

Appendix 1: Mosquito Trap Information Traps for Mosquito Surveillance

Adult Mosquito Surveillance

Gravid Traps (Figures 1,2,3)

Gravid traps use a small electric fan, typically powered by a 6-volt lantern battery (Fig. 1), to suck up the mosquitoes that visit the bait container and blow them into a collection bag. Gravid traps are baited with a tub of odorous infusion (tea or fermented brew) made from water and organic material (e.g. grass clippings, hay, dead leaves, yeast, pelleted rabbit chow, horse or chicken manure, etc.). Gravid traps have been modified. In the Frommer updraft gravid traps (Fig. 2) and Reiter-Cummings gravid traps (Fig. 3), mosquitoes suffer less damage than traditional gravid traps because the mosquitoes are drawn into the mosquito collection nets without passing through the fan and the collected mosquitoes are not exposed to precipitation.



- } Holding net
- } Fan draws mosquitoes up into holding net
- } Small amount of scented water

Fig. 1. Components and set-up of a gravid trap



Fig. 2. Frommer updraft gravid trap



Fig. 3. Reiter-Cummings gravid trap

When trapping *Culex* species, it is best to use a bait infusion made from a recommended formula. Gravid *Culex* mosquitoes are attracted to the smelly water infusion as a place to lay their eggs like the stagnant, high organic content water brew these mosquitoes normally breed in. There is a higher probability of collecting virus-infected mosquitoes in a gravid trap than in a light trap because gravid traps attract female mosquitoes that have already taken at least one blood meal and are ready to lay eggs. The species collected may vary by where the trap is set and/or what formula is used to make the infusion bait.

Traps are best set under bushes, under porches, in tall grass, or out of the wind in areas close to where target vector species may be seeking a place to lay eggs. General locations with some shade provided by a tree canopy or other source is desirable. When trapping any mosquito species, gravid traps are best set sometime between 2 - 4 p.m. and 4:00 PM and collected the next day around 8 - 9 a.m. To reduce viral decomposition and damage to the mosquitoes, gravid traps should only be run for a single day. Gravid traps collect live mosquitoes, which are to be used for identification and testing.

Formula for gravid trap bait infusion for *Culex* mosquitoes

Bait for gravid traps used in mosquito surveillance can be obtained through the public health entomologist or by obtaining the ingredients and following the directions below:

Ingredients

1. Large plastic trashcan (40-50 gallons) with a tight-fitting lid
2. 30 gallons of water
3. 1 pound of straw or hay
4. 5 grams of brewer's yeast
5. 5 grams of lactalbumin powder

Mixing Directions

1. Place the trashcan in a place where it will get direct sunlight for several hours per day
2. Fill the can with 30 gallons of water
3. Stir 1 pound of straw into the 30 gallons of water
4. Add 5 grams of brewer's yeast
5. Add 5 grams of lactalbumin
6. Stir the mixture
7. Place lid on trashcan and let the mixture brew for five days, stirring at least once each day

Bait Usage Directions

After a period of about five days, the bait will be ready to use. **Note:** This brew will have a foul odor (somewhat like that of sewage), but will be highly attractive to *Culex pipiens* and *Culex restuans*. If you use this bait and do not catch either of the above mentioned *Culex* species, there probably were not any active in that area where the gravid trap was set. Be careful not to leave the lid off the trashcan because the odor of this bait may offend neighbors, and may attract swarms of egg laying *Culex pipiens* every night.

It is convenient to pour the finished bait into a 2.5 gallon wide-mouth container to carry it to your trap sites (an empty 2.5 gallon plastic cat litter container works well for this purpose). After use, the bait can either be dumped or it can be poured back into the carrying container for repeated use. If the bait is to be reused repeatedly, add several granules of Altosid™ larvicide to the bait to prevent the development of mosquitoes from eggs that have been laid in it.

