Mosquito Surveillance Activities Plan

I. Introduction

Mosquito surveillance, along with bird-based surveillance, should be a mainstay of regional surveillance programs for West Nile virus (WNV). 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. Mosquito surveillance is best used in conjunction with avian surveillance because birds are often the first sign of WNV activity in any area. Concentrations of infected birds serve as an indication of where to target intensive mosquito surveillance activities.

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. In Virginia, mosquito control is administered locally, either by mosquito control commissions, public works departments, or by district health departments. Therefore, the capabilities and nature of vector control varies from locality to locality, and as mosquito surveillance is an integral part of control, it will also vary with program capabilities. The southeastern region of Virginia has had numerous established mosquito control programs for many years, and these programs have the most well developed and experienced surveillance programs in the state. Most surveillance programs in the southeastern region of the state have adopted arboviral surveillance methodologies. The methodologies used by these districts were designed initially to detect Eastern Equine Encephalitis (EEE) virus, but more recently have been adapted to WNV surveillance. This plan addresses practices that can be used by existing mosquito surveillance programs to expand and adapt their surveillance capabilities for WNV surveillance. It will also serve as an guide for the establishment of vector mosquito surveillance programs in regions of the state where little or no vector control work has been done.

II. Objectives

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

1) Identifying the mosquito species that are present in a region;
2) Identifying the mosquito species that are the cause of local citizen complaints, and determining whether they are important WNV vector species;
3) Identifying and mapping 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 to control adult mosquitoes;

5) Determining the population density and the desired threshold (mosquito population density) for initiating 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 by WNV and/or other arboviruses;
9) Determining the mosquito infection rate (MIR) for WNV or other arboviruses in a vector species population; and
10) Determining the seasonal activity patterns of local mosquito species.

III. Implementation Plan

A. General

The VDH Public Health Entomologist will coordinate and encourage collaboration among a network of mosquito control professionals, public health entomologists and vector control organizations in Virginia to carry out mosquito surveillance and to assist in the education of government officials and personnel involved in vector surveillance and control. The Entomology Department at Virginia Tech will provide technical advice and assist in surveillance activities in southwestern and western portions of Virginia. The Virginia Mosquito Control Association (VMCA) will offer technical, educational and organizational services to mosquito surveillance and control professionals throughout the state. VDH and the Virginia Interagency Arbovirus Task Force will develop and disseminate yearly updates of the WNV Response Plan. Regions of the state and their associated mosquito surveillance programs are listed below.

1. **Eastern Region** – Mosquito surveillance operations in this region are conducted by the following Health Districts and organizations: Norfolk Department of Public Health, Division of Vector Control; Virginia Beach Mosquito Control; Chesapeake Mosquito Control; Suffolk Mosquito Control; Portsmouth Mosquito Control; Hampton Mosquito Control; Newport News Mosquito Control; York County Mosquito Control; James City County Mosquito Control; Langley Air Force Base Mosquito Control; and the Eastern Shore Health District. Personnel from many of the above organizations belong to the Tidewater Regional Arbovirus Surveillance Team (TRAST), and/or the Virginia Mosquito Control Association (VMCA), which play key roles in coordinating and supporting vector surveillance and control activities in the southeastern region.

2. **Southwest Region** – Mosquito surveillance in this region is being conducted by the following Health Districts and organizations: New River Health District; Lenowisco Health District; Allegheny Health District; and Virginia Tech Department of Entomology (Medical Entomology Section).

3. **Northern Region** – Mosquito surveillance in this region is being conducted by the following Health Districts and organizations: Alexandria City Health Department; Arlington County Health Department; Fairfax County Health Department; Loudoun County Health Department; and Prince William County Mosquito Control.

4. **Northwest Region** – Mosquito surveillance in this region is being conducted by personnel from the Lord Fairfax Health District.

5. **Central Region** – Mosquito surveillance in this region is being conducted by the following Health Districts and organizations: Richmond City Health Department, Chesterfield Cooperative Extension Service; and the Henrico County Extension Service.

In addition to the above listed organizations involved in mosquito surveillance, the VDH - Office of Epidemiology hires two seasonal mosquito biologists each summer to conduct mosquito surveillance around the state. These biologists are under the supervision of David Gaines, Ph.D. (VDH-Public Health Entomologist), and operate out of a laboratory space within the State Division of Consolidated Laboratory Services (DCLS) in Richmond. They are available to conduct investigative surveys of mosquito populations and arboviral events in any jurisdiction that requests their assistance. Although they have the capability to investigate WNV events in any part of the state, they are only available for special investigations, and cannot serve as a substitute for a season-long, local mosquito surveillance program. VDH strongly recommends that jurisdictions needing mosquito surveillance capabilities work towards the development their own surveillance personnel.

B. Surveillance Methods

Mosquito Surveillance involves numerous different strategies and practices. A variety of different methods are used to trap mosquitoes in the field because different mosquito species have different behavior and biology and cannot all be collected by the same method. For example, some mosquito species are readily caught in traps whereas other species are rarely collected by use of traps. Different types of traps are used for different species of mosquitoes. Also, larval mosquitoes occupy a different environment than adult mosquitoes, so collection methods used for larvae are much different than those used for adults. For WNV surveillance, appropriate species of adult mosquitoes should be collected, pooled and submitted to the laboratory for arboviral testing. Surveillance should be utilized for determination of arboviral risk as well as for planning, execution, and evaluation of control practices.

