

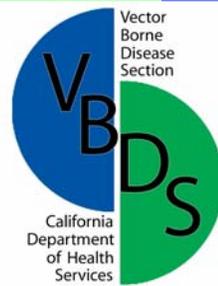


**Response**  
Biomedical Corp.



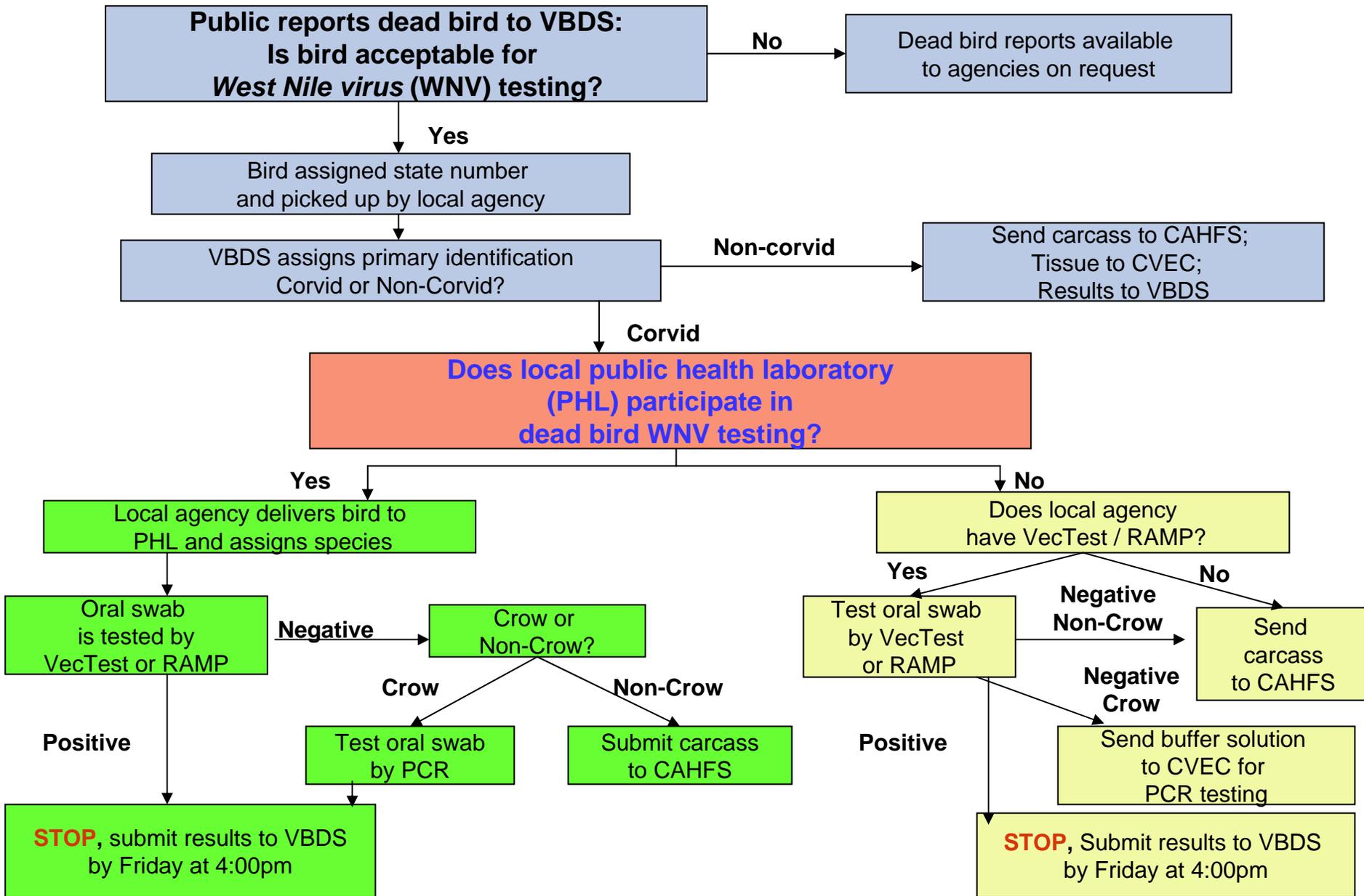
**WEST NILE VIRUS**

# **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



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

VBDS
  Public Health Labs
  Local Agencies

# 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.



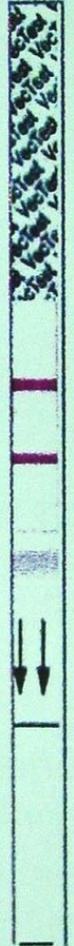
# VecTest<sup>®</sup>

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...



