

VecTest® Tutorial for Local Agencies



California Department of Health Services
and the
UC Davis Center for Vectorborne Diseases

What is the VecTest?

A simple rapid test for West Nile virus (WNV) in
crows or mosquito pools

Fancy name: Dipstick Immunochromatographic assay




Test strips in a sample solution

How does the VecTest work?

1. A dipstick is coated with special WNV proteins (monoclonal antibodies ) , some with with gold (★) attached.



2. If a sample has West Nile virus (●) in it, the virus particle will bind to the special proteins, making a “complex” ().



3. The complex migrates through the stick “test zone” where it binds to other WNV proteins. This shows up as a positive reddish-purplish line on the dipstick.



4. A “control” line is formed where extra gold-antibody is deposited (for both positive and negative samples).



How likely is the VecTest to detect WNV infection (*Sensitivity*)?

- **VecTest can detect a very small number of virus particles (titers of $> 3.8 \log_{10}$ PFU/ml WNV).**
- **VecTest can detect a single positive mosquito in pooled sample of 50 mosquitoes**
- **Sensitivity = 70-85% for crows (the proportion of crows with WNV that test positive)**

Ryan, J et al., 2003. J. Med. Entomol., 40: 95-99.

Lindsay et al., 2003. EID 9:1406-1410.

How likely is the VecTest to be negative when a bird is not infected with WNV (*Specificity*)?

- **Specificity = 90-95% for crows (the proportion of crows without WNV that test negative; 5-10% of crows will be “false positives”).**
- **When a bird is infected with EEE or WEE (Togaviruses), the WNV VecTest is negative.**
- **A separate kit is available for SLE (a different Flavivirus).**

Ryan, J et al., 2003. J. Med. Entomol., 40: 95-99.

Lindsay et al., 2003. EID 9:1406-1410.

When do I use the VecTest?

- **Crows may be tested by the VecTest when WNV has been found in a region***

** The size/ boundaries of a region will be discussed and determined on a case-by-case basis (options: zip code, ecological zone, city, county, etc.)*

Important Changes to Protocol!

As of August 11, 2004:

- **CROW CARCASSES** tested by VecTest should **NO LONGER** be submitted to California Animal Health and Food Safety Laboratory (CAHFS).
- State dead bird submission numbers are still required for birds tested by VecTest (call hotline).
- Send VecTest results to arbovirus@dhs.ca.gov.
- A duplicate oral swab in viral transport medium (VTM) should be sent to CAHFS for future testing.

Important Changes to Protocol!

- **For safety reasons, DHS now recommends that technicians swirl swab in viral transport medium (VTM) for 15 seconds instead of snipping or breaking off the end.**
- **The swab should be removed from conical tube and disposed with bird (i.e. double bag and place in garbage or incinerate).**

What's in the kit???

- Antigen Assay dipsticks
- Grinding solution
- Copper-coated BB's*
- 50 culture tubes
- 50 conical tubes
- Tube racks



***BB's are not necessary for testing dead bird swabs.**

What DHS will supply...



- One VecTest kit if local resources are insufficient to purchase kit “in house.”
- Conical tubes (eppendorf tubes) filled with 1ml Viral Transport Medium (VTM) (*keep refrigerated at 4° C*).
- Swabs.
- Freezer boxes.



**How Do I Use the VecTest to
Test Dead Birds?**

Conduct tests in a biosafety cabinet with appropriate personal protection



Ideal Personal Protection:

- Eye protection
- Surgical mask
- Disposable water-resistant clothing
- Double glove with disposable gloves
- Perform test in biosafety cabinet

1. Dispense 2.5 ml of grinding solution into plastic test tube.



2. Using 2 cotton or polyester swabs, scrape oropharyngeal cavity of crow (i.e. mouth, throat, and tongue).



- 3. Place first swab into conical tube with 1ml VTM and swirl for 15 seconds. Press swab on edge of tube to release VTM from swab. Snap tube closed and label top of tube with state bird number and date.**



4. Crows: Send only VTM sample to CAHFS.

Other bird species: Send VTM sample *and* bird carcass to CAHFS.

- Place labeled VTM samples in white freezer box. Keep refrigerated until ready to ship with bird; do not freeze/thaw sample.
- Pack freezer box with folded paper towels and place in plastic bag before sending with bird carcass.

5. Place second swab into grinding solution in test tube provided with VecTest kit; break or cut off cotton (polyester) tip near top of shaft.



6. VecTest swab should be incubated in grinding solution for 30 minutes at room temperature; it may be vortexed intermittently. **BBs are not necessary*



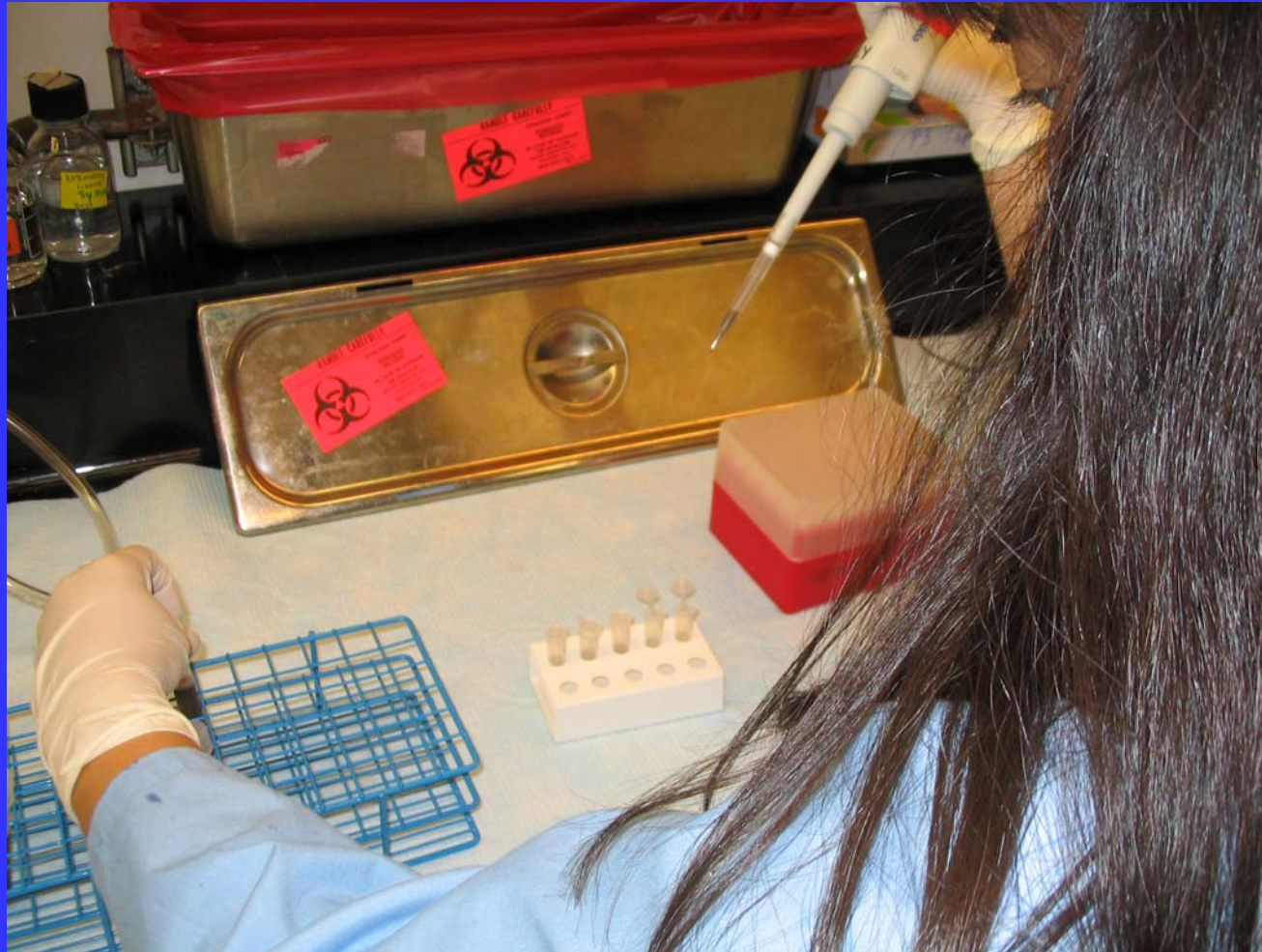
7. If sample is full of debris, tubes can be centrifuged for 5 minutes at 4,000 rpm.



8. Dispense 250 μ l of grinding solution from test tube into conical tube.



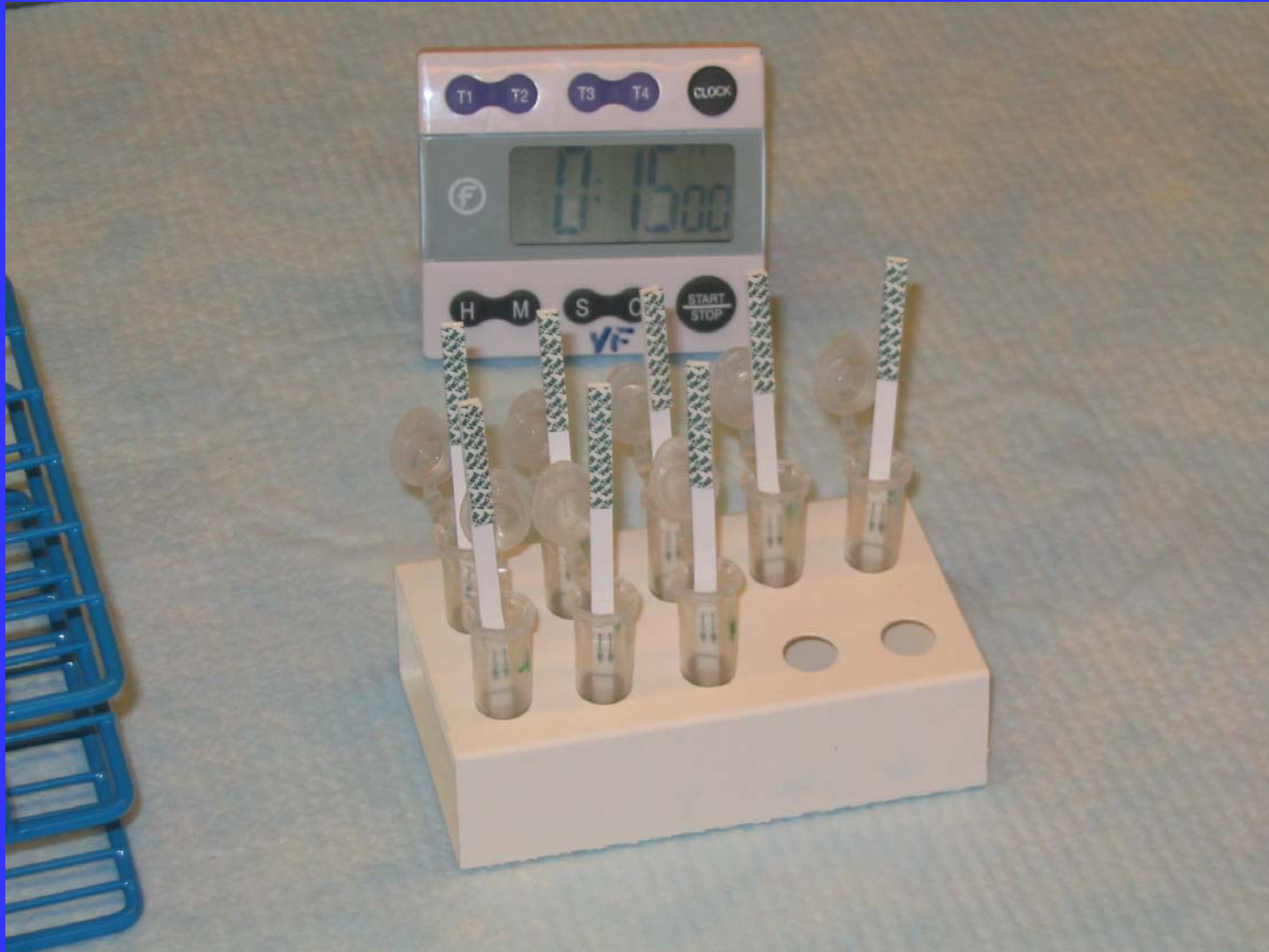
9. Place conical tubes into tube stand.