When this bait is freshly made, it is not attractive to *Aedes albopictus* or container-breeding *Aedes* species. After about three weeks of usage, this bait becomes slightly less attractive to *Culex pipiens* or *Culex restuans*, and becomes more attractive to the *Ae. albopictus* and container-breeding *Aedes* species. If collection of *Culex pipiens* and *Culex restuans* is your primary goal, you should start a fresh batch of bait every month. You can keep the older bait and use it when trapping specifically for the container breeding *Aedes* species.

Other bait mixtures include but are not limited to:

1. Large plastic trashcan (40-50 gallons) with a tight-fitting lid
2. 30 gallons of water
3. 1 pound of straw or hay
4. 1 pound of grass clippings
5. 1 Tablespoon of brewer's yeast
6. 3 lbs. dried chicken manure (available at garden centers)

Follow mixing and usage directions for the above formula. To save time in mixing bait, the straw can be spread on the lawn prior to a mowing. After mowing, rake the chopped straw and grass mixture and freeze in two pound blocks.

Light Traps (Figure 4)

For special surveillance of short duration, a light trap is an efficient, reliable surveillance tool. This trap can be used to assess a citizen's complaint, check the success of an adulticide, or gather arbovirus information. The light trap is portable, battery powered, and efficient.

The light trap uses a small light source to attract and capture mosquitoes seeking hosts for a blood meal. Different light wavelengths can attract different mosquito species. Since light traps use only a small light source, these traps attract relatively few non-mosquito insect species, such as beetles and moths, that can damage the trapped mosquitoes. Unfortunately, using only a light will make this trap ineffective due to competing light sources (ex. full moon). Baiting the trap with CO₂ increases both the number of mosquitoes and range of species collected as compared to traps using light as the sole attractant. Use of CO₂ to bait the trap requires a supply of dry ice or canisters of compressed CO₂. A trap baited with CO₂ may require 2-3 pounds of dry ice or compressed gas per night. Light traps may also be baited with octenol and/or a human skin non-toxic chemical lure, which are designed to attract blood-seeking females. The chemical lure should be attached to the outside of the tube containing the fan assemblage with a rubber band. The combination of the chemicals and CO₂ may make the trap more attractive to some mosquitoes.

Container holds dry ice that melts and produces carbon dioxide

Light and fan draws mosquitoes into holding net

Holding net



Fig. 4. Light trap

Guidelines for CDC Light Traps: The following guidelines are offered to minimize variability in the use of CDC light traps for mosquito surveillance.

1. Whenever possible, use the CDC trap with a dry ice supplement. A quantity of 2.5 to 3.0 lbs. of pelletized or block dry ice in an insulated container (2-quart cooler) will mimic a large mammal's respiration and last long enough to cover the usual mid-afternoon to dawn trapping period.
2. If the capture of excessive non-mosquito insect species is a problem, or vandalism or theft of the trap is a concern, remove the light source when dry ice is used as an attractant; the absence of light will eliminate other photopositive insects from the collection, increasing the efficiency of identification. It will also make the trap less visible to vandals and thieves.
3. Hang the dry ice directly above, or adjacent to, and slightly below the aluminum lid of the CDC trap to draw mosquitoes as close as possible to the collection fan. Or, if supplied, place the dry ice into the container of the trap or attach the CO₂ hose, depending on what make of trap you are using.

4. Trap at least one hour prior to dusk until one hour after dawn to ensure surveillance is conducted during the primary host-seeking periods for most species. Setting traps earlier in the afternoon will result in the capture of day-biting species. This is especially important when collecting container breeding *Aedes* species. These would be the most important species in communities where La Crosse encephalitis has been a public health problem.
5. To prevent viral decomposition and mosquito damage, traps should only be left running for a single day.
6. Hang the trap so its light is 5-6 ft. from ground level unless specific information is needed on canopy dwellers. For most species, this height will provide a reliable indication of activity. For *Ae. Albopictus*, the lid of the trap should be at waist height when the trap is hanging.
7. Try to set the traps along the edges of habitats to increase trapping efficiency. A trap located strictly in one ecosystem/habitat may exclude certain species; trapping along the edge of a swamp, for example, will provide a picture of those species found not only in the swamp, but also in the nearby upland.
8. Consider two traps as the minimum number per site in most situations and compare your data to detect differences that may have been due to outside influences.
9. Be aware that differences do exist in the host seeking behavior of some species and that alterations from these general guidelines may be necessary to get complete surveillance data (record all trap settings and deviations on the mosquito surveillance field form). Strictly daylight feeding species will not be accurately represented in dusk-dawn collections. A species seeking hosts in tree canopies will not be accurately sampled by a trap that is suspended 5 ft. from the ground. Whenever possible, become familiar with the host seeking habits of the mosquitoes being surveyed.