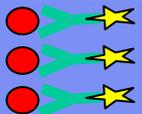
- **One VecTest kit if local resources are insufficient to purchase kit “in house.”**
- **Conical tubes (eppendorf tubes).**
- **Swabs.**
- **Freezer boxes.**



# How does the VecTest work?

1. A dipstick is coated with special WNV proteins (monoclonal antibodies , some with 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**





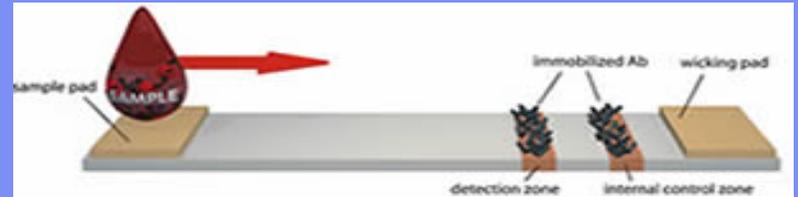
# What is the RAMP Assay?

- **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).**

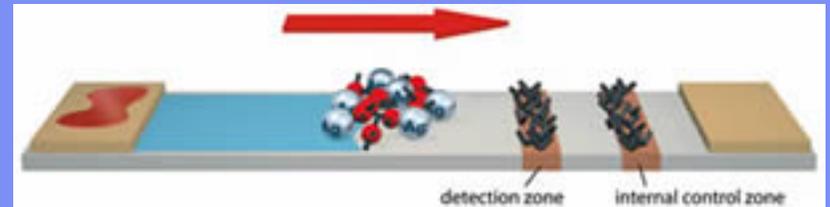


# How does RAMP work?

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



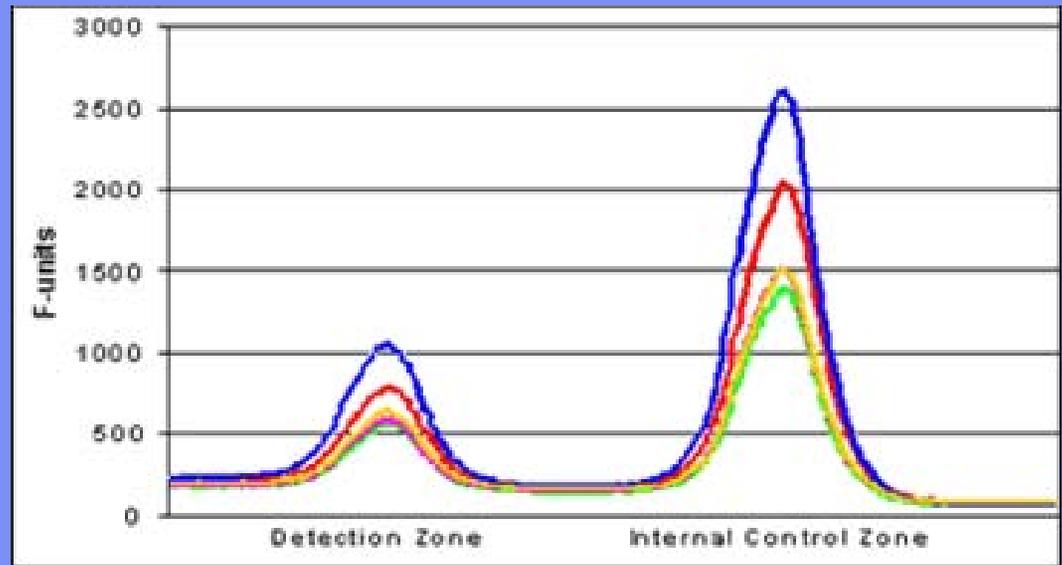
When Sample migrates through membrane, fluorescent latex particles are carried by capillary flow.



