West Virginia Mosquito Surveillance Plan and Generalized Sampling Instructions

(Adapted from the Virginia Mosquito Surveillance Plan)

I. Introduction

Mosquito surveillance should be the mainstay of regional surveillance programs for arboviruses. An effective mosquito surveillance program provides an estimate of vector species abundance and distribution. This data is used to estimate risk levels, guide control operations, and to evaluate control methods. Laboratory testing of mosquitoes for arbovirus infection provides information on the relative risk to humans and animals. This plan addresses practices that can be used to aid in the establishment of vector surveillance programs in regions of the state where little or no vector control work is being done.

A universally applicable arbovirus surveillance system does not exist, thus, local mosquito surveillance systems should be tailored according to (1) the probability of arbovirus activity, and (2) the resources available for surveillance (CDC, 2003). Because resources for arboviral surveillance are currently limited in the State of West Virginia (one public health entomologist, 5 local health departments) and temporary college student interns. Based on suggestions from CDC DVBID Arboviral branch we are not trapping mosquitoes around La Crosse cases in 2007 with the exception of occasional trapping for academic purposes. Follow-up of La Crosse cases will include community clean-ups and public outreach to the community surrounding the positive case. West Nile surveillance will include setting an array of gravid traps in suburban areas. Good places to set traps include areas surrounding sewage lagoons and sewage treatment facilities (including small package plants and large municipal systems). Traps will be set on Mondays and collections will be made on Tuesday, Wednesday and Thursday. All samples from an individual trap will be pooled from a week's collection. All *Culex* mosquitoes will be pooled together, regardless of species and submitted for virus testing. For Chikungunya virus (CHIKV) it is important to monitor populations of *Stegomyia albopicta* (*Aedes albopictus*). This particular species can be difficult to monitor populations because it breeds primarily in containers and may not exist in high populations. Initial sampling to assess population sizes of St. albopicta will consist of setting CO^2 traps baited with an additional synthetic human skin lure. These traps would be set in areas with large numbers of artificial containers such as salvage yards and, facilities that sale and collect large numbers of automobile tires. If the virus is detected in the state, ovitraps could be deployed to add to the surveillance data.

II. Objectives

This plan for mosquito surveillance, will serve as guidance for local jurisdictions that are developing mosquito and arboviral surveillance. The goals of mosquito surveillance are numerous and serve to obtain the following information about their local mosquito populations:

1. Identification of the mosquito species that are present in a region;
2. Identification of mosquito species that are the cause of local citizen complaints, and determining whether they are important vector species;
3. Identification and mapping of mosquito breeding habitats for larval control purposes;
4. Defining the geographic area affected by mosquitoes originating from identified

habitats and the geographic area that needs to be treated for adult mosquito control;
5. Determining the population density and the desired threshold for control of a local mosquito species;
6. Determining when local mosquito populations are at an appropriate developmental and/or behavioral stage to apply control measures;
7. Determining the effectiveness of local mosquito control measures;
8. Determining whether vector mosquito species are present in an area, and whether they are infected with arboviruses;
9. Determining the mosquito infection rate for arboviruses in a vector species population; and
10. Determining the seasonal activity patterns of local mosquito species;

III. Surveillance Plan

The West Virginia public health entomologist will coordinate and encourage collaboration among the Local Health Departments (LHDs) to carry out mosquito surveillance and will assist in the education of government officials on aspects of mosquito control. In addition, after a confirmed case of arboviral disease (humans or animals) has been identified or there is a complaint of substantial mosquito activity, and the public health entomologist has been contacted, a site visit consisting of an inspection and adult and/or larval surveillance (to include identification of the species present and their testing for arboviruses) will be conducted.

Citizen Complaints

If the public is informed about whom to call, citizen complaints about adult mosquito activity or about potential breeding habitats are useful in mosquito surveillance. Maintaining records of citizen complaints can contribute toward identification and mapping mosquito problem areas. When areas are identified through citizen complaints, the public health entomologist is to be notified. The use of citizen complaints can be especially useful when establishing a new surveillance program in an area where the mosquito breeding habitats and/or areas within adult mosquito flight range have not yet been identified. Citizen complaints can be investigated through visitation and direct observation, trapping, aspiration of adult mosquitoes and larval dipping in identified habitats.

