VecTest™ and RAMP ® Tutorial for Local Agencies
What is covered in this tutorial:

• Algorithm for WNV avian oral swab testing
• Brief Description of rapid WNV tests:
  – VecTest
  – RAMP
  – Sensitivity and Specificity
• How to swab birds – detailed protocol
• Bird species
• VecTest – detailed protocol
• RAMP assay – detailed protocol
• Algorithm for testing birds for WNV
  – How to report testing results
  – Shipping
Public reports dead bird to VBDS:
Is bird acceptable for West Nile virus (WNV) testing?

Yes

Bird assigned state number and picked up by local agency

VBDS assigns primary identification Corvid or Non-Corvid?

Non-corvid

Send carcass to CAHFS; Tissue to CVEC; Results to VBDS

Corvid

Does local public health laboratory (PHL) participate in dead bird WNV testing?

Yes

Local agency delivers bird to PHL and assigns species

Oral swab is tested by VecTest or RAMP

Crow or Non-Crow?

Crow

Test oral swab by PCR

STOP, submit results to VBDS by Friday at 4:00pm

Non-Crow

Submit carcass to CAHFS

STOP, submit results to VBDS by Friday at 4:00pm

Negative

Crow or Non-Crow?

Crow

Test oral swab by PCR

STOP, submit results to VBDS by Friday at 4:00pm

Non-Crow

Submit carcass to CAHFS

STOP, submit results to VBDS by Friday at 4:00pm

Negative

Does local agency have VecTest / RAMP?

Yes

Test oral swab by VecTest or RAMP

Positive

Send carcass to CAHFS

Positive

Send buffer solution to CVEC for PCR testing

STOP, submit results to VBDS by Friday at 4:00pm

Negative

Non-Crow

Send to CAHFS

Public reports dead bird to VBDS: Is bird acceptable for West Nile virus (WNV) testing?

Dead bird reports available to agencies on request

VBDS
Public Health Labs
Local Agencies

CVEC = Center for Vectorborne Disease Research
VBDS = Vector-Borne Disease Section, California Department of Health Services
PHL = Public Health Laboratory
CAHFS = California Animal Health and Food Safety Laboratory
State Algorithm

- Test only corvids using VecTest or RAMP (submit non-corvid carcasses to CAHFS).
- If corvid tests positive, submit results to VBDS.
- If crow tests negative, send VecTest or RAMP buffer to CVEC.
- If other corvid (non-crow) tests negative, submit carcass to CAHFS as usual.
VecTest®
Medical Systems, Inc., Camarillo, CA
Description of the VecTest

• Quick and easy dipstick test for WNV
  – Detects virus in a sample (mosquito or avian oral swab).

• Can be used by local vector agencies.

• Qualitative ("yes" or "no" result).
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).
• Swabs.
• Freezer boxes.
How does the VecTest work?

1. A dipstick is coated with special WNV proteins (monoclonal antibodies), some 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).
RAMP®
Response Biomedical Corp. Burnaby, B.C. Canada
What is the RAMP Assay?

• RAMP stands for “Rapid Analyte Measurement Platform.”
  – Tests for virus in sample (mosquito or avian oral swab).
  – Uses disposable cartridge and fluorescence reader.
• Can be used by local vector agencies.

• Quantitative (higher the number reflects higher viral load in specimen).
What’s in the RAMP kit?

- Test cartridges
- Assay tips
- Lot card
- 1.5mL dilution vials
- Sample buffer
- 0.6 mL Vials
- Copper ball bearings
- RAMP reader
- 70µL pipette
How does RAMP work?

Sample is mixed with fluorescent-labeled latex spheres (○) coated with WNV-specific antibody (←) before loading into test cartridge sample well.

When Sample migrates through membrane, fluorescent latex particles are carried by capillary flow.

WNV antigen (virus) (○) bound to latex is immobilized by second WNV-specific antibody at detection zone. Unbound latex particles are immobilized by third antibody (anti-mouse Ab) at control zone.
Test cartridges are scanned in the RAMP Reader to measure the fluorescence bound at the detection zone and at the Internal Control Zone. Using a ratio between the two fluorescence values, a quantitative reading is calculated and reported.

A reading of $\geq 50$ units is considered positive for avian oral swabs (14.9 is cut off for mosquitoes).
Sensitivity and Specificity
How likely is the VecTest to detect WNV infection (*Sensitivity*)?

- VecTest can detect a very small number of virus particles (titers of $> 3.8 \log_{10} \text{PFU/ml WNV}$).

- 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).