1. **Larval Surveillance -** Surveys of immature mosquitoes are an important aspect of any surveillance program, and for certain species larval surveillance may be a more accurate measure of mosquito population

density than adult trapping. Larval surveillance is essential for the appropriate targeting of larval control methods. Larval surveillance should begin early in the season, even before adults are active to help identify the breeding sites of vector species so that larval control efforts can be targeted. Larval surveillance can be conducted as part of inspection and complaint investigation activities and is often done in conjunction with the application of larvicides for control. In areas where there is no baseline mosquito surveillance data, larval samples can be used to identify and map vector-breeding sites. This information can then be used to help in determining appropriate trap locations for monitoring adult mosquito populations.

a. Yard Inspections: Whenever an environmental health person or mosquito control staff member is in a private yard around a home, 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 can be dipped for larval samples. Samples can be poured into whirl-pack or zip-lock bags and returned to the office for larval identification or maturation to adult stage for identification. Larval habitats can be dumped, but if homeowners are home, habitats and larval samples should first be shown to the homeowner for their own edification. This provides an opportunity for educating the property owner or occupant on how to eliminate a potential or existing mosquito problem.

b. Equipment, Techniques and Record Keeping: 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. Larval surveillance may require the use of different dipping techniques depending on the target species and habitat (see Attachment 3.A). Accurate records should be kept of when and where larvae are collected (see Larval Surveillance Data Form; Attachment 3.A).

2. **Adult Surveillance** - Because it is the adult female mosquito that carries and transmits disease, many surveillance techniques have been devised to collect adult female mosquitoes 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. There are also

certain mosquito species that will not be attracted to traps and which 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 different habitats in different parts of a jurisdiction. Trapped mosquitoes that have been identified can either be logged into the computerized mosquito database (see section B.5 below), or may be logged onto a paper data sheet for future data entry (see Weekly Adult Mosquito Trap/Collection Form, Attachment 3.D). The most common trapping and adult collection and monitoring methods used include:

a. Reiter gravid trap – The Reiter gravid trap is designed to collect gravid mosquitoes and is among the most important mosquito traps used for WNV surveillance. Gravid mosquitoes are mosquitoes that are carrying eggs and are seeking a place 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 *Aedes* species captured in gravid traps include: the Asian tiger mosquito (*Aedes albopictus*), 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 manure, etc.). When trapping *Culex* species it is best to use a bait infusion made from a recommended formula (see Attachment 3.F for a formula used to make a highly effective gravid trap bait for *Culex* species). 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. 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, and fresh specimens are preferred for arboviral testing; virus isolation by tissue culture works best in mosquitoes that have been dead for less than a day.

b. CDC light trap (Attachment 3.B) – CDC light traps are one of the standard tools for arboviral surveillance. Like the gravid trap, this trap is very portable because it is lightweight 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 a host for a blood meal. Unlike the gravid trap, a CDC type light trap attracts a relatively wide variety of species and because of this, is the best trap to use for identifying the species composition of a locality. 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 some 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. Mosquitoes are trapped live, and this feature helps maintain the freshness of mosquito specimens that are being tested for arboviruses. CDC light traps use only a small light source that attracts relatively few non-mosquito insect species such as beetles and moths. When large beetles and moths get collected in a trap, they can damage the trapped mosquitoes. The CDC-light trap collects mosquitoes that are mostly undamaged and this makes them easy to identify.

c. New Jersey light trap (See Attachment 3.C) - This trap has historically been a major component of mosquito abatement programs, but is not very useful for arboviral surveillance. These traps use a 25-watt light bulb as an attractant, and a fan draws the insects into a collection jar, which is usually equipped with a vapona strip as a killing agent. A disadvantage of New Jersey light traps is that they are large and heavy, and require standard electrical current. That limits trap placement to locations where there is availability of electricity. Another disadvantage is that

many large, non-mosquito insects are attracted to the light bulb used in the New Jersey trap, and these larger insects often damage the collected mosquito specimens badly enough that identification is impossible. Unidentifiable, dead specimens are not useful for arboviral testing. Two important target species for WNV surveillance (C*ulex pipiens* and *Aedes vexans*) are attracted to New Jersey traps, and in areas where these species are the predominant mosquito, a New Jersey light trap can be used to monitor their relative population density over time. New Jersey traps are best used in areas where only a few predominant species occur (e.g., near a salt marsh). In such locations the collected species do not need to be identified and the trap catches only need to be counted to provide relative mosquito numbers, from week to week as a means of directing adult mosquito control activities.

d. 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. Blood fed or gravid mosquitoes are much more likely to be infected with WNV. 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.

e. Citizen Complaints - If the public is informed about whom to call, citizen complaints about adult mosquito activity or about potential breeding habitats are useful for mosquito surveillance. Maintaining records of citizen complaints, can contribute toward identification and mapping mosquito problem areas. 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 affected by adult mosquito activity 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 sizable portion of citizen complaints are unfounded or misidentify the source of the mosquitoes, and so it may take a person with some knowledge of mosquito biology to question the complainant and get the complete and pertinent facts. Obtaining detailed information 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 visitations/investigations. For example, adult mosquitoes are relatively small and are generally difficult to observe, so people do not normally notice them unless they are biting or trying to bite. There are thousands of small flying insects species that might be mistaken for mosquitoes, so if the insects noticed by the complainant were not trying to bite or biting, it is probably unlikely that they were mosquitoes. Questioning the complainant about the time of day mosquitoes were 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 most complaints in Virginia are related to Asian tiger mosquito activity, determining that the mosquitoes are biting during daylight hours will indicate that the problem mosquitoes are most probably 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 have originated 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 are biting during the daylight hours and that they originate from a nearby pond or ditch, the person taking the complaint will know that the identified habitats are an unlikely source.