Note: Trap should have contact information and warning related to dry ice attached when placed in the field.

BG Sentinel Traps (Figures 5 and 6)

The BG Sentinel trap attracts and captures mosquitoes using visual cues, olfactory stimuli, and air convection currents associated with vertebrate hosts (Fig. 5). The trap is a collapsible container with white gauze covering the opening. In the middle of the white gauze cover is a black catch pipe with an electric fan at the base. Air is drawn through the pipe by this electric fan, and mosquitoes caught in the air current are captured in the collection bag (Fig. 6). Air exits through the white gauze and generates an ascending air current, mimicking convection currents generated by human hosts. Lures containing chemicals generated by living vertebrate hosts are available. Chemical attractants include octenol and BG-Lure (ammonia, lactic acid, and fatty acids found on human skin). Carbon dioxide will act synergistically with these chemical lures to attract more blood-seeking mosquitoes. The black and white color scheme of the BG Sentinel trap has

proven useful in other successful mosquito sampling traps. And the low ground design is conducive to the lower strata feeding habitats of certain mosquito species, such as the Asian tiger mosquito (*Aedes albopictus*).



Fig. 5. BG Sentinel trap

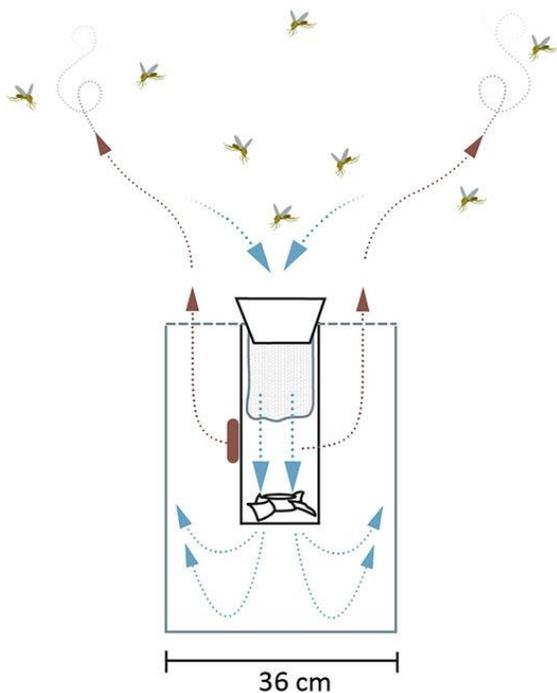


Fig. 6. BG Sentinel Diagram

Aspirators (Figure 7)

Like a vacuum cleaner, the aspirator uses air suction to collect small insects into a collection vial (Fig. 7). Power aspirators range in size from small hand-held battery powered units to larger battery or gasoline powered backpack units. Aspirators are quick,

active, and non-obtrusive, and can be used to capture adult mosquitoes which elude gravid and light traps. Aspirators are very effective for quick environmental assessments near the residence of arboviral patients.