10. Insert test strip into solution with arrows pointing DOWN.



11. Wait 15 minutes for the test to be completed.



12. Determine test results by removing test strip and checking for presence of two lines (positive control and test line).



Incinerate or Autoclave

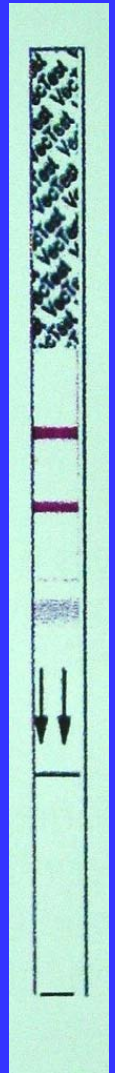
All Dead Birds and VTM Swabs

(if not possible, double-bag and put in trash)



Interpretation of Results

- Control line: should be **reddish purple** to indicate test is working.
- A second **reddish-purple** line that corresponds to WNV-positive location (below control line) indicates a positive result.



Subjectivity of results

- **Sometimes it is difficult to determine if sample is positive or negative (light bands).**
- **DHS suggests that several people independently interpret results.**
- **Remember to include borderline or questionable (“low positive”) VecTest results in weekly report to DHS.**

What to do if a bird tests positive?

- **Call the hotline (877 WNV-BIRD) to report bird as VecTest positive.**
- **For all birds – with the exception of CROWS -send carcass to CAHFS.**
- **Send the swab in VTM in labeled conical tube (see steps 3 and 4) for parallel confirmatory testing by Center for Vectorborne Diseases (CVEC). *Oral swabs in VTM will be tested by RT-PCR and Viral Isolation to assess whether oral swabs are potential surrogate for bird carcass.***

Crows are only bird species approved for WNV VecTest

Crows acceptable for testing...

- **Have no blood in mouth**
- **Are not desiccated**
- **Died recently**



Yaremych et al. 2003. EID 9: 1319-1321.

Birds that are not currently approved for VecTest...



Steller's Jay



Magpies



Western Scrub Jay

Birds that are NOT currently approved for VecTest...

Photo by Peter S. Weber



Blackbirds



Hamblin

Sparrows



Finches

Birds that are NOT good candidates for VecTest...



Hawks



Owls

Report ALL VecTest results weekly to DHS!

- **Send weekly report of VecTest results (both positive and negative) to DHS at arbovirus@dhs.ca.gov**
- **Information from local agencies on the VecTest will determine usefulness of assay for future years (e.g. number of false positives and false negatives from field results).**

VecTest Advantages

Simple

Rapid

Portable

VecTest Disadvantages

Not as sensitive as RT-PCR

Results are subjective (difficulty interpreting bands)

Expensive (\$8.00 per test)

To purchase VecTest & Supplies:

VecTest: MAS Medical Analysis Systems, Inc.
5300 Adolfo Road, Camarillo, CA 93012
Telephone: (805) 987-7891; FAX (805) 987-6442
www.mas-inc.com

Eppendorf Tubes: PCR-clean safe-lock 2.0ml
(* Cat No. 2236-335-2)

Swabs: Fisher polyester fiber tipped
applicators (* Cat. No. 14-959-90)
*Fisher Scientific (Telephone: 1-800-766-7000)
<https://www1.fishersci.com/index.jsp>

Viral Transport Media (VTM):

Pending: Invitrogen (Melody Savea) 1-800-955-6288 (Ext: 66533)

Any Questions?

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