A portion of citizen complaints misidentify the source of the mosquitoes, or are unfounded and so it may take a person with some knowledge of mosquito biology to question the complainant and get the complete or pertinent facts. Obtaining detailed information (enter on complaint form) from the caller (e.g., what time the mosquitoes were active, whether they were biting, what their biting behavior was, how large the mosquitoes were, what the mosquitoes looked like, whether there are any suspected breeding grounds near by, etc.) will help screen complaints and avoid unnecessary investigations. For example, adult mosquitoes are relatively small and are generally difficult insects to observe, so people do not normally notice them unless they are biting. Therefore, if the insects noticed by the complainant were not trying to bite or biting, it is unlikely that they were mosquitoes. Questioning the complainant about the time of day mosquitoes are biting is a useful screening tool. For example, Asian tiger mosquitoes are one of relatively few mosquito species that bite during the daytime (daylight hours) and because some complaints in West Virginia are related to Asian tiger mosquito activity, determining that the mosquitoes are biting during daylight hours will provide an indication that the problem mosquitoes are likely

Asian tiger mosquitoes. Also, Asian tiger mosquitoes only breed in containers (not in puddles or ground pools) and because they generally do not move far, they probably originate from a container on the complainants property, or from one that is on a neighbor's property. Therefore, if the complainant is indicating that the mosquitoes originate from a nearby farm pond or ditch, the person taking the complaint will be able to know that is unlikely.

Site Visits

Whenever an environmental health person or the public health entomologist is visiting the dwelling of the person with a probable or confirmed case of arboviral disease, or area suspected to be that at which transmission occurred, that person should observe the area looking for all potential mosquito **breeding habitats** (i.e. barrels, buckets, tarps, boats, ornamental ponds, old appliances, toys, trash, or any other item that may hold **as little as a tablespoon of water**). When infested habitats are encountered they should be noted on the mosquito surveillance field form and their location recorded and a GPS reading (latitude longitude and reference datum) taken for the site. Larval habitats should be dumped after sampling. If homeowners are home, habitats and larval samples should be shown to the homeowner. This provides an opportunity for educating the property owner or occupant on how to eliminate a potential or existing mosquito problem.

Surveillance

Mosquito surveillance involves the application of numerous different strategies and practices. A variety of methods are used to trap mosquitoes in the field because different mosquito species have their own characteristic behaviors and biology and cannot all be collected by the same method. Also, larval mosquitoes occupy a different habitat than adult mosquitoes, so collection methods for larvae are much different than those used for adults. Appropriate species (see list of vectors of adult mosquitoes will be collected, pooled and submitted to the West Virginia Office of Laboratory Services (OLS) for arboviral testing. Surveillance should be utilized for determination of risk as well as for planning, execution, and evaluation of control options.

Adult Surveillance

As it is usually the adult female mosquito that carries and transmits disease, many surveillance techniques have been devised to collect adult female mosquitoes and to monitor or record their activities. Techniques include the use of trapping, mechanical aspirator collections, and documentation of mosquito activity through citizen complaints. Trapping is widely used, but day-to-day success may be variable due to variation in environmental conditions such as wind, air temperature, and rainfall and/or trap location. Several different types of traps are used and each type is used to trap certain species of mosquitoes. Some mosquito species are not attracted to traps and must be collected by some alternative means. It is often advisable to use several types of traps (e.g., gravid and CDC-light traps) at a single trap site to collect a representative sample of the species active at that location. Data on the trapped mosquitoes should be maintained to create a historical record of mosquito species found in association with a variety of habitats in different parts of a jurisdiction. Although many methods for collecting adult mosquitoes exist, the initial surveillance program in West Virginia will rely on only two sampling

methods (CDC light trap and Reiter gravid trap). However, it may be useful to collect adults by aspirating.

A. **Reiter gravid trap**: The Reiter gravid trap is designed to collect gravid mosquitoes and is among the most important mosquito traps used for surveillance. Gravid mosquitoes are mosquitoes that are carrying eggs and are seeking a site to lay them. The gravid trap was originally developed for monitoring mosquitoes in the *Culex pipiens* complex, but will also work for trapping several of the *Aedes* and *Ochlerotatus* species that breed in containers. Gravid traps are the most effective means of collecting *Culex pipiens* and *Cx. restuans* which are the most important "primary vectors" of WNV. Primary vectors are those species responsible for transmitting WNV to the bird population. The container breeding *Ochlerotatus* and *Stegomyia* species captured in gravid traps include: the Asian tiger mosquito (*Stegomyia albopicta*), the Eastern tree-hole mosquito (*Ochlerotatus triseriatus*), and the newly introduced Asian rock-pool mosquito (*Oc. japonicus*). These species are potentially among the most important "bridge vectors" for WNV. Bridge vectors are those species which can bite birds, and commonly bite humans or other mammals and serve as a bridge for the virus to move from bird to mammal)

   Gravid traps use a small electric fan, typically powered by a 6-volt lantern battery 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 smelly 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.). 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. 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 2:00 and 4:00 PM and collected the next day around 8:00 or 9:00 AM.** 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 brewers 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 brewers 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 particular brew will have a foul odor (somewhat similar to 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 particular bait is freshly made, it is **not** attractive to *Stegomyia albopicta* or the *Ochlerotatus* species of container breeding mosquitoes. However, after about three weeks of usage, this bait becomes slightly less attractive to *Cx. pipiens* or *Cx. restuans*, and becomes more attractive to the *St.albopicta* and the *Ochlerotatus* species that breed in containers. If collection of *Cx. pipiens* and *Cx. 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* and *Ochlerotatus* species.