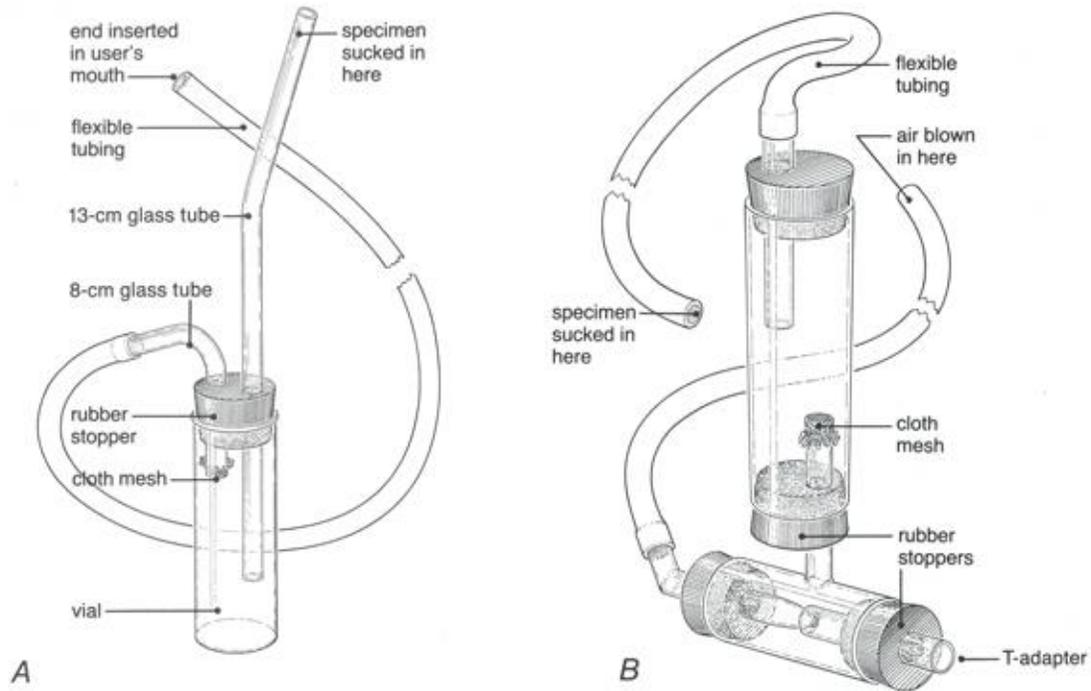


Fig. 7. Aspirator components

Larval Surveillance

Like fly larvae, mosquito larvae lack legs and wings. In addition, mosquito larvae have a cylindrical body with a developed head and no knobs or protuberances from the thorax. Learn to recognize different microhabitats within an area; each one of these should be sampled to obtain a comprehensive picture of the area's species composition.

Basic tools

1. Standard, white 400 ml-capacity dipper (Fig. 8)
2. Eyedropper; turkey baster, tea strainer, white enamel or plastic pan
3. Boots
4. Vials, plastic bags, or some other form of container for collecting larvae including labels for the collections, sharpies for labeling bags, and preservative
5. Mosquito surveillance field form
6. Pencil
7. GPS receiver - used to obtain data for GIS



Fig. 8. Mosquito dipper with turkey baster

Collection Methods: When searching for mosquito larvae, it is necessary to proceed slowly and carefully. Approach the area to be inspected with caution, as heavy footfalls will create vibrations that disturb larvae and cause them to dive to the bottom. Likewise, avoid disturbance of the water, as this will have the same result. Approach the area to be sampled with the sun in one's face; this prevents shadows that also disturb larvae and cause them to dive. If wind is of significant magnitude, sampling should be done on the windward side of the habitat where larvae and pupae will be most heavily concentrated.

The kind of mosquito one is looking for, as well as the type of habitat one is working in, will determine the technique used. If field personnel are familiar with the general breeding habits of the major species found within their county, they will be able to choose the most appropriate technique to obtain the most reliable results. Dipping is one of the most effective methods for sampling mosquito larvae. The following seven techniques for sampling mosquito larvae and pupae with the standard pint dipper are effective:

1. The Shallow Skim - *Anopheles* larvae are normally found at the surface of the water among aquatic vegetation or floating debris. *Anopheles* larvae do not have siphons. They can be collected with a shallow, skimming stroke along the surface, with one side of the dipper pressed just below the surface. End the stroke just before the dipper is filled, to prevent overflowing.
2. Partial submersion - Around emergent vegetation, logs and tree stumps, larvae may be drawn into the dipper by submerging one edge so that the water flows rapidly into the dipper. In this method, the dipper is stationary within the water.
3. Complete submersion - Certain Culicine larvae (such as species of *Aedes* and *Psorophora*) are very active and usually dive below the surface when disturbed. In this case, a quick plunge of the dipper below the surface of the water is required, bringing the dipper back up through the submerged larvae. Bring the dipper back up carefully, to avoid losing the larvae with overflow current.
4. Dipper as a background - This is an especially useful technique in woodland pools, for early season species. Submerge the dipper completely within the woodland pool, going down into the bottom litter if necessary. Use the white dipper as a background against which larvae and pupae can be spotted. Come up underneath

the larvae with the dipper. Once again, bring the dipper up carefully, to avoid losing its contents.

5. Flow-in method - This method is useful in situations where the water is shallow, with mud, leaf litter, or other debris on the substrate. Specimens can be collected by pushing the dipper down into the material on the bottom and letting the shallow surface water and mosquito larvae flow directly into the dipper.
6. Scraping - This method is used in permanent or semi-permanent habitats containing clumps of vegetation, such as reeds or tussocks. Dip from the water in, towards the tussock, and end by using the dipper to scrape up against the base of the vegetation to dislodge any larvae present.
7. Simple scoop - This is the technique which seems to be most commonly used by field personnel for larval surveillance and is the one referred to in much of the literature as "the standard dipping procedure." The technique involves simply scooping a dipper full of water out of a habitat. It is useful in a wide variety of habitats, especially for collecting *Culex*.

Note: Several species of mosquito are difficult to collect by dipper because their aquatic habitats often occur in containers or other depressions that are too small to sample with a dipper. These include:

- *Aedes albopictus* - tires
- *Aedes atropalpus* - rock pools, tires
- *Aedes japonicus* - rock pools, treeholes, tires, containers
- *Aedes triseriatus* - treeholes, tires, containers
- *Anopheles barberi* - treeholes, tires, containers
- *Coquilletidia perturbans* - permanent water with emergent vegetation
- *Culiseta melanura* - cedar and red maple swamps, occasionally tires
- *Orthopodomyia signifera* - treeholes, tires, containers
- *Toxorhynchites rutilus septentrionalis* - treeholes, tires, containers
- *Wyeomyia smithii* - pitcher plants

The turkey baster is an inexpensive, readily available tool that is very useful for sampling tires, containers, and tree-holes. A small white plastic soup ladle will also work well. The tea strainer can be used to concentrate and sort samples. Material collected can then be emptied into a white enamel pan (if preserving or concentrating samples in the field), from which the mosquito larvae are then removed, or poured into a plastic bag or water-holding container if being returned live to the laboratory.

Note: It is important to recognize that whenever dipping for immature mosquitoes, regardless of the technique used, it is important to look for actual presence of larvae before dipping, and to proceed carefully and pay attention to what you are doing.

Oviposition Trap (Figure 9):

Collections of mosquito eggs in oviposition traps are used to detect and monitor container-breeding mosquitoes such as *Aedes triseriatus*, *Aedes japonicus*, *Aedes albopictus*, and the yellow fever mosquito *Aedes aegypti* (not currently found in West Virginia). The oviposition trap can be easily made from food cans (3 lb. coffee cans), pint jars or 16 oz. aluminum cans painted black inside and outside. The traps are placed in shaded areas at a height no greater than 1.2 m (around 3 ft.) above the ground and filled with water. An oviposition substrate (seed germination paper, muslin, formica, balsa wood, wooden tongue depressor) is then placed vertically inside the container with the water covering about half of the substrate. Gravid females use this substrate to lay eggs just above the water level. Traps are checked every 10 to 14 days to maintain water levels or prevent them from becoming breeding sources. If larvae are found in the trap, then the water should be removed and the trap reset. The ovipositional substrate is periodically collected and returned to the laboratory in a plastic bag. Samples are kept cool and moist during transportation, taking care to avoid too much moisture. Excessive moisture will cause the eggs to begin hatching. Eggs or emergent larvae are then identified. Human pathogen screening is usually not conducted on adults reared from eggs. Eggs can be stored and reared to adulthood later.