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  $> 3.8 \log_{10}$  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.*

*Stone et al., 2004. EID 10:2175-2181.*

# VecTest: Sensitivity and Specificity of Avian Oral Swabs, California 2004

<i>Birds tested</i>	<i>Number</i>	<i>Sensitivity</i>	<i>Specificity</i>
<b>American Crow</b>	<b>347</b>	<b>70%</b>	<b>98%</b>
<b>Common Raven</b>	<b>22</b>	<b>75%</b>	<b>100%</b>
<b>Western Scrub Jay</b>	<b>98</b>	<b>54%</b>	<b>97%</b>
<b>Raptors</b>	<b>30</b>	<b>50%</b>	<b>100%</b>
<b>Non Corvids:</b>	<b>53</b>	<b>47%</b>	<b>100%</b>

*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  $> 3.17 \log_{10}$  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

<i>Birds tested</i>	<i>Number</i>	<i>Sensitivity</i>	<i>Specificity</i>
<b>American Crow</b>	<b>91</b>	<b>64%</b>	<b>95%</b>
<b>Western Scrub Jay</b>	<b>55</b>	<b>37%</b>	<b>100%</b>
<b>YB Magpie</b>	<b>21</b>	<b>69%</b>	<b>100%</b>
<b>Raptors</b>	<b>21</b>	<b>0%</b>	<b>100%</b>

*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”).**

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

*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...