Note: A mosquito surveillance field form should be completely filled out for each collection. In addition, all collections need to be handled appropriately and labeled with pertinent information (when setting a trap, a label should be partially completed and inserted into the trap bag, and then completed upon retrieval of sampler). Send a copy of the completed field form to the public health entomologist in Charleston, along with voucher specimens once identified.
Note: Trap should have contact information attached when placed in the field.

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 brewers 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.

B. CDC light trap: CDC light traps are one of the standard tools for arboviral surveillance. Like the gravid trap, this trap is very portable because it is light-weight and can be powered by a 6-volt lantern battery. The CDC light trap uses a small light source to attract and capture mosquitoes that are seeking hosts for a blood meal. Unlike the gravid trap it attracts a relatively wide variety of species and is the best trap to use for identifying the species composition of a locality and for monitoring them. The CDC light trap is highly effective for trapping and monitoring various species of floodwater and marsh mosquitoes, but may only be marginally or poorly attractive to other species including many of those that are attracted to gravid traps. Baiting the trap with CO_2 increases both the number of mosquitoes and range of species collected as compared to traps using light as the sole attractant. Use of CO_2 to bait the trap requires a supply of dry ice, or canisters of compressed CO_2. A trap baited with CO_2 may require 2-3 pounds of dry ice or compressed gas per night. CDC light traps use only a small light source that attracts relatively few non-mosquito insect species such as beetles and moths that can damage the trapped mosquitoes. This feature insures that the collected mosquitoes remain undamaged and this makes them easy to identify. Additionally traps may be baited with octanol and/or a human skin non-toxic chemical lure. 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 CO_2 may make the trap more attractive to some mosquitoes.

CDC Light Trap Procedures
(Modified from McNally, 1989)

The control of adult mosquitoes begins with proper surveillance. For special surveillance of short duration, the dry ice baited CDC trap is an efficient, reliable surveillance tool for mosquito surveillance. This trap can be used to assess a homeowner's complaint, check the success of an adulticide or gather arbovirus information. The CDC trap's portability, battery power, and efficiency add versatility to the surveillance program.

Guidelines for CDC Trapping: The following guidelines are offered to minimize variability in the use of CDC 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 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 CO^2 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 insure that 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 container breeding mosquitoes (*Ochlerotatus spp.*, *Stegomyia spp.* and *Aedes spp*.). These would be the most important species in communities where La Crosse encephalitis has been a public health problem.

5. 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 *Stegomyia albopicta* the lid of the trap should be at waist height when the trap is hanging.

6. 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.

7. 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.

8. 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 that host seeks 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: A mosquito surveillance field form should be completely filled out for each collection. In addition, all collections need to be handled appropriately and labeled with pertinent information (when setting a trap, a label should be partially completed and inserted into the trap bag, and then completed upon retrieval of sampler). Send a copy of the completed field form to the public health entomologist in Charleston, along with voucher specimens once identified.

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

C. **Mechanical Aspirators:** – Powered aspirators are useful tools for collecting adult mosquitoes. Some species of mosquitoes (e.g., certain species in the *Anopheles* and *Culex* genus) do not readily come to traps and aspirating them from their resting areas is the only way to collect them in significant numbers. All mosquito species rest after taking a blood meal and the only way to capture certain mosquito species while they are

blood fed or gravid is to seek out their resting shelters and aspirate them. Mosquito resting places include: foliage of certain plants; building walls, ceilings and eaves; the undersides of bridges; the insides of hollow trees and logs; rodent burrows; and the insides of culverts or sewer pipes. Mosquitoes can also be collected with aspirators when they enter vehicles, or swarm around personnel during trap setting activities. Power aspirators range in size from small hand-held battery powered units to larger battery or gasoline powered backpack units.

Note: A mosquito surveillance field form should be completely filled out for each collection. In addition, all collections need to be handled appropriately and labeled with pertinent information. Send a copy of the completed field form to the public health entomologist in Charleston, along with voucher specimens once identified.

Larval Surveillance Procedures (Modified from O'Malley, 1989)

Larval surveillance requires the use of minimal and inexpensive equipment. Equipment should include: **a dipper, a small soup ladle, a small white, plastic or enamel pan, a turkey baster, larval collection bags, a tea strainer and a shoulder bag**. When infested habitats are encountered, they can be dipped for larval samples. Samples can be poured into whirl-pack, zip-lock bags or urine specimen cups and returned to the office for larval identification or maturation to adult stage for identification. Larval surveillance may require the use of different dipping techniques depending on the target species and habitat. Accurate records (on the mosquito surveillance field form) should be kept of when and where larvae are collected.