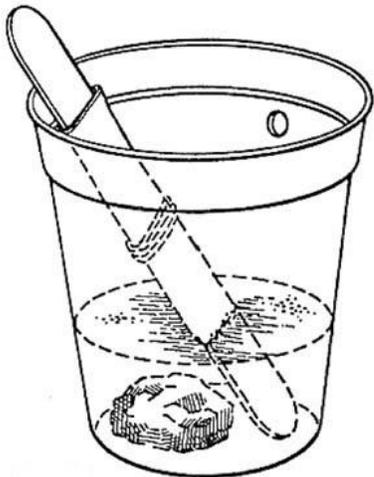


Fig. 9: Oviposition trap

Mosquito Surveillance Collection Form

Collecting Agency

Name of Surveyors

Surveyor's Agency

Collecting Locality

County

City

Nearest Street Address

Latitude (37.2 to 40.5)

Longitude (-82.64 to -77.72)

Habitat

- Business Construction Site Industrial Residential Rural School
 Tire Dump Urban Public Place Vacant Lot Other (Specify)

Collecting Date

Start Collecting Date

Time of Day

End Collecting Date

Time of Day

Collecting Method

Collection Method

- Aspirator BGS Trap Gravid Trap Landing Count Larval/Pupal Collection
 Light trap New Jersey Trap Omni-Directional Fay Prince Trap Ovicup/Ovitrap
 Sweep Net UV Light Trap Other (Specify)

Attractants Used (Check All That Apply)

- None Light Lure Octenol Hay or Grass Infusion
 Other (Specify)

Number of Traps Used

THE IMPORTANCE OF ENVIRONMENTAL ASSESSMENTS

Environmental assessments are often used by public health to reduce the spread of mosquito-borne diseases. The information collected during an assessment can be used to guide public health action. An important reason for conducting environmental assessment is to prevent outbreaks or clusters of mosquito-borne diseases. For example, *Aedes albopictus* mosquitoes are not only able to transmit many different diseases (e.g. La Crosse encephalitis and Zika), but they are also aggressive biters, meaning that if they are infected, they can spread disease to many people in a short amount of time. Mosquitoes do not travel long distances and remain within the same area throughout their entire lives.

Environmental assessment(s) should be conducted at the location(s) (e.g. home, day care, etc.) of suspected and confirmed mosquito-borne cases. Environmental assessments may be impacted by capacity, cooperation of the case, and time of year.

Capacity: a member of the public health workforce (e.g. county sanitarian, district sanitarians, state public health entomologist) should have the ability to visit the case's home. This is based on workload, training, and other factors (e.g. safety).

Willingness of the case: public health is given authority by the case or head of the household to conduct an environmental assessment.

Time of year: environmental assessment should be done during active mosquito biting season (May to September).

INSTRUCTIONS FOR CONDUCTING AN ENVIRONMENTAL ASSESSMENT

1. Obtain contact information about the (suspected) case from West Virginia Electronic Disease Surveillance System or from Regional Epidemiologist/Public Health Nurse. **It is important to ensure that the case has been informed of their case status by public health prior to the assessment.**
2. Make sufficient (at least three) attempts to contact the patient. Try to call at different times of the day.
3. Once the case has agreed to allow an assessment at its home, set up a date (preferably within three days of the call) to assess the area around the home. The "Case Information" and Case Clinical and Exposure History" sections of the Environmental Assessment Form may be collected during the telephone interview/appointment (in the event an assessment is done when the case is not present) or this information may be collected during the assessment.
 - a. If the case does not agree to an assessment, **still attempt to share mosquito-borne disease prevention literature with the case.** Giving a physical copy of literature is preferred (e.g. drop off the literature in the case's mailbox or mail it). **If the case has been symptomatic within the past two weeks, remind them to practice mosquito bite prevention strategies** (e.g. wearing mosquito repellent, wearing long sleeves and pants when outdoors, using air conditioning vs. keeping windows open).
4. Complete the "Assessment of Outdoor Environment" on the Environmental Assessment Form.
5. Once an on-site assessment is completed, share the "Environmental Assessment Actions and Recommendations" page with the case or homeowner. This should preferably be done in person, but can also be mailed to the case or homeowner.
6. Inform Jessica Shiltz, Vectorborne Disease Epidemiologist by email (jessica.l.shiltz@wv.gov) that an attempt was made to conduct the assessment. Fax Assessment Form to Jessica Shiltz in DHHR's Division of Infectious Disease Epidemiology at (304) 558-8736.