Photo by Peter S. Weber



**Blackbirds**



Hamblin

**Sparrows**

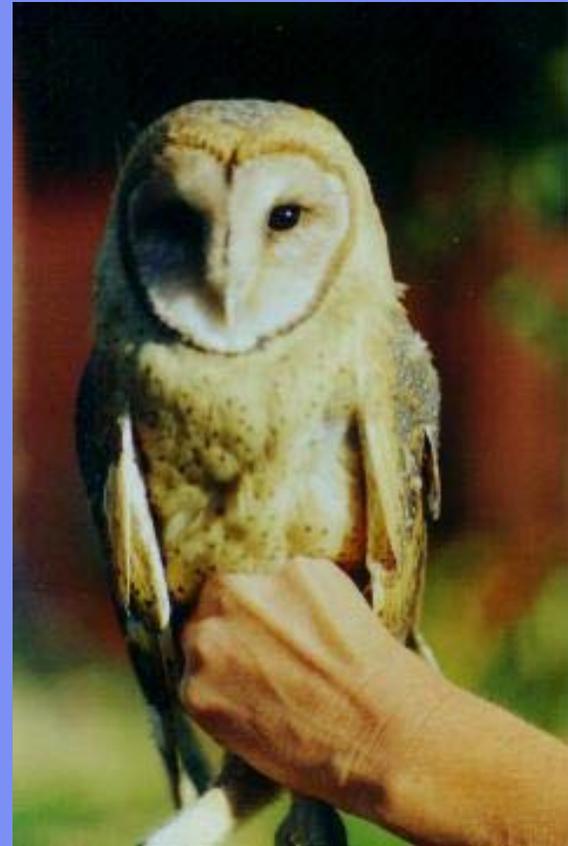


**Finches**

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