3. **Mapping and Analysis of Mosquito Surveillance Data** - Surveillance activities should include locating mosquito breeding habitats and defining the geographic range (area) affected by adult mosquitoes from an identified habitat. Habitats and areas of adult activity can be marked on paper maps used for reference when planning control activities. The use of Global Positioning System (GPS) devices is recommended for accurate mapping, and is indispensable for mapping with computer based Geographic Information Systems (GIS) software. Use of GIS requires good surveillance data management. It also requires adequate computer hardware and software. GIS mapping allows the incorporation of many map layers that include such information as: road layout, jurisdictional boundaries, human population density, aquatic and/or wetland habitat types, topography, aerial photography indicating vegetation zones, etc. These many map features can aid in the analysis of mosquito data, or in

the planning of control programs. Some mosquito control programs use GPS devices linked to GIS maps and to spraying equipment to plan, control, and monitor their insecticide coverage during control operations. Many local governments have planning departments with GIS capabilities, and these departments may be useful as a GIS mapping resource.

4. **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 Attachment 3.E). Collected mosquitoes should be pooled for testing. Pools of most approved mosquito species consist of 25 to 50 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. The labs will not test pools containing fewer than 10 mosquitoes unless the submitter has obtained prior approval to submit them. Approval can be obtained by contacting Dr. David Gaines, (Public Health Entomologist, VDH-Office of Epidemiology, Tel. [804] 786-6261), and/or by contacting personnel in charge of testing at the laboratories (see Attachment 3.E).

   Pooled mosquitoes should be accurately identified and grouped by species, site, and week of collection. Depending on the county, city or geographic location of your surveillance program pooled mosquitoes should either be sent to the Norfolk Department of Public Health Laboratory (NDPHL) or to the State Division of Consolidated Laboratory Services (DCLS) laboratory in Richmond for testing (see Attachment 3.E). Pools should be frozen until shipment or delivery to the laboratory, and shipped in insulated containers packed with dry ice. Target species for laboratory submission vary by disease/pathogen of concern. For WNV, 19 species are currently given priority for testing. Within the *Culex* genus, *Cx. pipiens*, *Cx. salinarius*, *Cx. restuans*, and *Cx. erraticus* are tested. The mosquito *Culiseta melanura* is also tested. The *Aedes* species tested include *Aedes albopictus* (the Asian tiger mosquito) and *Ae. vexans*. *Ochlerotatus* species that breed in containers (i.e., *Oc. atropalpus*, *Oc. triseriatus,* and *Oc. japonicus*) are considered important to test. Several other salt marsh and floodwater *Ochlerotatus* species are also recommended for testing (see Attachment 3.E). The *Culex* species and the *Culiseta melanura* mosquito might all act as important primary vectors (bird feeding species that amplify WNV in the bird population). All of the above species except *Cx. restuans* and *Cs. melanura* may also act as bridge vectors (species that can transmit WNV from birds to humans or other mammals). Except for *Cs. melanura*, and *Cx. erraticus*, all of the above listed species have been tested and proven to have WNV vector competence in laboratory trials. Field collected pools of each of the above listed species have tested positive for WNV in the United States. Early season pool submissions should concentrate on primary vector species

involved in amplification of the virus in the bird population. Once WNV has been detected in the local bird or primary vector population, pooling and testing efforts should also concentrate on bridge vector species of mosquitoes.

5. **Surveillance and Testing Data** – The use of a **standardized** database by Virginia's mosquito surveillance programs facilitates compilation of data for the entire state, and comparison of mosquito data from one region to another. When collected mosquitoes are pooled for testing, the use of a standardized database to report those pools to the laboratory facilitates the entry and tracking of that data by laboratory personnel as well as the accurate analysis and reporting of test results to the collecting agency, VDH and the CDC.

   A database for the management of adult mosquito surveillance records and submitted mosquito pool records will be available from the VDH in May of 2003. The database written for MSDE (SQL Server software) will be provided free of charge, to all surveillance programs statewide, on a CD-ROM. The operating software (MSDE) the database software and database operating instructions will be included on the CD-ROM. Newly organized surveillance programs are encouraged to obtain and use this database for their own mosquito data management. Programs needing a copy of the database should contact Dr. David Gaines, (Public health Entomologist, VDH-Office of Epidemiology, Tel. [804] 786-6261). Programs using the database should e-mail copies of their weekly mosquito pool data to their mosquito-testing laboratory on the same date that pools are sent to the lab. Sending an electronic copy of each week's pool data to the laboratory will facilitate laboratory management of test data. Surveillance programs that do not have computer capabilities may use the Weekly Adult Mosquito Trap Collection, and Pool Submission Forms (found in Attachments 3.D and 3.E). Programs conducting larval surveillance should compile larval surveillance data using the larval surveillance form (Attachment 3.A).

C. Phased Response Plan

The phased response plan provides recommended levels of surveillance activity, and recommended surveillance activities for each level of WNV activity detected in a geographic region. Each jurisdiction should define its own geographic surveillance region based on human population density, topography, road layout or other locally important geographic features. (See the Avian Surveillance, Section C. 6. of the WNV Plan)

1. **Level Ia** – Winter weather, low likelihood of WNV epizootic activity, and little or no adult mosquito vector activity present.

a. Larval surveillance – Use maps, windshield surveys and walking surveys or aerial surveillance to identify and map the locations of wet or flood prone areas, tire piles and other potential mosquito breeding habitats. The absence of foliage on trees or shrubs during the winter season facilitates the observation and identification of potential mosquito breeding habitats from the road or from the sky.

b. Adult mosquito surveillance – Analyze and map previous year's citizen complaint calls and surveillance records of mosquito activity to help determine future target locations for surveillance and control activities.

2. **Level Ib** – Early mosquito breeding season (April – May), adult mosquito activity present, no current evidence of WNV epizootic activity.

a. Larval surveillance – Use investigative surveillance (larval dipping) in suspected habitats to identify mosquito breeding sites. Identify neighborhoods where container breeding mosquitoes are a problem.

b. Adult mosquito surveillance – Use investigative trapping and collections in suspected and identified problem areas to identify the species present and to monitor for vector species. Target surveillance to detect primary vector species (*Culex* species) by extensive use of gravid traps and CDC-light traps. Commence pooling and testing of mosquitoes, focusing on primary vector species only. There is little need to be testing bridge vector species at this time.

3. **Level II** – Early Summer (June – July), mosquito larvae and adults present, initial evidence of WNV epizootic activity in region (limited to birds or mosquitoes).

a. Focus mosquito surveillance efforts in areas close to where WNV infected birds have been found. Clusters of WNV infected birds are an indication of considerable WNV activity in primary vector species. Primary vectors include *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius*, (some other species such as *Culiseta melanura* or even *Cx. erraticus* might also merit investigation even though their importance as WNV vectors has not yet been established). Surveillance efforts should focus on detecting the habitats of the primary vectors for control purposes. Collected adults of primary vector mosquito species should be tested to measure their WNV infection rates.

b. Larval surveillance: Seek out habitats and conduct investigative larval dipping to identify primary vector habitats in the vicinity of WNV positive bird finds. If possible, continue to conduct regularly scheduled (weekly) larval mosquito surveillance in other identified breeding habitats.

c. Adult Surveillance: Use both gravid traps and CDC-light traps to detect and test primary vector species in areas close to WNV positive bird finds. Gravid traps are the best means of capturing *Cx. pipiens* or *Cx. restuans*, and CDC light traps are the best means of collecting the other potential primary vector species (*Cx. salinarius*, *Cx. erraticus* and *Cs. melanura*). If possible, continue to conduct regularly scheduled (weekly) adult mosquito surveillance at other identified problem areas using light traps and gravid traps. Commence to focus attention on suspected important bridge vector species such as *Aedes albopictus*, *Ae. vexans*, *Ochlerotatus triseriatus* and *Oc. japonicus*. Submit pooled vector mosquito species to laboratory for testing.

4. **Level III** – Mid-summer to Fall, moderate WNV epizootic activity in mosquitoes and birds with initial evidence of WNV in a horse or human.

   Increase larval and adult mosquito surveillance in identified areas of WNV epizootic activity. Continue use of both gravid and CDC type traps and increase pooling and testing of both primary vector and important bridge vector species. Investigate and test mosquito populations in the vicinity of all equine and human cases.

5. **Level IV** – Mid-summer to Fall, WNV epizootic activity suggesting high risk of human infection (i.e., high dead and/or positive bird densities, high mosquito infection rates). confirmed human or horse case, abundant adult bridge vectors.

   Maintain heightened levels of larval and adult mosquito surveillance and adult mosquito testing. Focus more adult surveillance efforts on populations of potential bridge vector species in areas where WNV activity has been found in primary vector species, horses and humans.

6. **Level V** – Mid-summer to Fall, multiple human cases of WNV and conditions favoring further transmission to humans.

   Maintain heightened levels of larval and adult mosquito surveillance and adult mosquito testing. Where possible, investigate mosquito populations (species and infection rates) in the vicinity of where identified human cases were thought to have been exposed to mosquitoes.

Larval Surveillance Procedures

(Modified from O'Malley, 1989)

Basic tools: Standard, white 400 ml-capacity dipper; an eyedropper; turkey baster, tea strainer, modified bilge pump, white enamel or plastic pan, boots, vials, 6 oz. plastic bags or some other form of container for collecting larvae; labels for the collections; and a pencil. A GPS receiver should also be used to obtain data for GIS.

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.

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 dipping should be done on the windward side of the habitat where larvae and pupae will be most heavily concentrated.

Mosquito larvae are usually confined to the margins of a body of water and will not be found in open, deep water. Dipping 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 dip.

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.

Collection Methods: The kind of mosquito one is looking for, as well as the type of habitat one is working in, will determine the dipping 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. The following eight 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*.

8. Salt marsh - As the name indicates, this is a procedure to utilize when conducting salt marsh larval surveillance. In the case of salt marsh potholes, dip in a number of spots around the edge of the pothole, dipping in toward the edge. Sample the middle of the pothole, using either a skimming or scooping stroke. In areas containing numerous potholes, make sure several are sampled, not just one or two. Use the same combination of techniques to sample a salt marsh pan.

It is important to recognize that there are different techniques which can be used in different habitat types. 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.

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:

- *Ae. albopictus* - tires
- *Ae. atropalpus* - rock pools, tires
- *Ae. triseriatus* - treeholes, tires, containers
- *An. barber* - treeholes, tires, containers
- *Cq. perturbans* - permanent water with emergent vegetation
- *Cs. melanura* - Cedar and red maple swamps, occasionally tires
- *Or. alba* - treeholes, tires, containers
- *Or. signifer*: - treeholes, tires, containers
- *Tx. r. septentrionalis* - treeholes, tires, containers
- *Wy. 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. Modifying a hand-operated bilge pump by removing the intake valve converts the pump to a syringe capable of drawing up a column of water (Walker and Crans, 1986). The modified bilge pump can also be used to sample treeholes, tires and various other containers. Material collected by bilge pump or baster can be emptied into a white enamel pan, from which the mosquito larvae are then removed.

Measurement of Density: 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.

Data Collection: Due to the current lack of an adequate computerized database for larval surveillance data, a “Larval Surveillance Data Form” should be completely filled out for each collection. Consult the attached “Code Sheet” for breeding site description and control options.

LARVAL MOSQUITO SURVEILLANCE LOG

Collection Date

County/City

Town/Borough

Property Owner

Map Coordinates

Latitude

Longitude

Address/Location

Telephone

Mosquito Aquatic Habitat (circle one)

1. Lake/Pond Margin
2. Permanent Wetland/Marsh
3. Seasonal Wetland/Marsh
4. Saltmarsh/Tidal Pool
5. Vernal Pond
6. Forest Flood Pool
7. Field Flood Pool
8. Puddle
9. Receding River/Creek Pool
10. Rock Pool
11. Sluggish Stream
12. Flooded Ditch
13. Storm-sewer Pool (underground)
14. Storm-water Pond (BMP)
15. Large Artificial Container (>30 gal.)
16. Small Artificial Container
17. Tree Hole
18. Livestock Wallow

Larvae Present (circle one) Yes No

Pupae Present (circle one) Yes No

Eggs Present (circle one) Yes No

Predominant Instars (circle one)

1. 1st Instars
2. 2nd Instars
3. 3rd Instars
4. 4th Instars
5. Pupae
6. Mixed 1st & 2nd instars
7. Mixed 2nd & 3rd instars
8. Mixed 3rd & 4th instars
9. Mixed 4th instars & Pupae

Average # Larvae Per Dip

Number of Dips

Control Measure Applied (circle) Yes No

Control Measure Applied (circle one)

1. Dumped/Drained
2. Agnique
3. Surface oil
4. Larvicide (B.t.)
5. Larvicide (B.s.)
6. Larvicide (Altosid)
7. Larvicide (Abate)
8. Mosquito Fish

Notes

Characteristics of Aquatic Habitat (circle one or more)

1. Muddy
2. Clear
3. Salty
4. Fresh
5. Polluted, Organic Waste
6. Polluted, Mineral Waste
7. Polluted Organic/Mineral
8. Floating Debris/Trash
9. Algae Turbid (green water)
10. Algae Crusted (bottom/sides)
11. Algae Floating
12. Aquatic Emergent Grasses
13. Aquatic Floating Vegetation
14. Peat choked (black water)
15. Live Grass Choked
16. Dead Grass Tea
17. Live Vegetation Choked
18. Dead Vegetation Tea
19. Tree Leaf Tea

CDC Light Trap Procedures

(Modified from McNelly, 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.

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.

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.

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. 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.

New Jersey Light Trap Procedures

The New Jersey light trap depends on a 110-volt source of electric power, which somewhat restricts its use. Most often, the collection is funneled into a collection jar. This makes the collection suitable for relative abundance studies, but unacceptable for arbovirus studies that require live specimens. The traps can be very useful in monitoring changes in abundance and species diversity in an area. They can also be used to document efficacy of control efforts.

Not all mosquito species are attracted to or collected by New Jersey light traps. There is considerable variation in the relative attractiveness of different mosquito species to light. Generally, light traps do not reflect the abundance or presence of species that are negatively phototaxic or only active during the day. In addition, mosquito species that inhabit wooded areas are less attracted to light traps then those that prefer open areas. The following species **can** be accurately monitored by the New Jersey light trap:

Ae. vexans	*Oc. trivittatus*	*Cq. perturbans*
Oc. cantator	*An. bradleyi*	*Ps. columbiae*
Oc. solicitans	*Cx. pipiens*	
Oc. taeniorhynchus	*Cx. salinarius*	

The following species **cannot** be accurately monitored with a New Jersey trap:

Ae. albopictus	*Oc. excrucians*	*An. quadrimaculatus*
Oc. canadensis	*Cx. restuans*	*Cs. melanura*
Oc. stimulans	*Cx. territans*	*Ps. ferox*
Oc. triseriatus	*An. punctipennis*	

Guidelines for New Jersey Trapping: The following guidelines are offered to minimize variability in the use of CDC traps for mosquito surveillance.

1) Select a location with little or no competing light source. The area must have an electrical source to power the trap.

2) Hang the trap on a pole or tree limb sturdy enough to hold 20 lbs. The height should be around 1.5 meters. Once the trap is hung the automatic timer (if the unit does not have a photo-sensor) must be set to turn the unit on at dusk and off at dawn. The collection jar with a vapona strip as a killing agent and a 7oz. Dixie cup with small holes in the bottom are placed inside the jar to capture the mosquitoes.

3) Samples are collected from the New Jersey traps three (3) times per week by taking an extra sample jar to empty the contents of the kill jar in and returning the sample to the lab for enumeration and species identification. These mosquitoes are not used for viral testing but all data is recorded and maintained on file.

Weekly Adult Mosquito Trap / Collection Form

County/City:	**Address/Location**
Town/Borough	
Collection Site ID *(see footnote):	

Week #	**Sample Dates: From_____ To_____**	**Year**
Trap Type		

Genus species	**Abbreviation**	**Collection Dates in Sample Week**				**Total Females Per Week**
		Day 1	**Day 2**	**Day 3**	**Day 4**	
***Aedes* species**						
Ae. aegypti	*Ae. aeg*					
Ae. albopictus	*Ae. albo.*					
Ae. cinereus	*Ae. cin.*					
Ae. vexans	*Ae. vex.*					
***Ochlerotatus* species (formerly *Aedes*)**						
Oc. atlanticus/tormentor	*Oc. atl/tor.*					
Oc. atropalpus	*Oc. atro.*					
Oc. aurifer	*Oc. aur.*					
Oc. canadensis	*Oc. cana.*					
Oc. cantator	*Oc. cant.*					
Oc. cinerus	*Oc. cin*					
Oc. dupreei	*Oc. dup.*					
Oc. fulvus pallens	*Oc. f. pal.*					
Oc. grossbecki	*Oc. gros.*					
Oc. hendersoni	*Oc. hen.*					
Oc. infirmatus	*Oc. inf.*					
Oc. japonicus	*Oc. jap.*					
Oc. mitchellae	*Oc. mit.*					
Oc. sollicitans	*Oc. sol.*					
Oc. sticticus	*Oc. stic.*					
Oc. stimulans	*Oc. stim.*					
Oc. taeniorhynchus	*Oc. tae.*					
Oc. thibaulti	*Oc .thib.*					
Oc. triseriatus	*Oc. tris.*					
Oc. trivittatus	*Oc. triv.*					
Oc. _____						
***Anopheles* species**						
An. atropos	*An. atro.*					
An. barberi	*An. barb.*					
An. crucians/bradleyi	*An. cr/br.*					
An. perplexens	*An. perp.*					
An. punctipennis	*An. punc.*					
An. quadrimaculatus	*An. quad.*					

An. walkeri	*An. walk.*					
An. _____						
***Coquilletidia* species**						
Cq. perturbans	*Cq. per.*					
***Culex* species**						
Cx. erraticus	*Cx. err.*					
Cx. nigripalpus	*Cx. nig*					
Cx. peccator	*Cx. pec.*					
Cx. pilosus	*Cx. pil.*					
Cx. pipiens	*Cx. pip.*					
Cx. restuans	*Cx. res.*					
Cx. pipiens/restuans	*Cx. pip/res.*					
Cx. salinarius	*Cx. sal.*					
Cx. tarsalis	*Cx. tar.*					
Cx. territans	*Cx. terr.*					
Cx. _____						
***Culiseta* species**						
Cs. inornata	*Cs. inor.*					
Cs. melanura	*Cs. mel.*					
Orthopodomyia						
Or. alba	*Or. alba*					
Or. signifera.	*Or. sig.*					
***Psorophora* species**						
Ps. ciliata	*Ps. cil.*					
Ps. columbiae	*Ps. col.*					
Ps. cyanescens	*Ps. cyan.*					
Ps. discolor	*Ps. disc.*					
Ps. ferox	*Ps. fer.*					
Ps. horrida	*Ps. hor.*					
Ps. howardii	*Ps. how.*					
Ps. mathesoni	*Ps. math.*					
Ps. varipes	*Ps. var.*					
Ps. _____						
***Toxorhynchites* species**						
Tx. rutilus septentorialis	*Tx. r. sep.*					
***Uranotaenia* species**						
Ur. lowii	*Ur. low.*					
Ur. sapphirina	*Ur. saph.*					
***Wyeomyia* species**						
Wy. smithii	*Wy. smit*					
TOTAL						
Mutilated (Unidentifiable) Specimens						
Male Mosquitoes						
Predominant Male Species (Abbreviate)						

* See footnote for Collection Site ID codes below. I

* Collection Site ID = two letter County/City code + four digit site number (e.g., VB-0012 would be Virginia Beach collection site #12; once used to describe a collection site, the Collection Site ID should be permanently assigned and should not be used again for another site (Use the two letter County/City code list below).

County/City	Alpha Code	FIPS Code	County/City	Alpha Code	FIPS Code	County/City	Alpha Code	FIPS Code	County/City	Alpha Code	FIPS Code
Accomack Co.	AC	001	Dickenson Co.	DK	051	Lancaster Co.	LR	103	Pulaski Co.	PU	155
Albemarle Co.	AL	003	Dinwiddie Co.	DN	053	Lee Co.	LE	105	Radford City	RF	750
Alexandria City	AX	510	Emporia City	EM	595	Lexington City	LX	678	Rappahannock Co.	RA	157
Alleghany Co.	AY	005	Essex Co.	EX	053	Loudon Co.	LN	107	Richmond City	RM	760
Amelia Co.	AM	007	Fairfax City	FA	600	Louisa Co.	LS	109	Richmond Co.	RI	159
Amherst Co.	AH	009	Fairfax Co.	FX	059	Lunenburg Co.	LU	111	Roanoke City	RK	770
Appomattox Co.	AP	011	Falls Church City	FC	610	Lynchburg City	LY	680	Roanoke Co.	RO	161
Arlington Co.	AR	013	Fauquier Co.	FQ	061	Madison Co.	MD	113	Rockbridge Co.	RB	163
Augusta Co.	AU	015	Floyd Co.	FD	063	Manassas City	MS	683	Rockingham Co.	RH	165
Bath Co.	BA	017	Fluvanna Co.	FV	065	Manassas Park City	MP	685	Russell Co.	RU	167
Bedford City	BF	515	Franklin City	FN	620	Martinsville City	MV	690	Salem City	SA	775
Bedford Co.	BE	019	Franklin Co.	FR	067	Mathews Co.	MT	115	Scott Co.	SC	169
Bland Co.	BL	021	Frederick Co.	FK	069	Mecklenburg Co.	MB	117	Shenandoah Co.	SH	171
Botetourt Co.	BO	023	Fredericksburg City	FG	630	Middlesex Co.	MX	119	Smythe Co.	SM	173
Bristol City	BR	520	Galax City	GX	640	Montgomery Co.	MY	121	Southampton Co.	SO	175
Brunswick Co.	BK	025	Giles Co.	GI	071	Nelson Co.	NL	125	Spotsylvania Co.	SP	177
Buchanan Co.	BN	027	Gloucester Co.	GL	073	New Kent Co.	NK	127	Stafford Co.	SD	179
Buckingham Co.	BM	029	Goochland Co.	GO	075	Newport News City	NN	700	Staunton City	ST	790
Buena Vista City	BV	530	Grayson Co.	GR	077	Norfolk City	NO	710	Suffolk City	SK	800
Campbell Co.	CA	031	Greene Co.	GN	079	Northampton Co.	NH	131	Surry Co.	SU	181
Caroline Co.	CE	033	Greensville Co.	GV	081	Northumberland Co.	ND	133	Sussex Co.	SX	183
Carroll Co.	CR	035	Halifax Co.	HX	083	Norton City	NT	720	Tazewell Co.	TZ	185
Charles City Co.	CC	036	Hampton City	HN	650	Nottoway Co.	NW	135	Virginia Beach City	VB	810
Charlotte Co.	CT	037	Hanover Co.	HR	085	Orange Co.	OR	137	Warren Co.	WA	187
Charlottesville City	CV	540	Harrisonburg City	HB	660	Page Co.	PA	139	Washington Co.	WN	191
Chesapeake City	CK	550	Henrico Co.	HO	087	Patrick Co.	PT	141	Waynesboro City	WB	820
Chesterfield Co.	CD	041	Henry Co.	HY	089	Petersburg City	PB	730	Westmoreland Co.	WE	193
Clarke Co.	CL	043	Highland Co.	HI	091	Pittsylvania Co.	PI	143	Williamsburg City	WM	830
Colonial Heights City	CH	570	Hopewell City	HL	670	Poquoson	PQ	735	Winchester City	WR	840
Covington City	CN	580	Isle of Wight Co.	IW	093	Portsmouth City	PM	740	Wise Co.	WI	195
Craig Co.	CG	045	James City Co.	JC	095	Powhatan Co.	PH	145	Wythe Co.	WY	197
Culpeper Co.	CP	047	King and Queen Co.	KQ	097	Prince Edward Co.	PE	147	York Co.	YK	199
Cumberland Co.	CU	049	King George Co.	KG	099	Prince George Co.	PG	149			
Danville City	DV	590	King William Co.	KW	101	Prince William Co.	PW	153			