A. **Basic tools:** Standard, white 400 ml-capacity dipper, an eyedropper; turkey baster, tea strainer, white enamel or plastic pan, boots, vials, plastic bags or some other form of container for collecting larvae including labels for the collections, sharpies for labeling bags, preservative, mosquito surveillance field form, and a pencil. A GPS receiver should also be used to obtain data for GIS.

B. **Potential Breeding Habitat:** Mosquitoes will breed anywhere there is standing water such as: tires, bird-baths, plant pots, storm drains, and neglected, un-chlorinated swimming pools. Natural breeding habitats include: temporary flooded areas, ditches, tidal or freshwater wetlands, and other areas with temporary or seasonal standing water. Permanent bodies of water such as lakes or stream pools may also contain larvae of a few mosquito species in shallow areas, areas of emergent vegetation or areas with floating debris or vegetation. Flowing water or bodies of water subject to wind or wave action are not suitable breeding habitat for mosquitoes.

   Mosquito larvae are usually confined to the margins of a body of water and will not be found in open, deep water. Sampling should be done around floating debris, aquatic and emergent vegetation, logs and tree stumps in the water, and grasses around the margins. Look for the presence of larvae and pupae before beginning to sample.

   One must also recognize that each area to be checked may contain a number of different microhabitats, and each may contain the larvae of different species. Learn to recognize different microhabitats within an area; each one of these should be sampled in order to obtain a comprehensive picture of the area's species composition.

C. **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. 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:

- *Stegomyia albopicta* - tires
- *Oc. atropalpus* - rock pools, tires
- *Oc. triseriatus* - treeholes, tires, containers
- *Anopheles barberi* - treeholes, tires, containers
- *Coquilletiddia perturbans* - permanent water with emergent vegetation
- *Culiseta melanura* - Cedar and red maple swamps, occasionally tires
- *Orthopodomyia signifer*: - 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 preservation or concentrating samples in the field), from which the mosquito larvae are then removed, or poured into a plastic bag 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.

Note: A mosquito surveillance field form should be completely filled out for each collection. In addition, all collections need to be handled appropriately and labeled with the pertinent information. Consult the attached “code sheet” for breeding site description and send a copy to the public health entomologist in Charleston, along with voucher specimens once identified. In addition, it is important to keep samples from different habitat types separate so that a density estimate can be made.

D. **Measurement of Density:** It is highly recommended that larval density be calculated. However, it is not required at this time. Larval density is almost always expressed as numbers of larvae and pupae per dip. Density should be expressed in real numbers. That way, one knows exactly what one is dealing with in terms of population size.

Belkin (1954) developed a simple index for determining larval densities that some may prefer to use:

BI = TLP/ND x BP

BI = the breeding index

TLP = the total number of larvae and pupae taken

ND = the number of dips

BP = the number of breeding places

A "breeding place" is defined as each separate microhabitat or station within a site from which one to three positive dips are obtained.

Note: Data required for the breeding index can be entered on the mosquito surveillance field form.

Preservation and identification of mosquitoes

All specimens, whether larval or adult, live or dead, should be treated in a manner to limit damage to the specimens. Damage can occur to the specimens through jarring, grinding, or handling. In addition, direct sunlight and extremes in cold and heat can damage specimens.

Adults

The trap bag from the sampler should be handled in a manner so as not to destroy the mosquitoes. The mosquitoes, after being trapped, usually die quickly. The specimens do not need to be submitted to the OLS alive, as the method of analysis does not require live specimens. A short time in a field vehicle is enough to ensure the demise of the mosquitoes on a warm summer day. After the mosquitoes have been sacrificed, they should be put into a container to avoid damaging them or exposing them to harsh temperature conditions and direct sunlight.

Procedures:

1. Empty the catch and place the label from the trap bag into a white plastic tray for sorting or into a container for transporting and sorting later. It may be necessary to sort the mosquitoes using a dissecting scope.

2. Sort the mosquitoes into piles of similarly looking kinds. You may discard other organisms caught in the trap (mosquitoes have a proboscis).

3. **The mosquitoes can then be identified** using the appropriate taxonomic key and placed in containers. The recommended keys are, "**The Key to the Mosquitoes of North Carolina and the Mid-Atlantic States**" and "**Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico**". The identification of mosquitoes should be done in consultation with the public health entomologist. All identifications can be confirmed by the public health

entomologist. Once identified, the data should be recorded on the mosquitoes surveillance field form and copies forwarded to the public health entomologist along with voucher specimens of the species collected. Ensure that all specimen containers have been labeled with **site name/number, collection date, county, collector, and species**. On page 2 of the mosquito surveillance field form, indicate if the specimens have been archived and/or vouchered (either on site or with the public health entomologist) and their physical location. The public health entomologist can help you with the appropriate storage methods for archival (vouchers specimens are different from archives).