**Hawks**



**Owls**

**How do I swab birds?**

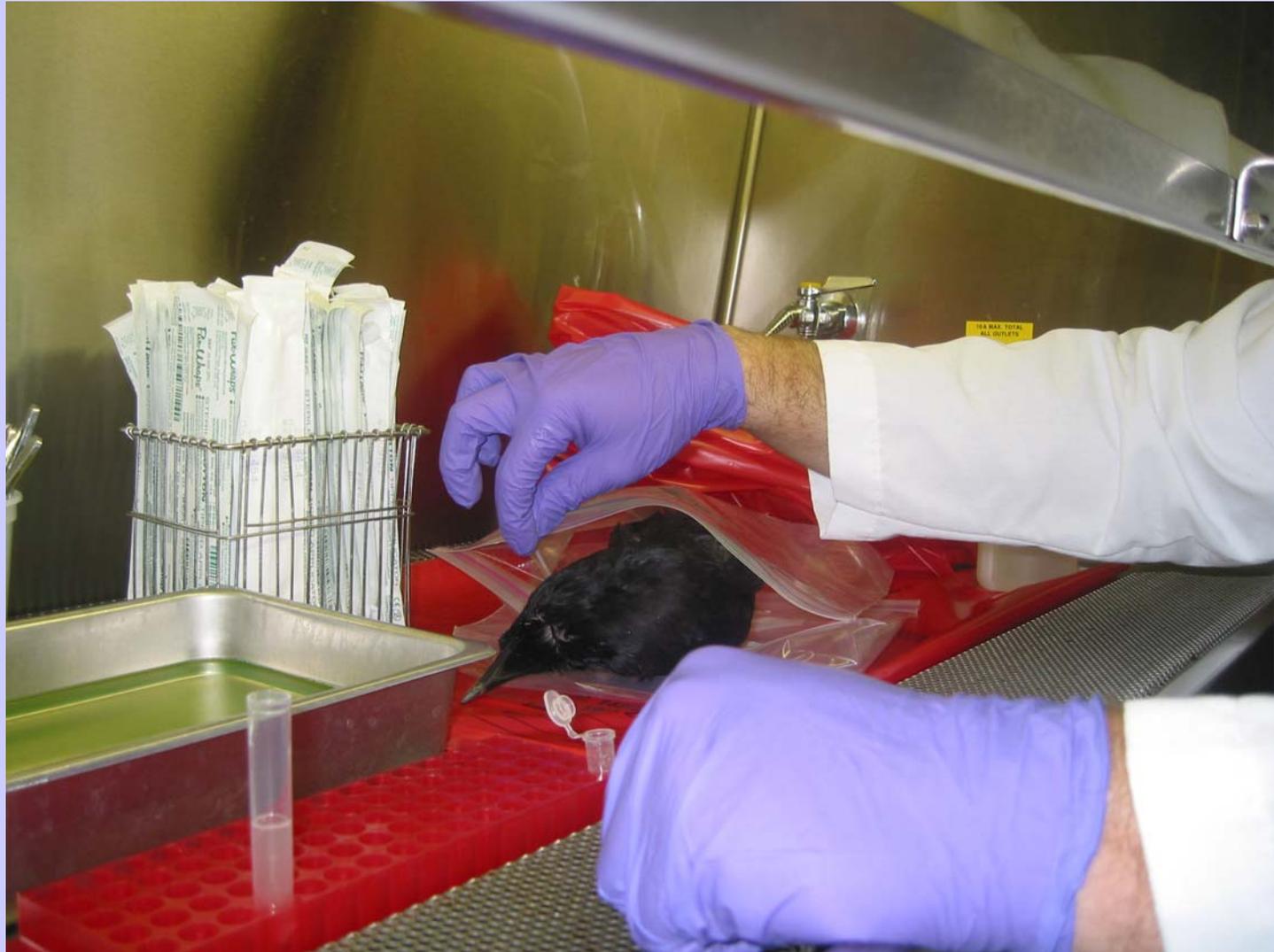
# Swab birds in a biosafety cabinet with appropriate personal protection



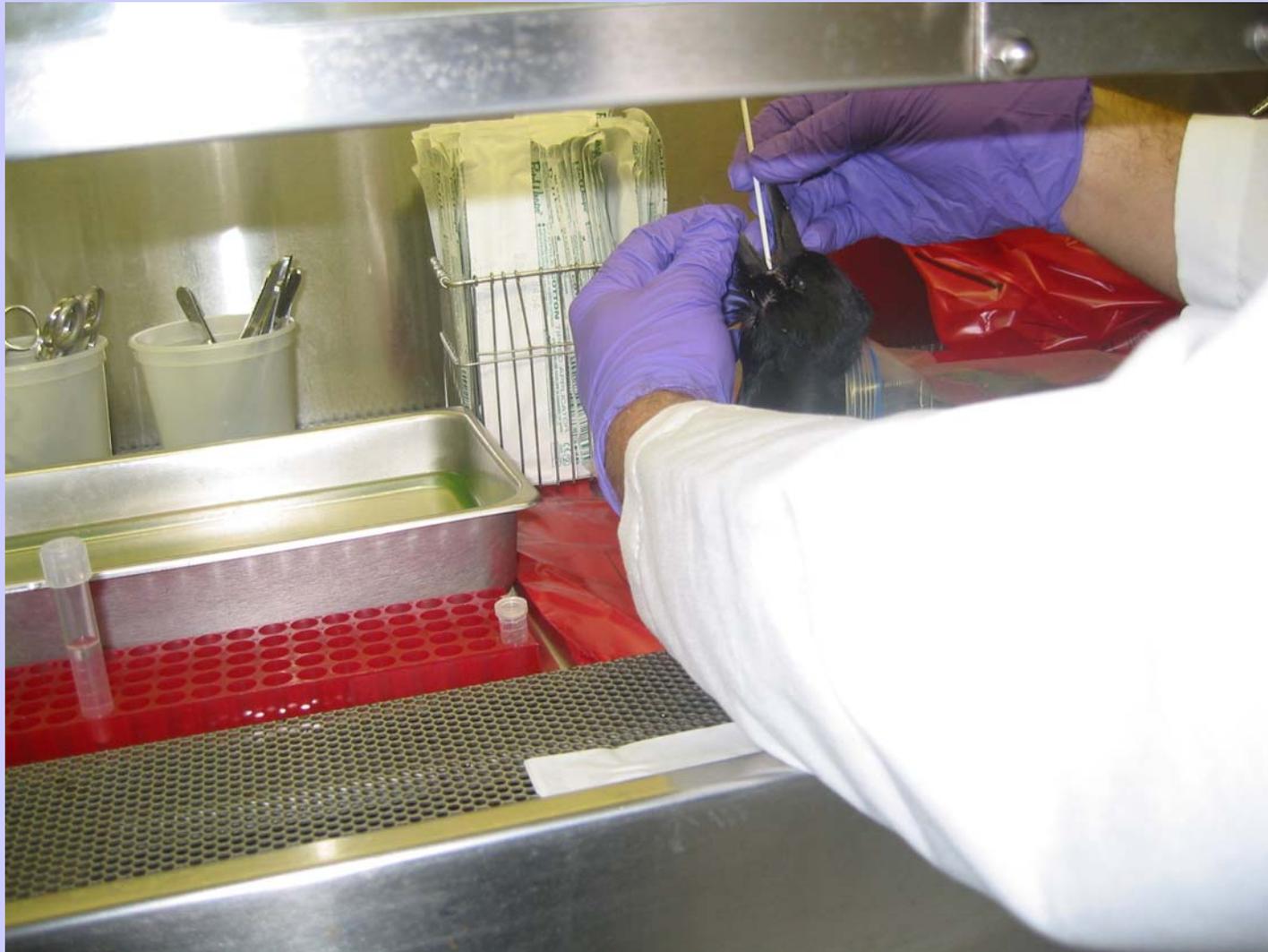
## Ideal Personal Protection:

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

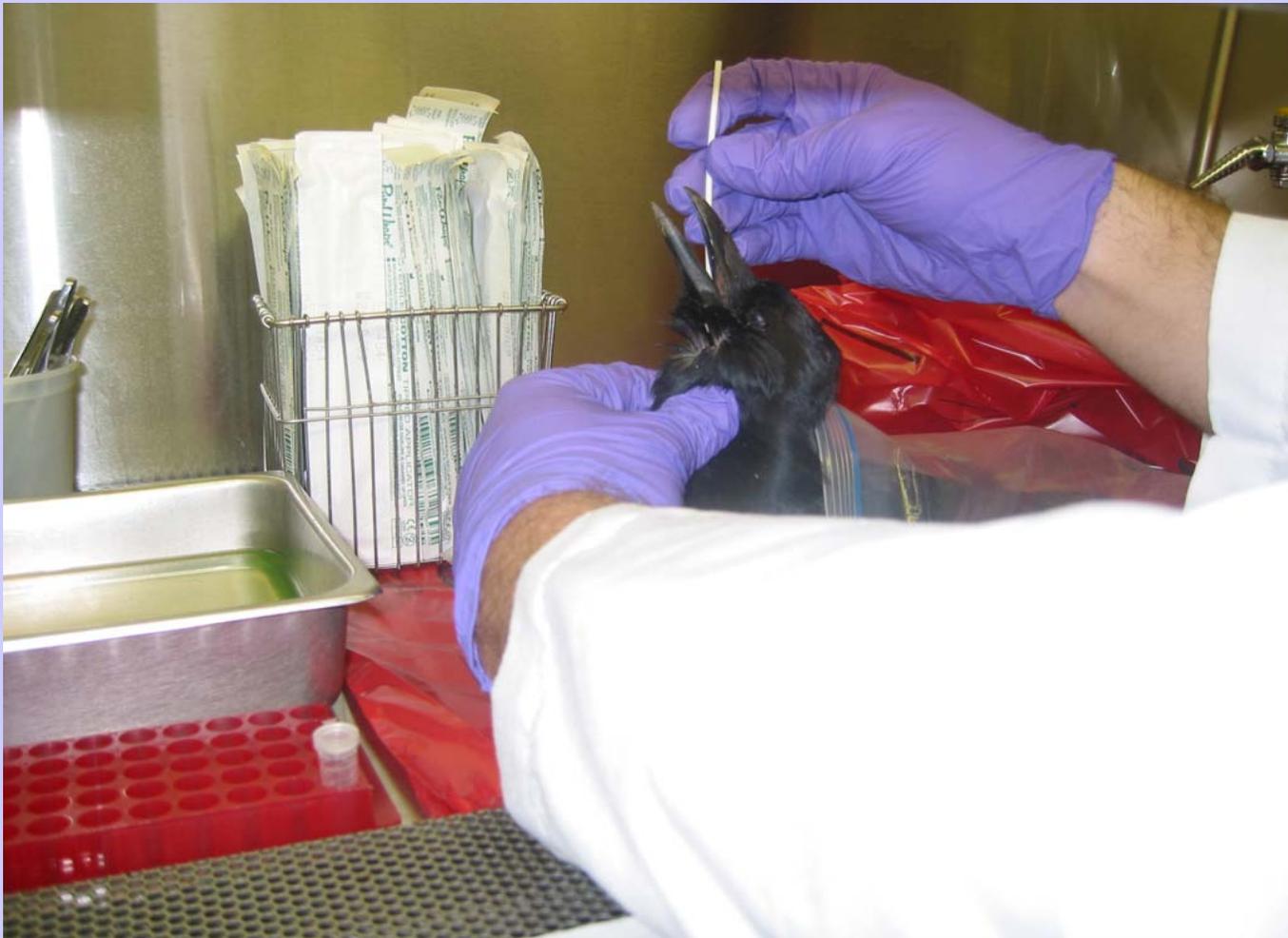
**Inside biosafety cabinet, remove bird's head, keeping body within plastic bag.**



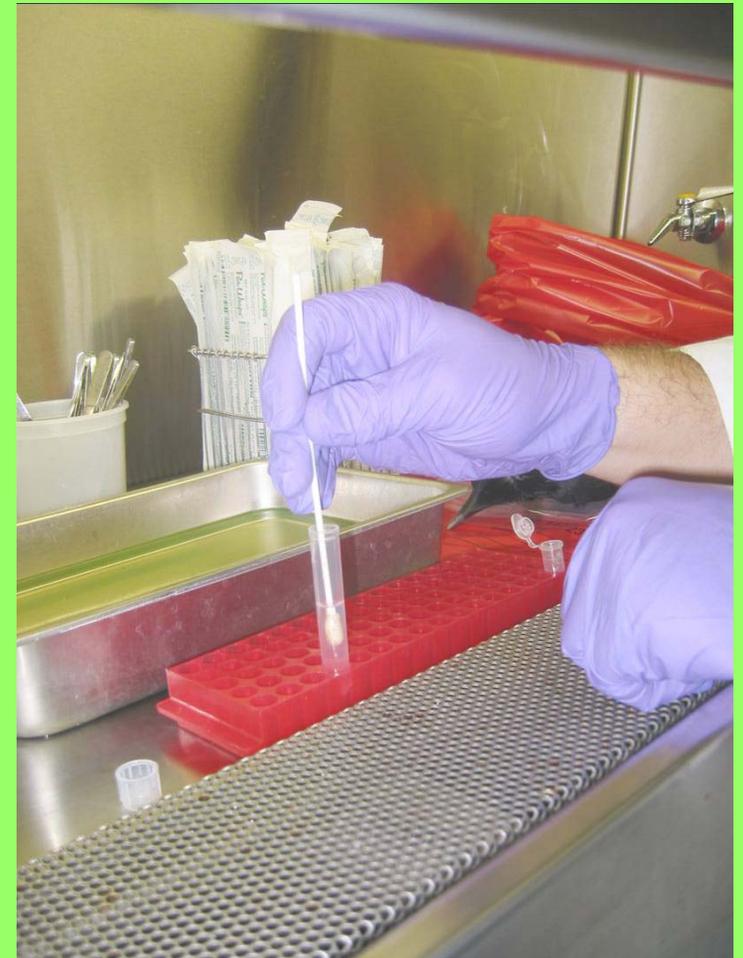
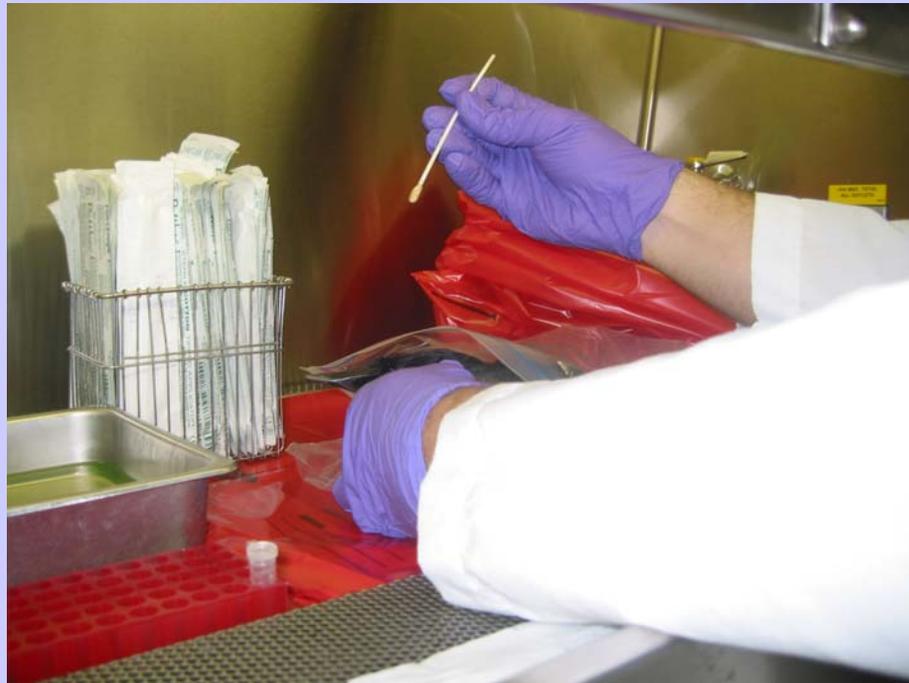
**Open bird's beak and place swab into oral cavity.**