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

Mosquitoes tested for West Nile virus (WNV) or any of the other arboviruses should be collected live and maintained in that condition until they have been identified and pooled; WNV degrades quickly in dead mosquitoes; any mosquitoes that are tested by the tissue culture method for virus isolation should be fairly fresh. To maintain the live virus in mosquitoes, they should be frozen soon after death. WNV will degrade and not grow on tissue cultures when it is in mosquitoes that have been dead and exposed to room temperatures for more than one day. After identification and pooling, it is ok to kill the mosquitoes by freezing. Materials needed for processing and pooling mosquitoes are as follows:

Item Description	**Source ***	**Part Number**
Triethylamine - (500 ml bottle)	Fisher Scientific	BP 616 500*
Disposable polypropylene culture tubes 12x75mm (1000. case) **Required for pools tested by the Norfolk Public Health Laboratory**	VWR Scientific	60818-281*
Plug type caps for tubes (1000/case) **Required for pools tested by the Norfolk Public Health Laboratory**	VWR Scientific	60819-070*
Fisherbrand Microfuge Tubes (2.0 ml conical screw cap tubes with caps and O-rings, sterilized (500 per case) **Required for pools tested by the State Division of Consolidated Laboratory Services (DCLS) Laboratory**	Fisher Scientific	Cat # 02-681-375

* Similar items may also be available from other suppliers.

Procedures: When possible, trapped mosquitoes should be returned to the laboratory alive. Trap bags or containers may be placed in a 48 qt, cooler chest so they do not become overheated in the vehicle after collection and during transport. Just prior to identification mosquitoes should be anesthetized with **Triethylamine (TEA)**. They can also be anesthetized by holding them in a closed cooler containing dry ice for 15 minutes. When using Triethylamine, trap bags or containers of live mosquitoes may be placed in a heavy-duty trash bag along with a cotton wad soaked with one bottle cap-full of Triethylamine. This operation should be performed outdoors in a well ventilated area, and rubber gloves should be worn to avoid dermal contamination with TEA. The trash bag should be held closed for approximately 8 minutes and then opened to check the condition of the mosquitoes. If some mosquitoes still have their wings buzzing, close the trash bag for an additional minute to achieve complete anesthetization. Anesthetization is an operation that requires precise timing and observation. An exposure of less than 8 minutes may not anesthetize mosquitoes sufficiently for sorting and identification. An exposure of 10 minutes or more may kill the mosquitoes. Some slight day-to-day, species-to-species variations may occur in the time required for anesthetization.

Anesthetized mosquitoes should be sorted, identified and pooled as quickly as possible. Mosquito pools should be made by **species**, **date collected**, and **location collected**. Each pool

identification label should contain a "Sample Number". If you are using the mosquito database (See Section B.5 of the Mosquito Surveillance Plan), the database will automatically create a Sample Number for each new pool entered into the database. Otherwise, the Sample number will appear as follows P-0784-HENR-29-03, with the P and the first four digits of the sample number being the pool number, the four letter code being the Surveillance Program ID [in this case it is HENR for the Henrico County program], the finally there is a two digit week number followed by a two digit year number. If you are not using the database which automatically numbers all entered pools, pools should be numbered consecutively (i.e., P-0001, P-0002, P-0003, P-0004, etc.) starting from the beginning of each year (surveillance season).

Correct identification of all mosquitoes in a pool is important. Mosquito pools of most species should contain from 25 to 50 mosquitoes. Certain important vector species are difficult to trap in large enough numbers to pool, and others have been deemed important enough that they may be submitted in smaller pools of from 10 to 25 mosquitoes (see[1] in list below. Vials containing pooled mosquitoes should be placed in a freezer and held until shipment to the testing laboratory. Tubes should be shipped to the testing laboratory in an insulated container containing dry ice.

Mosquito surveillance organizations are allowed to submit as many pools per week as they can collect. However, if the laboratory is busy they may not be able to test more than 40 pools per week. Additional pools will be held by the laboratory until there is time to test them. Programs that submit more than 40 pools per week should include their priority species among the first 40 pools.

The species tested for WNV are listed in the table to the left.

Status

♦ Mosquito species that should be tested in pools of from 25 to 50 mosquitoes.

♦♦ Mosquito species that may be tested in pools of containing from 10 to 50 mosquitoes.

[1] Mosquito species that are important vectors and/or may be difficult to trap in large enough numbers to make pools of 25 mosquitoes.

[2] Damaged *Culex pipiens* and *Cx. restuans* that cannot be distinguished from each other during identification may be pooled as *Cx. pipiens/restuans,* or if *Culex* specimens are un-identifiable as any species they may be pooled as *Culex spp*.

