

**WEST NILE VIRUS
SURVEILLANCE AND CONTROL GUIDE
FOR
U.S. NAVY AND MARINE CORPS
INSTALLATIONS
2014**



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Jacksonville, FL**

1. Background

West Nile Virus (WNV), first isolated in 1937, has been known to cause human illness in Africa, West Asia, and the Middle East. Symptoms of WNV range from asymptomatic infections to encephalitis. Human and animal infections were first documented in the Western Hemisphere in 1999 when outbreaks of WNV encephalitis in humans were reported in the New York City metropolitan area, New Jersey, and Connecticut. Since the initial outbreaks in New York City, there have been a total of 37,000 cases of WNV infections with 1,500 deaths in the United States.

Most people infected with WNV, especially healthy young adults and children, do not develop symptoms. Up to 20% of infected people develop mild symptoms such as flu-like illness characterized by fever, muscle aches, swollen glands and skin rash. Less than 1% of infected people develop a more severe illness including meningitis (inflammation of the spinal cord) and encephalitis (inflammation of the brain). Symptoms of these illnesses include headache, high fever, neck stiffness, stupor, disorientation, coma, tremors, convulsions, muscle weakness, and paralysis. Less than 1 in 1000 infections in those who develop meningitis or encephalitis result in death. Persons over age 50 or with suppressed immune systems are more likely to become severely symptomatic when infected. The risk of mortality due to WNV infection is extremely low in people under age 50.

WNV is transmitted to humans through the bite of an infected female mosquito. The principal vector species of WNV are *Culex pipiens*, *Cx. tarsalis* and *Cx. quinquefasciatus*. Additionally, WNV has been detected in many species in several genera, including *Culex*, *Aedes*, *Culiseta*, and *Psorophora*. Mosquitoes become infected when they feed on birds infected with WNV. Infected mosquitoes can then transmit WNV when they feed on humans or other animals. Although American corvids (crows and blue jays) are particularly susceptible to WNV infection, it has also been detected in more than 130 bird species, 22 mosquito species, and a variety of mammals. Among domestic animals, horses are especially susceptible and often serve as an indication of viral activity in a given area. The virus is not transmitted from person to person, and there is no evidence that handling live or dead infected birds can cause human infection. However, the Center for Disease Control (CDC) warns that dead birds should not be handled with bare hands in order to minimize potential WNV transmission. West Nile virus is transmitted by mosquitoes that acquire the virus from infected birds. These infected mosquitoes then feed on non-infected birds, and spread the virus. In this way, wild birds serve as the WNV reservoir, while humans are dead-end hosts that cannot infect mosquitoes and cannot become infected when feeding on an infected human. WNV transmission can be significantly reduced or eliminated by controlling mosquitoes, thereby limiting or reducing viral transmission to humans and domestic animals.

2. Purpose of this Guide

The purpose of this guide is to explain the importance of WNV surveillance, describe the steps involved in establishing a surveillance and control program, and provide a threshold when control measures can be carried out, based on observed WNV infection in birds, mosquitoes and humans. The potential health threat for military personnel developing illness from WNV is low, but isolated cases cannot be excluded.

a. The Navy Entomology Center of Excellence (NECE), Jacksonville, Florida, provides guidance for disease vector surveillance and control programs for Navy and Marine Corps installations in the continental United States. This document is intended as general guidance concerning WNV surveillance for these installations. This guide will assist installations in their WNV surveillance and prevention efforts. Because each military installation is unique, this guide is meant only to provide general guidance for shore installations.

b. Due to the rapid spread of WNV throughout the US, Navy policy concerning WNV states that “all installations in States where WNV has been found shall conduct surveillance for mosquitoes, and control measures as needed, in conjunction with local/State public health departments” (GENADMIN/SECNAV/271542ZSEP2002). Since then, WNV activity has now been reported in every state in the continental United States, and therefore this guide applies to all CONUS U.S. naval installations.

c. Per OPNAV Instruction 6250.4C, the medical department on each installation is ultimately responsible to the Commanding Officer for personnel health and to inform the CO of risks (including vector-borne disease risk). Therefore, each installation’s medical personnel should assess WNV risk, conduct ongoing WNV surveillance during the mosquito season appropriate to their region, and take preventive and responsive action to reduce WNV risk to active duty, government employees and family member populations.

d. Guidance for establishing mosquito surveillance and/or control programs, WNV surveillance training, and other technical assistance can be obtained by contacting Navy entomologists at NECE or at the closest Navy Environmental Preventive Medicine Unit located in Norfolk, VA (NEPMU2) or San Diego, CA (NEPMU5). Dead birds should be collected and processed by installation veterinarians. Contact the installation veterinarian facility before collecting dead birds to verify proper collecting procedures and ensure the facility is able to process the samples. Mosquitoes collected from Navy and Marine Corps installations in the Eastern U.S. (east of the Mississippi River) will be tested by the U.S. Army Public Health Command Region North, Fort George G. Meade, MD (PHCR-North) while samples from installations in the Western U.S. will be tested at Public Health Command Region-South, Ft. Sam Houston, TX (PHCR-South). Contact a Navy Entomologist or the PHC if mosquito testing is planned. Contact information can be found in Appendix A.

3. WNV Surveillance and Protection Plan

OPNAVINST 6250.4C states that the responsibilities for surveillance and control of medically important insects and arthropods will be clearly delineated in the installation’s pest management plan. In addition, this instruction requires all Navy and Marine Corps installations to have an emergency plan for disease vector surveillance and control (Emergency Vector Control Plan, EVCP). This plan is normally included as an appendix to the installation’s Pest Management Plan. OPNAVINST 6250.4C also designates the medical department as having the responsibility of performing inspections and surveys to determine the species, source, location, and density of disease vectors on installations for which they are responsible. Therefore, the development and implementation of a WNV surveillance and protection plan is the responsibility of personnel tasked with disease vector surveillance and control. The WNV surveillance and protection plan should compliment installation pest management plan guidelines (to include the EVCP) recommended for any potential mosquito-borne disease

outbreak. The surveillance program must include identification of mosquito species present, baseline population density, larval breeding areas, habitation zones (e.g. adult mosquito resting sites), and effectiveness determination of vector control measures. It is recommended that preventive medicine personnel consult the EVCP; it is possible that the plan already contains information regarding mosquito-borne disease on the installation. If necessary, the EVCP should be modified to include the installation WNV surveillance and control plan.

A WNV plan should include dead bird surveillance, adult mosquito surveillance, and control, adult mosquito surveillance and control (if required), WNV testing, data collection and reporting, and program assessment. Regardless of how a WNV program is implemented, each installation should liaise with their local mosquito abatement or public health agency to notify them of their WNV plan and to establish communication.

An installation can adequately perform WNV risk assessment through coordination with a local department of health or mosquito abatement district. For instance, arbovirus testing data or sentinel chicken flock results conducted by county or state health departments or local university can be shared with nearby shore installations. If information obtained from these sources is sufficient and mosquito populations are low, then WNV surveillance conducted by these other agencies may be adequate for the installation. However, it is the responsibility of installation personnel to ensure that proper surveillance data are obtained, whether it is from the military medical department, public works department, pest management contractor or local/State public health agencies.

4. General WNV Surveillance Options

There are various options for implementing a WNV surveillance plan.

a. Local Public Health and/or Mosquito Control Agencies. If local, county, or state agencies are currently performing surveillance, testing and control measures that meet or exceed U.S. DoD WNV recommendations, then arrangements may be made with that agency to include the military installation as part of the local agency's operations.

b. Private Contractor. The installation may contract with civilian mosquito/pest control professionals that meet or exceeds DoD WNV recommendations.

c. DoD Personnel. DoD medical personnel and equipment may be used to implement the WNV Plan. Data should be reported as mentioned above.