4. Once mosquitoes have been identified and separated into different groups of species, they should be sent for testing (if the appropriate species) for arboviruses at the OLS (a voucher should be sent to the public health entomologist),

Larvae

Larval mosquitoes can be processed in one of two ways after sampling. The mosquitoes can be returned to the lab, put in rearing chambers, and then identified as adults, or they can be processed and preserved in the field for later identification in the laboratory as larvae.

A. **Laboratory rearing**: Larval mosquitoes (and the water (up to ½ gallon) from the larval habitat) to be reared in the laboratory should be placed in a plastic bag (1 gallon zip-lock), and then the bag labeled with appropriate collection information (**site name/number, collection date, county, collector, habitat type, number of dips**). The plastic bags can then be placed in a cooler for transportation to the lab. No ice is necessary, but the specimens should **not** be allowed to overheat or freeze in a vehicle. The mosquitoes and the water from the sample should be placed into a rearing chamber. If there is too much water, remove some using a turkey baster. A small amount of tropical fish food should be finely ground and put into the water with the mosquitoes, only a pinch is needed. The rearing chamber should then be labeled with the appropriate collection information **(site name/number, collection date, county, collector, habitat type, number of dips**). The rearing chambers should be placed out of direct sunlight, however they should get ambient lighting from outside in order to receive the appropriate cues for emerging as adults. Once adults have emerged, they can then be identified. The adults can be aspirated from the rearing chambers, and place under a hot lamp (normal light bulb place very close to the specimens) for a very short amount of time, this will quickly subdue the active mosquitoes before identifying them. Once identified, the data and mosquitoes should be processed as indicated in the identification section of adult mosquitoes.

B. **Non-reared larvae**: Mosquitoes to be preserved in the field or laboratory can be removed from the sample water and placed into a small container/vial containing 70 percent ethanol or rubbing alcohol for preservation. Mosquito larvae must be in their fourth instar (stage) to be properly identified. The specimens then can be identified when needed. All specimens must be processed as described in the identification section for adult mosquitoes.

Voucher and Archive specimens

A. **Voucher specimens** are specimens (either larval or adult) that serve as a record of a species from a given area. They can (for example) be used in future taxonomic investigations, serve as proof that a species did exist in an area, or be used as sources of genetic material. Voucher specimens **must** be submitted from mosquito samples to the public health entomologist for long-term care. A central repository is much more easily maintained than is one that is dispersed. These specimens should be placed in containers supplied by the public health entomologist, then shipped to the public health entomologist with the appropriate information **site name/number, collection date, county, collector, habitat type, latitude, longitude, and reference datum).**

B. **Archived specimens** are those that are left over after pooling and vouchering. For example, if a large number of a single species are collected at a site, a number have been pooled, and a number of specimens vouchered, then the rest would be saved as archive specimens. Archived specimens can be utilized as teaching specimens for mosquito identification courses.

Virus Testing of Adult Mosquitoes

It is not appropriate to submit all mosquito species for arboviral testing. Surveillance programs should concentrate on trapping and submitting approved vector species for testing (see approved list of species and consult with the public health entomologist). Collected mosquitoes should be pooled for testing. Pools of approved mosquito species consist of 15 to 25 individual mosquitoes of the same species from the same location and collection date. Certain important vector species may be submitted in pools of as few as 10 mosquitoes (consult with the public health entomologist). Pooled mosquitoes should be accurately identified and grouped by species (except in the case of *Culex* which should be grouped as *Culex spp.),* site, and by day or week of collection. Pooled mosquitoes should be sent to the OLS using methods described below.

MATERIALS AND METHODS TO PREPARE AND SUBMIT ADULT MOSQUITO POOLS FOR ARBOVIRAL TESTING

Mosquitoes tested for arboviruses should be collected, identified, and pooled. The trap bag from the sampler should be handled in a manner not to destroy the mosquitoes. The mosquitoes, after being in the trap bag, usually die quickly. The specimens do **not** need to be submitted to the OLS alive, as the method of analysis does not require live specimens. A short time in a field vehicle is enough to ensure the demise of the mosquitoes on a warm summer day. After the mosquitoes have been sacrificed, they should be put into a container to avoid damaging them and exposing them to harsh conditions and direct sunlight.

Procedures:

1. Empty the catch and place the label from the trap bag into a white plastic tray for sorting or into a container for transporting and sorting later. It may be necessary to sort the mosquitoes using a dissecting scope.
2. Sort the mosquitoes into piles of similarly looking kinds. You may discard other organisms caught in the trap.
3. The mosquitoes can then be identified using the appropriate taxonomic key. The recommended keys are, "**The Key to the Mosquitoes of North Carolina and the**

Mid-Atlantic States" and "**Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico**". The identification of mosquitoes should be done in consultation with the public health entomologist.

