 Israel Road SE
Tumwater, WA 98501-6415
360-236-3385

Check FedEx package airbill as:

- Section 4 FedEx Standard Overnight
- Section 5 Other (ship mosquitoes back in small cooler)
- Section 6 Special Handling and Delivery Signature Options - None (Mosquito specimens, live or dead are not considered "dangerous goods" and do not requires special handling or delivery.)
- Section 7 Recipient, FedEx Account No. 4231-6308-9

For any questions about pack and shipping live mosquitoes, contact Anne Duffy at 360-236-3372 or anne.duffy@doh.wa.gov.

Section B. Dead Mosquitoes

Dead mosquitoes do not need to be shipped as Biological Substance-Category B, UN3373. Pack, label, and ship **dead** mosquitoes to DOH for identification and testing as follows.

HOW TO TRANSPORT Follow transport instruction of live mosquitoes on page 1.

HOW TO PREPARE AND PACK

When receiving mosquito catch bags check the primary and secondary containment to ensure both are securely closed. Do your check either by opening the cooler (tertiary containment) outside the building, or inside the building by opening the cooler in a sealed glovebox or safety cabinet intended for laboratory use. If the secondary containment has been compromised, re-clamp the bag or seal with tape.

Freeze-kill mosquitoes by placing the filter patch plastic bags (secondary containment) with the mosquito catch bags (primary receptacle) in the freezer for 90 minutes or longer until all mosquitoes are dead.

Prepare specimens for PCR testing

1. Remove from freezer, and keep dead mosquitoes cold to preserve virus RNA. Use a chill table during mosquito identification, or a metal baking sheet sitting on top of dry ice or frozen ice packs.
2. Use sterile forceps and pool vectors *Culex pipiens* and *Cx. tarsalis* into pools of up to 50 mosquitoes but not less than 10. For catches greater than 50, split into pools so that each pool has a strong representation of the catch. Example: split a catch of 60 *Cx. pipiens* into two pools of 30 females.
3. Place each pool into separate 2ml tubes containing DNA/RNA Shield buffer that have been provided. Keep tubes on ice in an ice bucket or in a frozen mini-cooler tube rack throughout the process.
4. Sterilize forceps with 70% isopropyl alcohol between each pool.
5. Record Tube ID on Mosquito Reporting Form under “For PCR Testing.”
    a. Tube ID - Location Code/Site #/Month/Day/Species Code/Pool #
        i. Location code should be one or two letters that represent the trap location name.
        ii. Site # should match the number assigned to the trap site at the location.
        iii. Month and day should not include “0” as placeholder (i.e., July 7 written as 77).
        iv. Species code should be “P” for *Cx. pipiens* and “T” for *Cx. tarsalis*.
        v. Pool number is the reporting form Pool #.
6. Example: Mosquito trap from Nisqually Woods (N) at Trap Site 2 (2) collected June 8, 2019 (68) yielded four pools for PCR testing; three pools of *Cx. pipiens* (P) and one pool of *Cx. tarsalis* (T): *Cx. pipiens* pool 1 reads as N2 68P1; *Cx. pipiens* pool 2 - N2 68P2; *Cx. pipiens* pool 3 - N2 68P3; and *Cx. tarsalis* pool 1 - N2 68T4.
7. Write the Tube ID on a provided label using a fine-tipped permanent marker, and place the label on its corresponding tube.
8. Freeze the tubes of vector pools in DNA/RNA Shield buffer before shipment, if possible. Whether frozen or not, ship tubes with frozen ice packs to keep cold during shipment.

Prepare specimens for identification

Gentle place freeze-killed mosquitoes for identification in petri trays with tissue. Place tissue over top of mosquitoes and overlap edges of petri tray. Please do not write on the petri trays. Instead write the site location, name, trap number, and collection date clearly on a sticky note and place on top of the tissue before placing the lid on petri tray. The lid should fit snuggly. Do not tape the petri tray closed. Refer to Figure 3.

Figure 3

Pack specimens

Pack the shipping cooler with two gel ice packs to keep tubes of vector pools cold. Place the frozen mini-cooler tube rack and petri dishes in the cooler. Place crumpled newspaper or similar absorbent material in the cooler to fill unused space, provide insulation, and absorb any liquids. Seal the field data reporting forms in a Ziploc bag then place in the cooler. Tape cooler shut with strapping tape. Stick FedEx airbill onto the cooler.

HOW TO SHIP Follow shipping instructions on page 2.