*Lindsay et al., 2003. EID 9:1406-1410.*
*Stone et al., 2004. EID 10:2175-2181.*
### VecTest: Sensitivity and Specificity of Avian Oral Swabs, California 2004

<table>
<thead>
<tr>
<th>Birds tested</th>
<th>Number</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>American Crow</strong></td>
<td>347</td>
<td>70%</td>
<td>98%</td>
</tr>
<tr>
<td><strong>Common Raven</strong></td>
<td>22</td>
<td>75%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Western Scrub Jay</strong></td>
<td>98</td>
<td>54%</td>
<td>97%</td>
</tr>
<tr>
<td><strong>Raptors</strong></td>
<td>30</td>
<td>50%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Non Corvids:</strong></td>
<td>53</td>
<td>47%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Results determined by comparing results from individual birds tested by VecTest (oral swab) at California vector agencies and by PCR at CVEC (kidney tissue).
How likely is the RAMP to detect WNV infection (*Sensitivity*)?

- RAMP can detect a very small number of virus particles (titers of $> 3.17 \log_{10} \text{PFU/ml WNV}$).

- RAMP can detect a single positive mosquito in pooled sample of 50 mosquitoes.

- Sensitivity = 64-94% for crows (the proportion of crows with WNV that test positive).

*DHS/CVEC, unpublished results
Burkhalter et al., poster 2003 AMCA Meeting*
### RAMP: Sensitivity and Specificity of Avian Oral Swabs, California 2004

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<th>Number</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Crow</td>
<td>91</td>
<td>64%</td>
<td>95%</td>
</tr>
<tr>
<td>Western Scrub Jay</td>
<td>55</td>
<td>37%</td>
<td>100%</td>
</tr>
<tr>
<td>YB Magpie</td>
<td>21</td>
<td>69%</td>
<td>100%</td>
</tr>
<tr>
<td>Raptors</td>
<td>21</td>
<td>0%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Results determined by comparing results from individual birds tested by RAMP (oral swab) at California vector agencies and by PCR at CVEC (kidney tissue).
How likely is the VecTest and RAMP to be negative when a bird is not infected with WNV (Specificity)?

- Specificity is excellent for these tests. For corvids, the proportion without WNV that test negative is 90-100% (0-10% of corvids tested will result in “false positives”).

Lindsay et al., 2003. EID 9:1406-1410.
DHS/CVEC, unpublished results
Recommendations for testing:
Bird Species
Birds acceptable for testing...

- Have no blood in mouth.
- Are not desiccated.
- Died recently (within 24-48 hours).
- Are not heavily infested with maggots.
Corvids are the only birds approved for VecTest and RAMP

Common Raven

American Crow
Other Corvids of California...

- Steller’s Jay
- Western Scrub Jay
- Yellow-Billed Magpies
Birds that are NOT currently approved for VecTest or RAMP...

Sparrows

Blackbirds

Finches
Birds that are NOT good candidates for VecTest or RAMP...

Hawks

Owls
How do I swab birds?
Swab birds 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
Inside biosafety cabinet, remove bird’s head, keeping body within plastic bag.
Open bird’s beak and place swab into oral cavity.
Move swab into proximal esophagus (throat); constrict throat and move swab up and down.
Immediately place swab into tubes containing either VecTest grinding solution or RAMP buffer.
After incubating swab for a minimum of 10 seconds, press swab against side of tube to release remaining fluid.
To dispose of swab – place swab into bag with bird to be disposed of appropriately.
Incinerate or Autoclave All Dead Birds and Swabs

(Positive birds must be treated as biohazard waste)
“Biohazardous Waste” as defined by the California Health and Safety Code

Section 117635:

Animal parts, tissues, fluids, or carcasses suspected by the attending veterinarian of being contaminated with infectious agents known to be contagious to humans.
How Do I Use the VecTest to Test Dead Birds?
1. Dispense 1.0 mL** of grinding solution into plastic test tube.

** Note: for mosquitoes, use 2.5mL
2. Using a polyester swab, swab oral cavity of bird completely as previously described.
3. Place swab into grinding solution in test tube provided with VecTest kit; Swirl for a minimum of 10 seconds.
4. Press swab against side of tube to release remaining fluid.
5. If sample is full of debris, tubes can be centrifuged for 5 minutes at 4,000 rpm.
6. Dispense 250 µl of grinding solution from test tube into conical tube.
7. Place conical tubes into tube stand.
8. Insert test strip into solution with arrows pointing DOWN.
9. Wait 15 minutes for the test to be completed.
10. Determine test results by removing test strip and checking for presence of two lines (positive control and test line).
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.
Interpretation of VecTest Results

Test Strip: 1 2 3 4 5

<table>
<thead>
<tr>
<th>Test Strip</th>
<th>Visual Scoring</th>
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<tbody>
<tr>
<td>1</td>
<td>Negative</td>
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<tr>
<td>2</td>
<td>Low Positive/ Negative?</td>
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<tr>
<td>3</td>
<td>Low Positive</td>
</tr>
<tr>
<td>4</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>Positive</td>
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</table>