4. Once mosquitoes have been identified and separated into different groups of species, the mosquitoes (25-50 of each species, less than twenty five can be submitted if agreed upon in consultation with the public health entomologist) should be placed into the appropriate type of vile (available from the OLS), two BBs placed inside, and the **vial labeled with the appropriate pool number** available from the public health entomologist. If the catches are extremely large (numbering in the hundreds), it will be necessary to increase pool size to 100 mosquitoes per pool. Only species from the following list should be submitted for testing, unless otherwise indicated through consultation with the public health entomologist.

Note: At this time, the OLS can test mosquito pools for WNV, LAC and SLE

Approved Species for Submission for Arbovirus Testing

WNV

Primary and bridge vectors to be tested:

Stegomyia albopicta
Aedes vexans
Anopheles punctipennis
Anopheles quadrimaculatus
Culex spp.
Culiseta melanura
Coquillettidia perturbans
Ochlerotatus atlanticus/tormentor
Ochlerotatus canadensis
Ochlerotatus infirmatus
Ochlerotatus japonicus
Ochlerotatus sollicitans
Ochlerotatus sticticus
Ochlerotatus taeniorhynchus
Ochlerotatus triseriatus
Ochlerotatus trivittatus

EEE

Primary and bridge vectors to be tested:

Stegomyia albopicta
Aedes vexans
Anopheles crucians
Anopheles punctipennis
Anopheles quadrimaculatus
Culex erraticus
Culiseta melanura
Culex salinarius
Coquillettidia perturbans
Ochlerotatus atlanticus/tormentor
Ochlerotatus canadensis
Ochlerotatus infirmatus

EEE

Ochlerotatus sollicitans
Ochlerotatus taeniorhynchus

LAC

Vectors tested during entire season:
Stegomyia albopicta
Ochlerotatus canadensis
Ochlerotatus japonicus
Ochlerotatus triseriatus

SLE

Primary vector and bridge vectors that can be tested:
Stegomyia albopicta
Culex erraticus
Culex pipiens
Culex restuans
Culex salinarius
Ochlerotatus japonicus
Ochlerotatus triseriatus

5. Voucher specimens must be taken from the pool and submitted to the public health entomologist. Voucher specimens are examples of the species identified that can be retained for further scientific investigation. These specimens should be placed in containers supplied by the state public health entomologist, then shipped to the public health entomologist with identification and location/collection information.
6. Once the vile containing the mosquito pool to be tested has been labeled with the appropriate pool number, it should be placed into a plastic bag, and the bag labeled with the appropriate pool number (available from the public health entomologist). In addition, the pool numbers should be recorded on the second page of the mosquito surveillance form.
7. Then, an OLS mosquito pool submission form should be completed. The form and bag containing the sample should be placed in a box and shipped to the OLS using their address on the submission form via U.S. Mail. Please inform OLS (Wendy Hess 304-558-3530 ext 2402 or wendychannell@wvdhhr.org) and the public health entomologist via e-mail that a shipment is on the way and let them know what the pool numbers are that are contained in the shipment.

Map Reference Datum

A reference datum is used to describe the location of an unknown point on the earth's surface, rather than using above or below sea level or using above or below the earths surface. This is done because sea level is different at different times and places, and the earth's surface is uneven. So, to avoid ambiguity due to potentially different points of reference, a reference datum is used in mapping. For example, a sphere with a radius equal to the earth's average radius. Such a sphere would provide a constant surface to which elevations on the earth's actual surface could be referenced. However, the radius of the earth is greater at the equator and less at the poles, not perfect sphere. The resulting shape is an 'oblate ellipsoid'. By using an oblate ellipsoid as a datum for the earth, we

have a shape that approximates the shape of the earth, and provides a datum to which points all over the earth's surface can be referenced (reference datum).

The North American Datum, 1927 (NAD-27), based on the Clarke ellipsoid of 1866, is still used on many of the 7.5 minute (1:24,000 scale) USGS maps, as the reference datum. Due to technological advances, more precise measurements of the earth have resulted in modifications of the Clarke ellipsoid, for example GRS-80 (Geographic Referencing System, 1980). The most recent maps commonly use the NAD-83 referencing system which is based on the GRS-80 ellipsoid. The datum used for a map is printed on the front of a map.

Because the ellipses are different in the different reference systems used, the same point on the earth's surface can have different coordinates depending on the reference datum used. For this reason, it is highly important to know and record what reference datum you are using when you record coordinates from your Global Positioning System (GPS) unit. If you do not, and you record a coordinate and then plot it on a map using a different reference datum, you will not necessarily be plotting the point in the accurate position on the map. Note: a datum correction can be used on any set of coordinates so that the point plots correctly on the map being used, regardless of datum, if both reference datum (map and coordinate) are know.