5. U.S. Navy WNV Plan Guidelines

a. Habitat Modification. Breeding site reduction is a fundamental control measure for reducing or eliminating mosquito populations. Control efforts should be concentrated on targeting the specific resources mosquitoes require for successful reproduction (water, a blood meal, and a place to rest and lay eggs). Specifically, drainage or insecticide treatments of mosquito breeding sites provide efficient and cost effective mosquito population control. Since larval mosquitoes are more highly concentrated than the adult stage, a greater number of mosquitoes can be eliminated when treating larval habitat than when spraying for adults. By finding, eliminating, or treating potential larval sites, mosquito populations can be reduced or eliminated before virus transmission to humans occurs.

Artificial breeding sites, such as containers that fill with water during rainfall, should be discarded. Standing water should be drained, and depressions that hold water should be filled when possible.

b. Immature Mosquito Surveillance and Control. Immature mosquito (larvae and pupae) control is an essential component of any effective WNV prevention program. Mosquito breeding site identification should begin early to mid spring by surveying the installation and noting actual and potential breeding sites on a base map. Global Positioning System (GPS) and Global Information System (GIS) tools should be used whenever possible to accurately document mosquito breeding areas on the installation. Breeding sites may or may not include larvae at the time of survey. Continual surveillance (i.e. weekly) should commence after mosquito larvae are found. Larval surveillance should continue throughout the mosquito season, since the species composition of an area will often change over time. Surveillance should include collection (using a simple plastic or enamel dipper on the end of a 3-4 foot pole), identification of larvae, mapping and monitoring of larval sites on the installation, and searching for new sites as weather or other conditions change. While immature mosquitoes can be identified to species, adults may be easier to identify. Immature mosquitoes can be reared to adults in larval rearing chambers and the adult mosquitoes identified upon emergence.

To assist in control measures throughout the mosquito breeding season, installation preventive medicine personnel should work with the Public Works department to eliminate mosquito-breeding sites, especially those that may be eliminated by filling or draining.

Several insecticides are available in the stock system to control immature mosquitoes, including microbial derived insecticides such as *Bacillus thuringiensis var israelensis* (Bti) briquettes (NSN 6840-01-377-7049) and insect growth regulators such as Methoprene briquettes (NSN 6840-01-424-2495) or liquid (NSN 6840-01-424-2493). Because of the environmental risks and legal limitations when applying pesticides to water, applications should only be conducted by certified pesticide applicators.

c. Adult Mosquito Surveillance and Control.

(1) **Trapping Methods.** Adult surveillance can be conducted using light traps such as the Solid State Army Miniature (SSAM) Hock Model 1012 (NSN 3740-01-106-0091), the CDC model 512 (NSN 3740-00-134-9229), and baited CDC gravid traps, available through commercial entomological supply sources such as the John W. Hock Co. (<http://www.johnwhock.com>) or Bioquip Inc. (<http://www.bioquip.com>). An effective surveillance program for adult mosquitoes should ideally use a combination of light traps and gravid traps. However, the CDC gravid trap should be the primary surveillance tool if the threat of WNV is high, as this trap targets blood-fed mosquitoes (those which may have acquired WNV). Mosquitoes collected in older style light traps, such as New Jersey Light Traps, are often not viable for WNV testing; however, mosquitoes usually remain alive and viable for WNV testing if collected in gravid traps, SSAM or CDC light traps. An adequate mosquito surveillance program should include at least 5 gravid traps operated at least 2 nights/week. More may be required depending on specific installation needs. Installations with housing, habitat variety, or areas of active WNV transmission may require a greater trapping effort. Any program should be sufficient to trap mosquitoes from all areas of concern (e.g. housing, bodies of water, horse stables, public use areas, wet areas), specifically targeting *Culex* species. Adult surveillance should begin when mosquitoes are observed emerging from breeding sites and continue until the first hard frost. If frost does not occur then, adult surveillance should be conducted year round. Generally, the

probability of WNV transmission in an area is increased if more than 25 *Culex* spp. mosquitoes are collected per gravid trap per night.