Status	Mosquito Species
♦♦	*Aedes albopictus* [1]
♦♦	*Aedes vexans* [1]
♦	*Anopheles crucians*
♦	*Anopheles punctipennis*
♦	*Anopheles quadrimaculatus*
♦	*Culex erraticus*
♦♦	*Culex pipiens* [1, 2]
♦♦	*Culex restuans* [1, 2]
♦♦	*Culex salinarius* [1, 2]
♦	*Culiseta melanura*
♦	*Coquillettidia perturbans*
♦♦	*Ochlerotatus atropalpus* [1]
♦	*Ochlerotatus canadensis*
♦♦	*Ochlerotatus japonicus* [1]
♦	*Ochlerotatus infirmatus*
♦	*Ochlerotatus sticticus*
♦	*Ochlerotatus sollicitans*
♦	*Ochlerotatus taeniorhynchus*
♦♦	*Ochlerotatus triseriatus* [1]

Any surveillance program that strongly suspects West Nile virus transmission from a species that is not on the above WNV tested list may consult with Dr. David Gaines (VDH-Office of Epidemiology; [804] 786-6261), and/or consult with the laboratory manager in the labs that the pools are being submitted to obtain approval for testing that species.

Mosquito pools should be submitted either to Dr. Dee Pettit at the **Division of Consolidated Laboratory Services (DCLS)** State Laboratory in Richmond, or to the Dr. Karren Loftin, or Deepak Phaltankar at the **Norfolk Public Health Laboratory** in Norfolk: Mosquito surveillance programs working outside of the Tidewater Region Should send their pools and samnples to the DCLS laboratory. Tidewater Region programs using the Norfolk Lab may originate from the following jurisdictions: Accomack Co., Chesapeake, Hampton, Isle of Wight Co., James City Co./Williamsburg, Newport News, Norfolk, Northampton Co., Portsmouth, Southampton Co./Franklin, Suffolk, Virginia Beach, York Co./Poquosin. To arrange for testing of mosquito pools contact the laboratory managers first. Contact information is as follows:

Division of Consolidated Laboratory Services
Attention: Dr. Dee Pettit
1 North 14th Street
Richmond, VA 23219
Tel. (804) 786-9715

Norfolk Public Health Laboratory
Attention: Dr. Karren Loftin
830 Southampton Ave
Norfolk, VA 23510
Tel. (757)-683-2746

A paper copy of the mosquito database entry form for the submitted mosquito pools ("Pool Log") should be sent along with each shipment of pools. Electronic copies of the Pool Log file (in spreadsheet format) should also be e-mailed to the appropriate laboratory on the day that pools are sent. Database users should e-mail the "Pool Log" file as an Excel attachment. Each program should use a consistent file naming convention will help the laboratory identify the e-mailed files from each surveillance program. Mosquito surveillance programs not having access to computers or unable to use the Virginia Mosquito and Arboviral Tracking System database may copy and use form on the following page to accompany their submitted mosquito pools.

Weekly Laboratory Mosquito Pool Submission Form

Program ID [1]:	Program Name	
Date Shipped to Laboratory	Sample Dates: From_____ To_____	Week #

Pool #	Collection Date [2]	Collection Site ID [3]	County/City	Mosquito Species	Number in Pool	Trap Type	Sample Number [4]
P-							
P-							
P-							
P-							
P-							
P-							
P-							
P-							
P-							
P-							
P-							
P-							
P-							
P-							
P-							
P-							
P-							
P-							
P-							
P-							
P-							

[1] Program ID = four letter code (e.g., Virginia Beach = VABE). To avoid duplication of Program Codes across jurisdiction, codes should requested and approved through Dr. Gaines at the VDH Office of Epidemiology (804-786-6261)

[2] Collection Date = mm/dd/yy

[3] Collection Site ID = two letter County/City code + four digit site number (e.g., VB-0012 would be Virginia Beach collection site #12; once used to describe a collection site, the Collection Site ID should be permanently assigned to that site and not be used again for another site (to create your own Site IDs, use the reference list for the two-letter County/City codes at the bottom of Attachment 3.D).

[4] Sample Number = the letter P + four digit consecutive pool number + the four letter Program ID code + two digit week number + two digit year number (e.g., P-0123-VABE-22-03 = Pool # 123 from the Virginia Beach Surveillance Program, collected on week# 22 of the year 2003).

Formula for Gravid Trap Bait Infusion for *Culex* Mosquitoes

Materials and Ingredients:

1 One each, 40 gallon plastic trashcan with a tight fitting snap-on lid (Rubermaid or other brand).
2 30 gallons of water
3 About 1 lb of dry straw or hay.
4 About 1 lb of freshly mowed grass clippings.
5 Five grams of brewers yeast.

Mixing Directions

(1) Place the plastic trash can in a location where it will get direct sunlight for at least several hours per day; make sure the trash can is not located in a place where odor from the finished bait will offend anyone. **(2)** Fill the plastic trashcan with 30 gallons of water. **(3)** Mix the dry straw or hay with the fresh grass clippings, and stir 2 pounds of this grass/straw mixture into the 30 gallons of water. **(4)** Add 5 grams of brewers yeast and stir it into the grass/straw/water mixture. **(5)** Place lid on the trashcan and let the mixture brew for five days; stir the mixture once every 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 *Cx. restuans*. If you use this bait and do not catch either of the above-mentioned *Culex* species, there probably were not any active in the area where the gravid trap was set. Be careful not to leave the lid off of 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 *Aedes albopictus* or the *Ochlerotatus* species of container breeding mosquitoes. However, after about three weeks of usage, this bait becomes slightly less attractive to *Culex pipiens* or *Cx. restuans*, and becomes more attractive to the *Aedes albopictus* 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.