In the setup menus of your GPS, you can find out what reference datum you are using. In addition, you can change the reference datum as well. Further information can be found at:

http://kartoweb.itc.nl/geometrics/Reference%20surfaces/body.htm

REFERENCES

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Centers for Disease Control and Prevention, 2003. Epidemic/Epizootic West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control, 3rd revision http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-aug-2003.pdf

O'Malley, Claudia, M., 1989. Guidelines for Larval Surveillance. Proceedings of the Seventy-Sixth Annual Meeting of the New Jersey Mosquito Control Association, Inc. 1989, pp 45-55.http://www-rci.rutgers.edu/%7Einsects/larvsurv.htm

McNelly, J. R., 1989. The CDC Trap as a Special Monitoring Tool. Proceedings of the Seventy-Sixth Annual Meeting of the New Jersey Mosquito Control Association, Inc. 1989, pp 26-33. http://www-rci.rutgers.edu/~insects/cdctrap.htm

Mosquito Surveillance Data

LaCrosse *or* West Nile?

(please circle the appropriate one)

Unique ID: _____ ☐ Check this box when data has been entered

Link to Human _____

Reason for Testing _____

GIS: Lat: _____ **Date:** _____

Long: _____ **County:** _____

Larval Surveillance:_____ **Reference Datum:**_____

CDC Gravid Trap:_____ **or** **CDC Light Trap:** _____

Habitat Description: ☐ Sun ☐ Shade **Site:** _____

(If multiple collection from the area)

Check if present:

Tires	☐ Yes	☐ No	#: _____	Distance from House_____
Overgrown Brush	☐ Yes	☐ No		Distance from House_____
Empty Containers	☐ Yes	☐ No	#: _____	Distance from House_____
Wooded Lot	☐ Yes	☐ No		Distance from House_____

Water Source _____

Other (specify)_____

Site Description:

Environmental Setting: _____ Habitat Type: _____

Permanence of Habitat: _____ Habitat Condition: _____

Size: _____ Depth: _____

Overall Habitat Description: _____

_____

_____

Codes for Mosquito Breeding Site Description

Environmental Setting

1. Business or Industrial
2. Urban
3. Suburban
4. Farm
5. Natural or Undeveloped
6. Park or Recreational
7. County, City or Town Property
8. State or Federal Property
9. Other

Habitat Type

1. Fresh Water, Marsh, Swamp, or Bog
2. Large Pond, Lake, or Ornamental Pond
3. Small Pond, Pit, or Hole (TH=tree hole)
4. Margin of Flowing Stream
5. Ditch or Sluggish Stream
6. Large Artificial Container (tank, boat, pool, etc)
7. Small Artificial Container (tire, drum, barrel, etc)
8. Other (describe)

Permanence of Habitat

1. Permanent - wetland, marsh, swamp
2. Winter-Spring Pond
3. Semi-permanent (water present more than 8 days after heavy rain)
4. Transient or Temporary (water present less than 8 days after heavy rain)
5. Stagnant, Land locked pond
6. Mostly standing but with outlet, overflow, or movement
7. Flowing Stream
8. Tidal
9. Other

Habitat Condition

(Use + or - for amount)

1. Open water - no vegetation and bare shoreline
2. Water polluted (oil, seqage, gas bubbles, film)
3. Solid trash or debris in water (tires, cans, etc.)
4. Water clean or nearly so
5. Woodland pool (no vegetation)
6. Vegetation present
7. Emergent Vegetation (CT=cattails, PH=phragmites)
8. Floating Vegetation
9. Much Marginal Vegetation
10. Other (describe)

West Virginia Bureau for Public Health
Mosquito Surveillance Form
page 1

West Virginia Bureau for Public Health
Mosquito Surveillance Form page 2 of 3

Unique ID ______________ Name______________ Date__________
Latitude______________ Longitude__________

Species	Number Present	Pool Number	Number In Pool	Tested Y	Tested N	WNV	LAC	SLE	EEE	Other

West Virginia Bureau for Public Health
Mosquito Surveillance Form page 3 of 3

Name_____ Date_____
Address_____
Phone _____
Latitude_____ Longitude_____
Reference Datum _____

Date Surveillance Conducted_____

Larval samples

Habitat Sampled	Sampling Method	Number of Dips	Volume Sampled	Number of Breeding Sites	Number of Larvae Sampled

Adult samples

Sampler Type	Attractant Used	Time/Date Set	Time/Date Retrieved	Height Above Ground	Comments

Notes/Recommendations:

Mosquito Collection Labels

Net Traps

Site Name________________ Date________
Sampler: Gravid CDC Light Other________
C0^2 Source: Dry Ice Tank Other________
Time Set ______ Time Retrieved_______
Collected by:____________ County:______________
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date______
Sampler: Gravid CDC Light Other________
C0^2 Source: Dry Ice Tank Other________
Time Set ______ Time Retrieved______
Collected by:____________ County:______________
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date________
Sampler: Gravid CDC Light Other________
C0^2 Source: Dry Ice Tank Other________
Time Set ______ Time Retrieved______
Collected by:____________ County:______________
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date______
Sampler: Gravid CDC Light Other________
C0^2 Source: Dry Ice Tank Other________
Time Set ______ Time Retrieved______
Collected by:____________ County:______________
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date________
Sampler: Gravid CDC Light Other________
C0^2 Source: Dry Ice Tank Other________
Time Set ______ Time Retrieved______
Collected by:____________ County:______________
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date______
Sampler: Gravid CDC Light Other________
C0^2 Source: Dry Ice Tank Other________
Time Set ______ Time Retrieved______
Collected by:____________ County:______________
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date________
Sampler: Gravid CDC Light Other________
C0^2 Source: Dry Ice Tank Other________
Time Set ______ Time Retrieved______
Collected by:____________ County:______________
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date______
Sampler: Gravid CDC Light Other________
C0^2 Source: Dry Ice Tank Other________
Time Set ______ Time Retrieved______
Collected by:____________ County:______________
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date________
Sampler: Gravid CDC Light Other________
C0^2 Source: Dry Ice Tank Other________
Time Set ______ Time Retrieved______
Collected by:____________ County:______________
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date______
Sampler: Gravid CDC Light Other________
C0^2 Source: Dry Ice Tank Other________
Time Set ______ Time Retrieved______
Collected by:____________ County:______________
Lat:__________ Lon:__________ Ref. Datum______

Larval Collections

Site Name________________ Date_________
Habitat: _______ Collected by: _________________
Number of dips:___ County: ______________
Number of Breeding Sites:______
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date______
Habitat: _______ Collected by: _________________
Number of dips:___ County: ______________
Number of Breeding Sites:______
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date_________
Habitat: _______ Collected by: _________________
Number of dips:___ County: ______________
Number of Breeding Sites:______
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date______
Habitat: _______ Collected by: _________________
Number of dips:___ County: ______________
Number of Breeding Sites:______
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date_________
Habitat: _______ Collected by: _________________
Number of dips:___ County: ______________
Number of Breeding Sites:______
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date______
Habitat: _______ Collected by: _________________
Number of dips:___ County: ______________
Number of Breeding Sites:______
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date_________
Habitat: _______ Collected by: _________________
Number of dips:___ County: ______________
Number of Breeding Sites:______
Lat:__________ Lon:__________ Ref. Datum______

Site Name________________ Date______
Habitat: _______ Collected by: _________________
Number of dips:___ County: ______________
Number of Breeding Sites:______
Lat:__________ Lon:__________ Ref. Datum______

Sampler Labels

DO NOT DISTRUB **WEST VIRGINIA BUREAU FOR PUBLIC HEALTH** **MOSQUITIO COLLECTON DEVICE** **1-800-423-1271**
CAUTION CONTAINS **DRY ICE** **CAUSES SKIN BURNS**
DO NOT DISTRUB **WEST VIRGINIA BUREAU FOR PUBLIC HEALTH** **MOSQUITIO COLLECTON DEVICE** **1-800-423-1271**
CAUTION CONTAINS **DRY ICE** **CAUSES SKIN BURNS**
DO NOT DISTRUB **WEST VIRGINIA BUREAU FOR PUBLIC HEALTH** **MOSQUITIO COLLECTON DEVICE** **1-800-423-1271**
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CAUTION CONTAINS **DRY ICE** **CAUSES SKIN BURNS**
DO NOT DISTRUB **WEST VIRGINIA BUREAU FOR PUBLIC HEALTH** **MOSQUITIO COLLECTON DEVICE** **1-800-423-1271**
CAUTION CONTAINS **DRY ICE** **CAUSES SKIN BURNS**

Note: These labels can be laminated and attached to trap, or they can be tape directly onto trap.

Note: This type of label can be modified to contain your specific contact information.

Mosquito Complaint Information Form

Name_____ **Date**_____
Address_____
Phone _____

Time of day mosquitoes active_____

Were the mosquitoes biting _____**Yes** _____**No**

What was the biting behaviour?

Aggressive _____**Yes** _____**No**

Biting on Face _____**Yes** _____**No**

Biting on Arms _____**Yes** _____**No**

Biting onLegs _____**Yes** _____**No**

How large were the mosquitoes?_____

What did the mosquitoes look like?_____

What was the flight pattern? _____

Are there suspected breeding grounds near by? _____**Yes** _____**No**

Other Comments:

West Virginia Bureau for Public Health
Mosquito Surveillance Results and Recommendations

Name_____ Date_____
Address_____
Phone _____
Latitude_____ Longitude_____
Reference Datum _____

Date Surveillance Conducted_____
Larval_____ CDC Light Trap_____ Gravid Trap_____

Number of pools submitted_____ Number of Pools Positive_____

Species Positive_____

Recommendations/Other information: