

# Arizona West Nile virus Surveillance

## Introduction

The West Nile virus & St. Louis encephalitis surveillance season begins in April in Arizona. The Arizona State Health Lab, University of Arizona Veterinary Diagnostic Lab, and the Arizona Department of Health Services-Vector-borne & Zoonotic Disease Program are prepared for seasonal testing of any environmental samples collected, such as mosquito pools. Dead bird and human specimens (blood/CSF) are tested year-round.

## Modes of Surveillance

- ✓ Mosquito trapping & testing
- ✓ Avian
- ✓ Equine
- ✓ Sentinel chicken flocks
- ✓ Other animal testing (e.g. squirrels, llamas, etc. with unexplained neurologic illness or death)
- ✓ Human case surveillance

## Mosquito Surveillance

This program conducts surveillance for at least two mosquito-borne encephalitis viruses: St. Louis encephalitis & West Nile virus. The goal of this surveillance program is to detect and monitor arbovirus activity throughout the state so as to identify areas of increased risk and to help guide risk reduction efforts.

- ✓ Protocol:
  - Supplies for Mosquito Trapping: CO<sub>2</sub> trap(s), screw cap vials, shipping boxes. We will provide this equipment (as long as supplies last). If possible, please collect mosquitoes in your area at least once per month from April through October. We will identify mosquito species in all samples received. We will prioritize lab testing for the Culex species. However, species within other genera will be selectively tested as well.
  - Bait the trap with at least 2 pounds of dry ice to last all night, and hang the trap close to sun down. Hang CO<sub>2</sub> trap(s) with the bait can and/or motorized fan at shoulder height. Hang it from a tree limb or other structure in a place that is likely to have some shade from the east side so that the samples are not blasted by the morning sun. Also, hang it in a safe place to avoid theft or vandalism. You have the option of turning off the mini-light to be more discreet. The capture net hangs below the fan. If the night is likely to be breezy, it may be necessary to secure the net with a rubber band.
  - Harvest your catch in the morning by pinching off the capture net while the fan is still running. Keep the mosquitoes cool and shaded until you are ready to process them into vials. When you are ready to process the mosquitoes, chill the mosquitoes briefly on dry ice (approximately 30 - 60 seconds) to immobilize them. Then aspirate them into the plastic capsule. If the mosquitoes are beginning to move within the capsule, chill them again briefly to immobilize them. Then hold the capsule with the tube over the vial and tap the mosquitoes down the tube

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into the vial. Please DO NOT over-pack the vials, as this will damage the mosquitoes and makes identification difficult. Screw the cap on securely.

- Label the vial with the following information:
  - Date
  - Location (GPS coordinates if possible)
  - Collector's Name
  - Seal the vial(s) inside a plastic bag so as to facilitate easier handling when removing the samples from the shipping box.
- ✓ Pack the vial(s) in a shipping box containing enough dry ice to keep the sample cold all the way to the Arizona Department of Health Services Vector Lab. It is important to remember that preserving mosquitoes in a cold state makes species identification easier and more accurate. If samples become warm or soggy while in transit to the Arizona Department of Health Services Vector Lab, the mosquitoes will deteriorate often to the point where species identification is not possible.
- ✓ Complete & include a [Mosquito submission form](#) inside the package.
- ✓ Pack the samples in enough dry ice to last 2 days.
- ✓ Do not ship mosquitoes on Thursday or Friday
- ✓ Ship via Overnight Delivery
- ✓ Notify Arizona Department of Health Services of the shipment's tracking number at [vbzd@azdhs.gov](mailto:vbzd@azdhs.gov) or (602) 364-4562
- ✓ Ship samples to:
  - Arizona Department of Health Services
  - Infectious Disease
  - Vector-Borne Diseases Program
  - 150 N 18th Ave. Ste. 140
  - Phoenix, AZ 85007-3237

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## Sentinel Chicken Surveillance

The Arizona State Health Lab and the University of Arizona-Veterinary Diagnostic Lab will be testing chicken bloods by IgG assay (IFA). Sentinel chicken surveillance will run from April through October. You have the option of bleeding once every two or three weeks. Please stop sampling the chickens when they seroconvert.