Internal control
Detection zone
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.
- Treat borderline results as negative and submit carcass or buffer for further testing.
RAMP
1. Add 1mL RAMP sample buffer to empty 1.5mL dilution vial. Secure lid.
2. Using absorbent end of swab, swab bird’s oral cavity.
3. Place corvid swab into sample buffer and rotate for at least 10 seconds.
4. After incubating swab for a minimum of 10 seconds, press swab against side of tube to release remaining fluid (Note: 1.5mL conical tube not shown here).
5. Remove 120 µL supernatant and transfer to empty 0.6mL sample vial*

*(recommended to use 2mL tube as shown below).*
6. Open foil pouch containing assay tip and test cartridge. Place on clean, dry surface.
7. Firmly place assay tip on Minipet or 70µL pipette. Make sure pink dot is visible in assay tip.
8. Depress plunger and place assay tip into sample vial. Mix sample by releasing and depressing plunger 10 times.

* Make sure only liquid, not air, is drawn into assay tip. This will avoid foaming.
9. Release plunger and remove 70μL of mixed sample and transfer to sample well of test cartridge. Discard assay tip.
10. Allow test cartridge to dry (> 90 minutes) at room temperature.
11. Turn RAMP reader on.

Press [Enter] to select RUN TEST

Follow instructions on screen to enter SAMPLE and USER ID
12. When prompted, insert test cartridge into RAMP reader as shown below. Result is displayed within 1 minute.
13. Remove test cartridge from reader. Print or write output results in laboratory notebook.
Interpreting RAMP Results

- RAMP WNV results are displayed in units within range of 0.0 – 640.0.
- Results of 0 – 49.0 units from corvids are considered NEGATIVE.
- Results above 50.0 units from corvids are considered POSITIVE.

* Note: the cut off level for mosquitoes is 14.9 units.
Reporting Results
Report ALL avian oral swab test results weekly to DHS!

• Submit weekly report of VecTest/ RAMP results (both positive and negative) on “Avian VecTest/ RAMP Results” form.

• Forms can be found at [http://westnile.ca.gov/publications.htm](http://westnile.ca.gov/publications.htm) and should be submitted to arbovirus@dhs.ca.gov each Friday by 4:00pm to be included in following week’s West Nile state update.
Avian VecTest/ RAMP Results

TO BE INCLUDED IN WEEKLY WNV REPORT, RESULTS MUST BE RECEIVED NO LATER THAN FRIDAY AT 4:00PM.

California Department of Health Services
Vector-Borne Disease Section
850 Marina Bay Parkway
Richmond, CA 94804
FAX: 510-412-6263   Email: arbovirus@swhs.ca.gov

REPORTING AGENCY NAME: ____________________________________________

AGENCY CODE: ______________________________________________________

AGENCY CONTACT: __________________________________________________

WEEK NUMBER: ______________________________________________________

SERVICE DATES:      FROM: _____________      TO: _____________

<table>
<thead>
<tr>
<th>Date</th>
<th>Bird Number*</th>
<th>Species</th>
<th>Address (street, city, zip code)</th>
<th>VecTest Results</th>
<th>RAMP Results</th>
<th>RAMP Units</th>
<th>Buffer to CVEC?</th>
<th>Carcass to CAHFS?</th>
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* Call WNV Hotline to receive dead bird number for all birds tested (1-877-968-2473); add “V” suffix to number.
NEW!!!

Alternatively, results can be entered online at:
http://cvecdata.ucdavis.edu/deadbid3.cfm

Nevertheless, results must be received by Friday at 4:00pm
To submit VecTest or RAMP buffer from negative crows

- Discontinue use of viral transport medium.
- Send 1mL of VecTest grinding solution or 800-880µl (remaining volume) RAMP buffer in 1.5mL or 2mL snap-top tubes.
- Label with dead bird number, including suffix “V”
- Place inside 5 inch² white box with dividers.
- Box should be placed in gallon zip lock bag and sent overnight to CVEC within padded envelope.
- Buffer does not need to be sent on ice.
To purchase VecTest & Supplies:

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

RAMP: Response Biomedical Corp.
8081 Lougheed Hwy, Burnaby, BC Canada V5A 1W9
Telephone: (888) 591-5577; FAX (604) 412-9830
www.mas-inc.com

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
Laboratory Addresses

**CAHFS (to submit carcasses)**

Central  
(Dr. Leslie Woods)  
Attn: WNV  
West Health Sciences Dr.  
UC Davis, Davis, CA 95616

San Bernardino  
(Dr. Deryck Read)  
Attn: WNV  
105 West Central Ave.  
San Bernardino, CA 92408

**CVEC (to submit buffers)**

Center for Vectorborne Diseases  
(Dr. Barbara Cahoon-Young)  
Old Davis Rd.  
UC Davis, Davis, CA 95616
Acknowledgements:

This tutorial was developed by Kerry Padgett (VBDS) with assistance from:

Aaron Brault, Emily Green, Barbara Cahoon-Young and Nicole Kahl (CVEC)

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