(2) **Lures.** The SSAM or CDC light traps should be augmented with 4-5 lbs of CO₂ (dry ice) per night. Gravid traps should be filled with about one quart of "lure" material, consisting of water with decaying vegetation added from the surrounding area. An effective mixture may also be prepared by combining one cup of rabbit pellet food and one gallon of natural-source water (do not use tap water, since the water found in the area contains microbes needed to make the rabbit food attractive for oviposition), and aging the mixture for two days. This mixture is then sufficient to use in the gravid trap to attract blood-fed mosquitoes seeking a water source in which to deposit eggs.

(3) **Adult Control.** Adult mosquito control methods are implemented based on trap surveillance data. Spray threshold information for adult mosquito control is included in the IPMP for each installation. Action thresholds may not be available or applicable in some areas. Insecticide spray operations are conducted until adult populations fall below the prescribed threshold. When necessary, adult mosquito control is often conducted using ground Ultra-Low Volume (ULV) sprayers and insecticides to include Sumithrin (NSN 6840-01-474-7751), Pyrethrins (NSN 6840-01-104-0780) or Resmethrin (NSN 6840-01-359-8533) by certified pest control operators. Often, insecticide applications are conducted for 3 to 4 consecutive days/nights (timed to coincide with peak activity periods of the target species) to reduce adult mosquito populations. Control covering large areas or when severe outbreaks occur may require aerial insecticide application. However, aerial application cannot be implemented unless validated by a Navy Entomologist. It is important to note that **no mosquito control plan should rely solely on spraying adulticides in response to mosquito complaints.** Surveillance, breeding site reduction, and larval control measures should continue until the first killing frost occurs. If frost does not occur then, mosquito control measures should be instituted year round.

d. Dead Bird Surveillance. Surveying for bird morbidity/mortality is the most sensitive and cost effective method for detecting WNV in an area. Any dead bird found on an installation must be reported a facility at the installation whose staff are trained to properly recover, assess, and prepare the bird for WNV testing by either a federal or state laboratory. Past research indicates that dead crow density (number of dead crows per square mile) can be used to assess the risk of transmission to humans. However, a total of 130 bird species have been found to harbor WNV, so any dead birds should be reported. In fact, WNV confirmation in an area could be delayed by several weeks if only dead crows are reported.

e. Public Education and Cooperation. Information dissemination to the general public, support communities (public health departments, health care providers, veterinary communities, etc.), and other governmental agencies is critical to ensure effective implementation of any WNV surveillance and control plan. News bulletins, interviews, web sites, and articles in base newspapers can all be used to inform local military and other DoD personnel about WNV risks and precautions.

f. Mosquito Identification. Identification of mosquito larvae can be difficult. However, larvae collected from surveillance can be reared to adults in larval rearing chambers (NSN 3740-01-454-2345) and identified as adults using keys such as the Identification and Geographical Distribution of Mosquitoes of North America, by Darsie and Ward. Adult mosquitoes collected from each gravid or

light trap should be separated from other collected insects and sorted by sex. Mosquitoes should be identified to species whenever possible, however if mosquitoes are being pooled for testing, genus level identification is acceptable. Entomologists at NECE or the closest EPMU can provide more detailed assistance for training personnel in mosquito identification.

g. Processing Mosquito Specimens. Mosquitoes collected for WNV analysis should be quickly immobilized by placing them in a freezer and thereafter kept at freezing temperatures. Mosquito identification should be conducted on a chill table or some improvisation whereby the specimens are continuously in contact with a cold surface (see Appendix B). The standard method for analyzing mosquitoes for WNV infection is the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), a procedure that detects the presence of WNV genetic material in mosquito samples. This methodology requires that the mosquito specimens do not degrade due to warm temperature. Therefore a cold-chain must be maintained at all times in order to keep the mosquitoes viable for testing. Once identified to at least the genus level, specimens should be sorted into vials (preferably cryovials) based on the species (or genus). Each vial is considered a “pool”. Each pool of mosquitoes should consist of a maximum of 50 individual specimens collected from a single species. For instance, at a given installation, collections from a night of trapping are found to be of the following mosquito species:

<i>Aedes vexans</i>	80
<i>Culex tarsalis</i>	5
<i>Ochleratatus taenorynchus</i>	39

The *Aedes vexans* collection will consist of 2 pools (1 pool of 50 and 1 pool of 30), and there will be one pool of *Cx. tarsalis* (5 mosquitoes) and one pool of *Oc. taenorhynchus* (containing 39 mosquitoes), for a total of 4 pools for that night of collecting. Mosquitoes must be pooled from only a single night of trapping; do NOT combine specimens from more than one trapping period to increase pool size. After identification, mosquito pools may be kept frozen at -20°C until shipped for analysis. Ice packs should be used when shipping specimens for WNV testing.

h. Submitting Specimens for WNV Testing, Documentation and Reporting

(1) U.S. Army PHCR-North and PHCR-South are currently supporting WNV testing for installations located east of the Mississippi River. Adult mosquitoes should be pooled and shipped to PHCR-North or PHCR-South weekly for WNV testing. Mosquito pooling is conducted per instructions in Appendix C. Samples should be kept frozen until they are sent overnight packed in PHCR-supplied shipping containers with ice packs. Specimen information (Mosquito Pooling Information form found in Appendix D) should accompany the specimens. Coordination with PHCR-North or South **MUST** be done prior to shipping of any samples. Assistance in coordinating with PHC can be obtained by contacting the Fleet Support Department at NECE.

(2) PHCR-North or South will compile and forward results electronically back to the installation, NECE, state public health department (who will send to the CDC, and to the Department of Defense's Global Emerging Infections System (DoD-GEIS). The U.S. Army PHC points of contact for WNV testing can be found in Appendix A.

(3) Ensure all phases of mosquito surveillance are documented in a logbook used only for mosquito surveillance in a manner similar to Appendix E.

(4) Suspected human WNV cases should be reported directly to the Navy Marine Corps Public Health Center (NMPHC) 757-953-0700. Suspected human WNV cases must be diagnostically confirmed by testing serum or cerebrospinal fluid (or other appropriate tissue) and results reported to NECE and NMPHC.

i. WNV Risk Levels and Response. WNV has been established in the continental United States for over a decade. Although local areas may be characterized by varying degrees of risk. WNV risk levels have been established to serve as a guide for prevention and action measures to respond to increasing risk levels as outlined in Appendix H.

Entomologists at NECE or NEPMU's can be contacted for technical assistance, consultation, and guidance regarding mosquito control on Navy and Marine Corps installations. It is highly recommended that an installation's WNV surveillance and control program be developed in consultation with entomologists at NECE, Navy Environmental and Preventive Medicine Units (NEPMU)'s or Naval Facilities Engineering Command (NAVFAC).

Appendix A.

Contact Information

Navy and Marine Corps WNV Surveillance Program Issues/Questions:

Navy Marine Corps Public Health Center, VA (NMPHC):
757-953-0700, DSN 377-07000

Navy Entomology Center of Excellence, Jacksonville, FL (NECE):
Officer in Charge (OIC), 904-542-2424, DSN 942-2424

Navy Entomologists:

Navy Entomology Center of Excellence (NECE):

Fleet Support (904) 542-2424 DSN 942-2424.

Navy Environmental and Preventive Medicine Unit Number Two, Norfolk, VA (NEPMU-2): (757) 953 6600 or DSN 953-6600

Navy Environmental and Preventive Medicine Unit Number Five, San Diego, CA (NEPMU-5): (619) 556-7070 or DSN 526-7070

WNV Mosquito Testing at PHCR-North and PHCR-South:

Public Health Command Region-North
ATTN: MCHB-AN
4411 Llewellyn Avenue
Fort George G. Meade, MD 21755-5225
(301)-677-3806 or DSN 622-3806

Commander, PHCR-SOUTH
MCHB-AS-EHED
TO: Dr. Farida Mahmood
2899 Schofield Road 2D Floor
Fort Sam Houston, Texas 78234-6233
(210)-221-5336

Other WNV Program Links:

CDC WNV Surveillance Program:

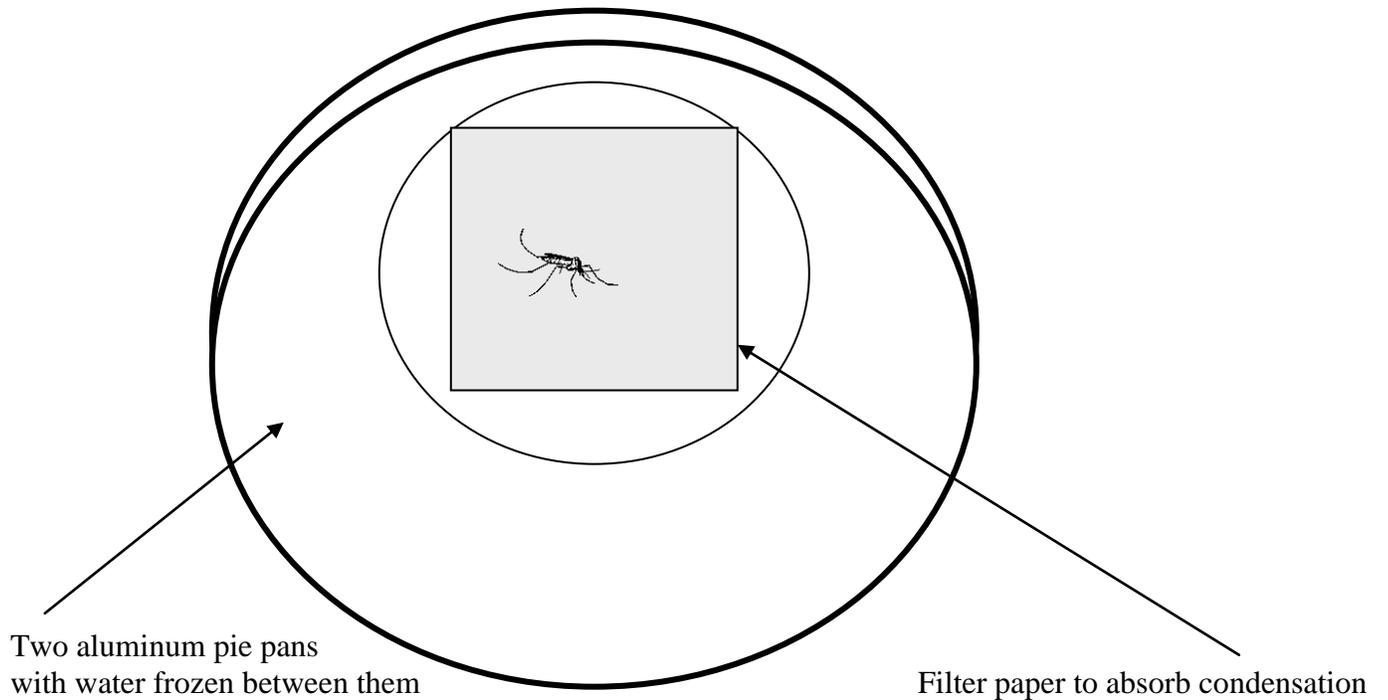
<http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-apr-2001.pdf>

USACHPPM WNV Mosquito Surveillance Program:

<http://chppm-www.apgea.army.mil/ento/westnile/South/Cover.pdf>

Appendix B.

Cold plate used to immobilize mosquito specimens while sorting and identifying.



Chill tables can be purchased through Bioquip, Inc (<http://www.bioquip.com/newproducts/1429.htm>).

Appendix C.