### ✓ Protocol

- Chicken samples will need to be collected on filter paper strips. The strips are cut to the size of 0.5 X 3 inches from standard Whitman filter paper which can be obtained through any laboratory/medical supply company. Filter paper can be obtained from the Arizona Department of Health Services.
- Label one end of the filter paper strip w/ the identification number of each bird & the bleeding date. Be sure to use a permanent ink pen. For consistency, please use the following ID number system:
- Example 10G15YUA=

|      |                           |           |                    |                          |
|------|---------------------------|-----------|--------------------|--------------------------|
| 10   | G                         | 15        | YU                 | A                        |
| Year | Leg Band Color<br>(green) | Leg Band# | Location<br>(Yuma) | Site Code<br>(unique ID) |

- Numbered leg bands can be recycled from last year, or new ones can be obtained from Arizona Department of Health Services.
- Perform a lancet prick to the comb or wing vein of the chicken and saturate a full inch of the strip with blood. It may be helpful to mark-off one inch on the strip prior to the bleeding. Do not soak the labeled end of the strip with blood. Air dry the blood soaked strip and be careful not to contaminate the sample with dirt, or blood from other strips, etc.
- Staple the strips in sequential order on a 5" X 7" card. The blood soaked end should point down. Do not staple the blood end.
- Label the card with: "CHICKEN BLOODS, COUNTY, AGENCY, SITE/LOCATION, DATE".
- Place the card with fully dried blood strips in a zip lock bag
- Fill out a [submission form](#)
- Mail the strips & submission form to:  
Arizona State Health Laboratory  
Attn: Virology  
250 N. 17th Avenue  
Phoenix, AZ 85007

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## Dead Bird Surveillance

- ✓ Dead Bird Submission Guidelines
  - Refer to the [Selection Criteria for Bird Submissions](#)
  - Submit recently dead birds only
  - Do not submit birds with chronic illness or non-neurological illness prior to death
  - It is preferable to submit corvids (crows, ravens, and jays), raptors, exotic species (especially from zoos), grackles, sparrows, and finches. Baby birds, pigeons, and indoor pet birds will not be accepted for West Nile virus testing.
  - Doves are not likely to be sick with West Nile virus.
  - Wear protective gloves, or an inverted plastic bag to collect dead bird(s).
  - Place each specimen in a separate sealable plastic bag and refrigerate as soon as possible. Do not freeze.
  - Fill out a [submission form](#), including information such as: Name, address, phone of submitting party, specific address where bird was found, history including clinical signs or evidence of illness in other birds, species, age if known, and date found.
  - Please fill out separate forms for each bird collected.
  - Ship the samples overnight in a Saf-T-Pak® with ice packs, as described on the Arizona Veterinary Diagnostic Lab website at <http://microvet.arizona.edu/AzVDL/index.shtml> (click on “packing and shipping”). Appropriate leak-proof shipping containers (ex. Saf-T-Pak®) must be used, which includes a sealable biohazard bag, a special white, absorbent envelope, and a shipping container consisting of a Styrofoam box which fits perfectly inside the provided cardboard box, with specific labeling:
  - Place bird in the provided clear, sealable biohazard bag (small and large sizes of these bags are available).
  - Place the bagged bird inside the provided white, absorbent envelope from Saf-T-Pak and seal this envelope (small and large sizes of these envelopes are available).
  - Place the bagged and enveloped bird(s), sealed ice packs, and submission form (in a separate Ziploc bag to keep dry), inside the shipping container. Do not use loose ice.
  - Shipping container is a styrofoam container encased in a cardboard box.
  - Properly seal the shipping container with heavy duty shipping tape, attach the shipping document (usually a Federal Express air-bill).
  - The outside of the shipping container should have affixed the following labels:
    - UN3373 (can be printed on box, not necessarily a label).
    - "Diagnostic Specimen packed in accordance with IATA Packing Instructions 650"
  - Ship package to:
    - Arizona Veterinary Diagnostic Laboratory
    - 2831 North Freeway
    - Tucson, Arizona 85705-5021
  - Do not ship on Friday. Hold specimen in a refrigerator until Monday and ship as above. If desired, use the Arizona Veterinary Diagnostic Lab's Federal Express account number and complete the shipping tag with "bill recipient". Call the University of Arizona Veterinary

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Diagnostic Lab at (520) 621-2356 to obtain the Federal Express account number. Designate the package as "Priority Overnight". Do not select the "FIRST OVERNIGHT" box.

- Saf-T-Pak containers will be returned, refurbished with biohazard bags and white absorbent envelopes, and condition of outside labels will be checked for condition.
  - The Arizona Department of Health Services and the University of Arizona Veterinary Diagnostic Lab support West Nile virus testing. Arizona Veterinary Diagnostic Lab staff will perform immunohistochemical staining and/or oral swabs testing specific for West Nile virus only. If additional diagnostic testing is desired, please contact Arizona Veterinary Diagnostic Lab staff at (520) 621-2356 to make arrangements for additional tests and for appropriate billing.
- ✓ Dead Bird/Other Animal University of Arizona Veterinary Diagnostics Lab-Submission Form: <http://microvet.arizona.edu/AzVDL/Submission%20Form.pdf>
- ✓ Selection Criteria for Bird Submission
- Listed below are criteria designed to assist in making the decision of whether or not to submit a dead bird to the Arizona Veterinary Diagnostic Laboratory (AzVDL) for West Nile virus (WNV) surveillance.
  - Is the carcass intact and fresh (dead less than 24 hours)?
    - Carcasses that are decomposed are usually of limited diagnostic value. If the carcass has an odor, is soft and mushy, has skin discoloration, feathers or skin that easily rubs off, or has maggots present, it is too decomposed for testing
    - An exception to the rule above: high priority specimens such as ravens, crows, grackles, sparrows, finches and raptors may still be submitted for testing even if they are already a few days (approximately 1-3 days) post mortem. Birds that commonly develop high viremias during infection may still be testable using an oral swab test.
    - If the carcass is very light in weight for the size of the bird and the wings are not moveable, then the carcass may be too dried out.
  - How old is the bird?
    - We are not accepting baby birds for WNV surveillance at this time. Dead baby birds are already too common and suffer high mortality due to other reasons. They do not make good sentinels for WNV surveillance.
  - Are there signs of avicide/pesticide use?
    - Were many birds found dead in close proximity to each other? If yes, then WNV is not likely to be the cause of death. Purposeful or accidental toxin exposure is a more likely cause of death in this setting.
  - What species is the bird?

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- Almost all species of birds will be accepted for WNV testing. Corvid species (e.g. crows, ravens, jays, magpies), raptors, and exotic avian species (if housed outside) are the most likely to die suddenly from WNV infection. Other species such as grackles, sparrows, finches, may also show considerable susceptibility/mortality. Doves are less likely to die from West Nile virus, so please be selective when submitting these.
- We are NOT accepting submissions of pigeons, as they do not appear to be good WNV sentinels. Birds housed indoors (with screened windows) will not be accepted for submission, as they would not be exposed to mosquito bites.
- Birds submitted for WNV surveillance will be tested with immunohistochemical staining (IHC) of tissues and/or an oral swab test specific for WNV.
- Costs for WNV testing are already covered under contract between the ADHS and AZ VDL. Any additional diagnostic workup (testing) must be requested, and will be billed to the submitter.
- If you have questions about whether to submit a bird for WNV surveillance, please contact:
  - AzVDL (520) 621-2356
  - Arizona Department of Health Services (602) 364-4562 [vbzd@azdhs.gov](mailto:vbzd@azdhs.gov)
- ✓ Dead Bird Reporting - Listing of County Phone Numbers
  - Environmental Health Agencies- Dead birds, green pools, and mosquito complaints
  - The Arizona Department of Health Services currently cannot test birds for influenza (bird flu). The USDA Wildlife Services and Arizona Game & Fish Department are doing some avian influenza surveillance in wetland birds (ducks, geese, shorebirds) and raptors (hawks, owls, eagles). The public can report ill or dead wetland birds or raptors found near any lake in Arizona by calling the toll-free bird monitoring hotline number at 1-877-97-AVIAN (1-877-972-8426) or by logging onto [www.azgfd.gov/ai](http://www.azgfd.gov/ai) and filling out the online form. The public can report ill or dead poultry to the Arizona Department of Agriculture Livestock and Poultry Hotline at 1-888-742-5334.

See next page for agency listing

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## ENVIRONMENTAL HEALTH STATEWIDE CONTACT LIST

|   |  |  |
|---|--|--|
| Apache  | (928) 337-7640   | <a href="http://www.co.apache.az.us">http://www.co.apache.az.us</a>  |
| Cochise<br>Bisbee<br>Benson<br>Douglas<br>Sierra Vista<br>Willcox                       | (520) 432-9440<br>(520) 586-8208<br>(520) 805-5601<br>(520) 803-3915<br>(520) 384-7111 | <a href="http://www.co.cochise.az.us">http://www.co.cochise.az.us</a>  |
| Coconino  | (928) 679-8750   | <a href="http://www.coconino.az.gov">http://www.coconino.az.gov</a>  |
| Gila<br>Globe<br>Payson   | (928) 425-5882<br>(928) 474-1210   | <a href="http://www.co.gila.az.us">http://www.co.gila.az.us</a>  |
| Graham  | (928) 428-1962   | <a href="http://www.graham.az.gov">http://www.graham.az.gov</a>  |
| Greenlee  | (928) 865-2601   | <a href="http://www.co.greenlee.az.us">http://www.co.greenlee.az.us</a>  |
| La Paz  | (928) 669-1100   | <a href="http://www.co.la-paz.az.us">http://www.co.la-paz.az.us</a>  |
| Maricopa  | (602) 506-0700   | <a href="http://www.maricopa.gov">http://www.maricopa.gov</a>  |
| Mohave  | (928) 757-0901   | <a href="http://www.co.mohave.az.us">http://www.co.mohave.az.us</a>  |
| Navajo Nation<br>Chinle<br>Tuba City<br>Window Rock<br>Fish & Wildlife<br>Navajo County | (928) 674-2069<br>(928) 283-4644<br>(928) 871-6615<br>(928) 871-6451<br>(928) 524-4750 | <a href="http://www.navajocountyaz.gov">http://www.navajocountyaz.gov</a>  |
| Pima  | (520) 243-7999<br>(866) 939-7462   | <a href="http://www.pimahealth.org">http://www.pimahealth.org</a>  |
| Pinal   | (520) 866-6200<br>(866) 287-0209 x6200   | <a href="http://www.pinalcountyaz.gov">http://www.pinalcountyaz.gov</a>  |
| Santa Cruz  | (520) 374-7900   | <a href="http://www.co.santa-cruz.az.us">http://www.co.santa-cruz.az.us</a><br><br>Bonnie Greenwald:<br><a href="mailto:bgreenwald@co.santa-cruz.az.us">bgreenwald@co.santa-cruz.az.us</a><br><br>Ricardo Maldonado:<br><a href="mailto:rmaldonado@co.santa-cruz.az.us">rmaldonado@co.santa-cruz.az.us</a> |
| Yavapai<br>Prescott<br>Cottonwood   | (928) 771-3149<br>(928) 649-5078   | <a href="http://www.co.yavapai.az.us">http://www.co.yavapai.az.us</a>  |
| Yuma  | (928) 317-4584 x1632 x1633   | <a href="http://www.co.yuma.az.us">http://www.co.yuma.az.us</a>  |

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## Equine Surveillance

Suspected and diagnosed cases of equine viral encephalitis are (immediately) reportable to the State Veterinarian's Office (602) 542-4293 or fax (602) 542-4290. Complete the "[Equine Central Nervous System Case Tracking Form](#)" and fax to the Office of the State Veterinarian. If you do NOT have a fax machine, you may include the Equine CNS Case Tracking Form with the specimen submission form that accompanies the samples to the Arizona Veterinary Diagnostic Lab.

### ✓ Protocol

- Blood for arbovirus testing: Red top tubes of whole blood, or separated serum (no preservatives or anticoagulants) should be refrigerated; do not freeze. Serum is used for IgM-capture ELISA testing. Acute Sample: within 7 days of onset of symptoms. If the result is negative on first specimen, you can submit a convalescent sample 14-28 days after onset of symptoms.
- Brain for rabies and West Nile virus testing: Submission of the head intact with the first two cervical vertebrae (C1 and C2) is preferable because: 1) the brain and cervical spinal cord are better preserved (anatomically) when left in the skull during transport; 2) If West Nile virus is present, is more likely to be detected in the anterior spinal cord; and 3) brain removal in field conditions may increase the risk of exposure to rabies. If you are sending only an extracted brain, include the lower brainstem, medulla and upper spinal cord. The intact head should be chilled immediately after removal. Prepare a leak proof insulated transporting container with ice or cold packs to keep the specimen chilled while in transit. The head should be double bagged. NOTE: Rabies is on the differential diagnosis list for horses with suspected viral encephalitis. Saliva, cerebral spinal fluid and neural tissue may be infectious, so barrier precautions are advised.
- Submission instructions and lab locations: Submit head and/or serum to Arizona Veterinary Diagnostic Lab as quickly as possible with a [submission form](#) and make sure to include:
  - Name of submitting veterinarian & veterinary clinic
  - Phone number, fax number, and address of veterinarian for results
  - Name, Address, phone number of owner
  - Physical address where horse resides (Physical address of where horse lives & date of onset is needed by the county health department for West Nile virus surveillance and response purposes)
  - Name, age and breed of horse
  - Date of onset of clinical signs
  - Date of sample collection
  - Date of euthanasia or death, if applicable
  - Brief summary of neurologic illness
  - Number of other animals on farm/ranch
  - Number of affected animals on farm/ranch
- The submittal information for each specimen should be placed in a leak proof plastic bag and attached to the corresponding container. Samples should be shipped so as NOT to arrive on the weekend at the Arizona Veterinary Diagnostic Lab. In the event that rabies is a more likely

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differential diagnosis (e.g. due to origin of horse, history of previous contact with potentially rabid animal, or other), rabies may need to be quickly ruled in or out. Consult with Arizona Department of Health Services Vector-Borne & Zoonotic Disease Section staff at (602) 364-4562 to determine if the sample should be shipped to the Arizona State Health Lab or the Arizona Veterinary Diagnostic Lab.

- Laboratories
  - AZ Veterinary Diagnostic Lab: 2831 N. Freeway, Tucson 85705, Phone: (520) 621-2356
  - AZ State Health Lab: 1520 West Adams St, Phoenix, AZ 85007, Phone: (602) 542-1190
- At this time, there is no charge for rabies testing, WNV IgM capture ELISA, or immunohistochemistry (IHC) on tissue, due to funding from the Centers for Disease Control and Prevention and the Arizona Department of Health Services, which is being provided to the Arizona Veterinary Diagnostic Lab.
- A fee is charged for more extensive diagnostic testing (e.g. culture, toxicology, etc.). Call the Arizona Veterinary Diagnostic Lab for specifics.
- [Complete the Arizona Department of Health Services Equine Investigation form](#) and submit it to [vbzd@azdhs.gov](mailto:vbzd@azdhs.gov)

### Other Animal Surveillance

In addition to humans and horses, West Nile virus (WNV) infection can cause illness and death in certain other mammals. WNV infection has been shown to cause illness and death in squirrels, chipmunks and some camelids (e.g. llamas and alpacas) in Arizona in recent years. The carcasses of small animals like rodents and rabbits can be sent whole to the UA Veterinary Diagnostic Laboratory in Tucson for WNV and other disease testing. Follow the same instructions and use the same form as you would to send dead birds (see [Dead Bird protocols](#)). For large animals like camleids, blood samples can be sent. Follow the same procedures and use the same form as for horses. If you have any questions about the animal submissions, please contact an Epidemiologist at the Arizona Department of Health at (602) 364-4562 or via email at [vbzd@azdhs.gov](mailto:vbzd@azdhs.gov)

### Human Surveillance

Surveillance for West Nile virus in humans is performed year-round. Please contact a Vector-Borne & Zoonotic Disease Epidemiologist at Arizona Department of Health Services if you have any questions (602) 364-4562 or [vbzd@azdhs.gov](mailto:vbzd@azdhs.gov) .

[Arizona Department of Health Services Investigation form](#)

[Blood Donor Investigation form](#)

[Pregnancy Registry form](#)