Environmental Assessment Form

Name of Assessor: _____ Assessment Date: ____/____/____

CASE INFORMATION

Last Name _____ First Name _____ Middle Name _____ Date of Birth ____/____/____

Home Street Address _____ City _____ Zip Code _____ County _____

(Coordinates should be in decimal degrees) Latitude _____ Longitude _____

Arboviral Disease of Concern: La Crosse Encephalitis West Nile Virus Zika Virus Other: _____

ASSESSMENT OF OUTDOOR ENVIRONMENT

Owner present during assessment? Yes No

Containers holding water visible on property (If checked, indicate types of containers)
 Tires Pool (type) _____ Animal watering containers Flower pots Containers without lids
 Other: _____

Leaf litter and organic debris in yard Assessor encountered mosquitoes while on property
 Assessor noticed larvae in containers on property Screens on doors and windows in disrepair/missing
 House gutters with visible debris
 Other: _____

CASE CLINICAL AND EXPOSURE HISTORY

Has the case been symptomatic within the past two weeks? Yes No

If yes, indicate symptom onset date: ____/____/____

Types of symptoms: Fever Rash Joint pain Conjunctivitis Headache Muscle ache Encephalitis

Other: _____

Has the case traveled outside of West Virginia in the past two weeks? Yes No *If yes, indicate place of travel and travel dates.*

City _____ State _____ Country _____ Arrival Date _____ Departure Date _____

ACTIONS OF PUBLIC HEALTH OFFICIAL

Mosquito traps were set on property Shared mosquito bite prevention literature Shared disease specific literature
 Drained water holding containers Disposed of litter/organic debris around home
 Conduct treatment for mosquito larvae Showed owner larval/pupae mosquito stages
 Recommended that case remain confined indoors/covered up (for symptomatic cases)
 Other: _____

ENVIRONMENTAL ASSESSMENT ACTIONS AND RECOMMENDATIONS

Dear Property Owner/Occupant:

Thank you for allowing [enter health department here] to conduct an environmental assessment at your home. Environmental assessments are often used by public health officials to help reduce the spread of mosquito-borne diseases. The information collected during an assessment can be used to identify mosquito breeding sites and provide education about preventing mosquito-borne disease.

The following are recommendations or actions by public health officials during an environmental assessment of your home.

- | | |
|---|--|
| <input type="checkbox"/> Mosquito traps were set on property | <input type="checkbox"/> Shared mosquito bite prevention literature |
| <input type="checkbox"/> Shared disease specific literature | <input type="checkbox"/> Drained water holding containers |
| <input type="checkbox"/> Showed owner larval/pupae mosquito stages | <input type="checkbox"/> Disposed of litter/organic debris around home |
| <input type="checkbox"/> Conducted larvaciding (e.g. placed mosquito dunks in areas where water collects over time) | |
| <input type="checkbox"/> Recommended that case remain confined indoors/covered up for at least seven days | |
| <input type="checkbox"/> Other: _____ | |

If mosquito traps were set on your property, public health entomologist will be available to identify the different species of mosquitoes that are living near your home and test them for endemic disease (e.g. West Nile and La Crosse encephalitis).

It is recommended that cases prevent mosquito bites by wearing mosquito repellent, staying indoors and covering up as much as possible (i.e. wearing long sleeves and pants, sleeping under bed nets) because human cases of mosquito-borne disease can spread infections to mosquitoes (usually during the first week of infection).

Please take time to read the mosquito-borne disease prevention literature that was provided to you. The **[enter health department here]** sincerely appreciates your cooperation in making public health work for you. If you have additional questions or concerns, feel free to contact us at **[enter health department number here]**.