**West Nile Virus Mosquito Surveillance
Pooling Instructions and Data Recording**

1. Refer to data form entitled "[Mosquito Pool Information, West Nile Virus Surveillance](#)" (Appendix D) and read "codes and explanations" at bottom of sheet
2. After freezing captured mosquitoes (at least 20 minutes at -20°C), empty the contents of each trap on a chill table (or pan with frozen substrate) and sort and discard non-mosquitoes and male mosquitoes (while noting the number of males).
3. Identify and count female mosquitoes while keeping them on the chill surface (Appendix B). Pool up to 25 female mosquitoes, of the same genus, from the same trap and place in a tube (preferably a cryovial).
4. Place a pre-printed log number label on the side of the sample tube. Write, with an indelible pen, the corresponding log number (e.g. 001) on the cap. You do not need to rewrite the installation identifier.
5. Write the log number (e.g., 001, 002, etc.) in the "Pool Log #" column of the data sheet.
6. **IMPORTANT:** Do not mix mosquitoes collected from different installations on the same data sheet. Do not duplicate log numbers. Always pick up with the next number that follows the one that ended the last trapping period. You can keep track of this by photocopying and retaining data sheets at the end of each collection period. It is OK to indicate "0" trap catches in the "number of mosquitoes" section, but **DO NOT ASSIGN A POOL LOG #** to it. Put a dash or XXXX in the "Pool Log #" column.
7. After pooling the samples and recording the data, place vials in the freezer and arrange for overnight delivery. It is usually most cost efficient to send mosquitoes collected from each weekly surveillance together in one shipment. Contact NECE to discuss shipping methods, if needed.
8. Pack samples, sandwiched between at least 5 frozen freezer packs (e.g. blue ice), in a cooler or insulated box. Include a copy of the data sheet with the samples. Overnight ship (e.g., FEDEX) the box to the appropriate address listed in Appendix A.

Appendix E.

Example of logbook entry for mosquito surveillance documentation

<u>Date</u>	<u>Collection Time</u>	<u>Method</u>	<u>Location</u>	<u>Larval/Pupal/Adult</u>	<u>#♀ / Genus</u>	<u>Collector</u>
25-26 Apr '02	1800-0600	CDC Lt & CO ²	behind hospital	A	17 <i>Culex</i>	HM3 Smith
25-26 Apr '02	1800-0600	CDC Lt & CO ²	behind hospital	A	4 <i>Aedes</i>	HM3 Smith
25-26 Apr '02	1800-0600	CDC gravid	walking trail pond	A	23 <i>Culex</i>	HM3 Smith
25-26 Apr '02	1800-0600	CDC gravid	horse stable pond	A	16 <i>Anopheles</i>	HM3 Smith
28-29 Apr '02	1800-0600	CDC Lt & CO ²	behind hospital	A	20 <i>Culex</i>	HM2 Darwin
28-29 Apr '02	1800-0600	CDC Lt & CO ²	behind hospital	A	8 <i>Aedes</i>	HM2 Darwin
28-29 Apr '02	1800-0600	CDC gravid	walking trail pond	A	23 <i>Culex</i>	HM2 Darwin
28-29 Apr '02	1800-0600	CDC gravid	horse stable pond	A	17 <i>Anopheles</i>	HM3 Smith

Appendix H. WNV Risk Levels

WNV Transmission

Potential	Condition	Action
none	Level 1A Off season, mosquitoes inactive, climate unsuitable	Determine if new WNV Plan measures have been issued, liaise with local agencies
	Level 1B Early Spring, adult mosquitoes not active	Begin larval breeding site mapping, surveillance, and source reduction
	Level 1C Adult mosquitoes active but not problematic (no complaints),	Begin adult surveillance
low	Level 2A Surveillance finds adult mosquitoes active, but no complaints	Public education and personal protection information should be provided
	Level 2B Complaints of mosquito biting or mosquito traps collecting numbers of mosquitoes but no WNV found on installation	Consider adult &/or larval control measures, increase mosquito and dead bird surveillance, use ground truth maps for controlling breeding sites
	Level 2C dead bird(s) found where cause of death is unknown	Have installation vets or local public health agency test for WNV, increase surveillance for dead birds, public announcement about what public should do if a dead bird is found
med	Level 3 WNV confirmed in dead bird(s) locally or 1 or more WNV+ mosquitoes in traps	Consult with local mosquito abatement agencies & NECE, get public involved with environmental sanitation, consider increased adulticides and/or change in adulticide(s)
high	Level 4 1 or more WNV+ person or 1 or more WNV+ horse in area	Greater public announcement, consider wider use of adulticides esp. in public use areas, consult with local mosquito abatement agencies, consult with NECE

Note: Once an activity is started, it should be continued until the end of season or risk decreases.