**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 know 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.

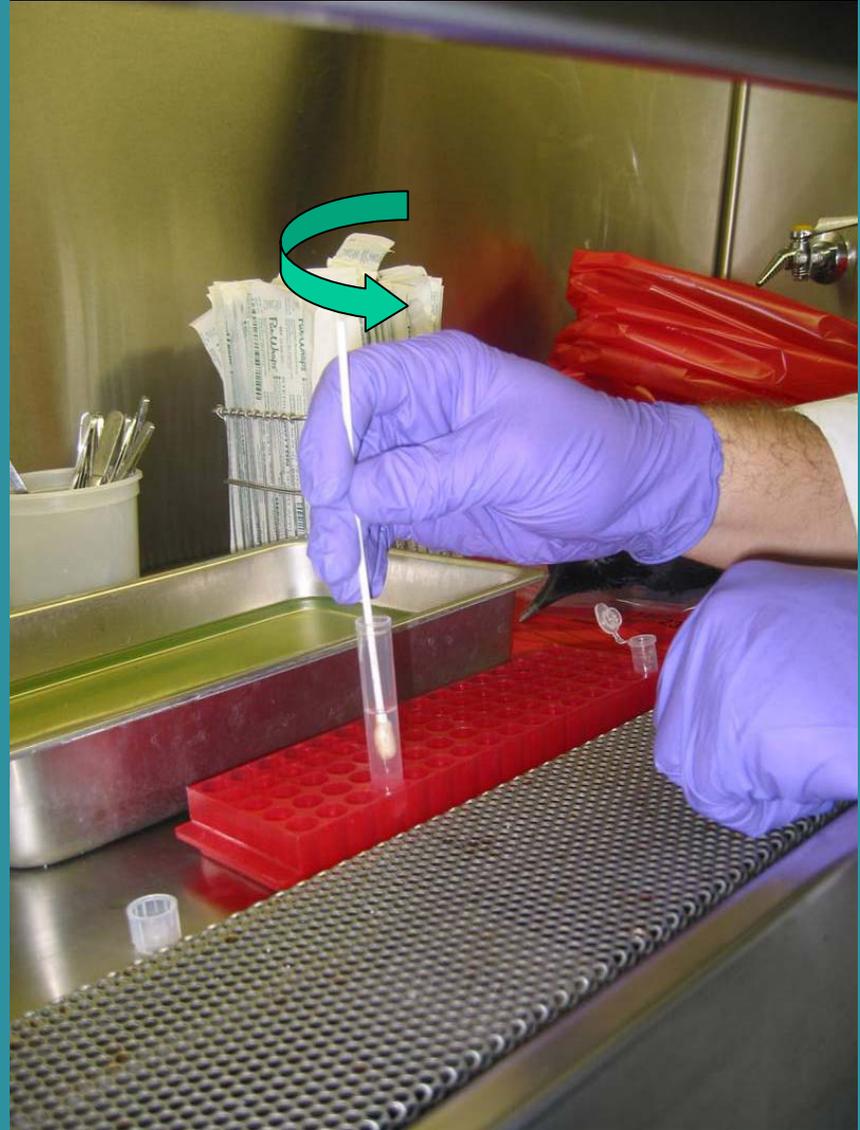


*\*\* Note: for mosquitoes, use 2.5mL*

**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.



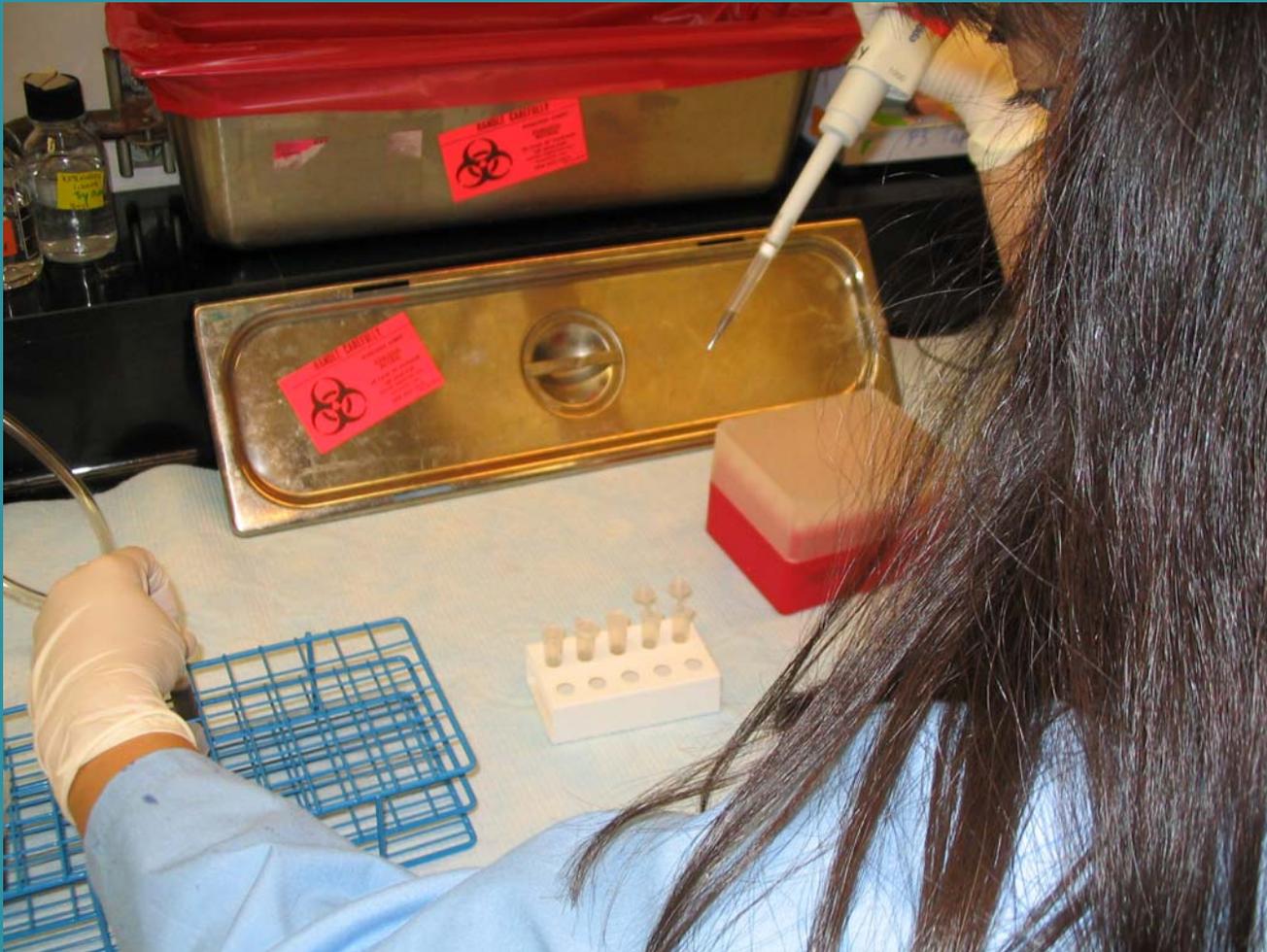
5. If sample is full of debris, tubes can be centrifuged for 5 minutes at 4,000 rpm.



**6. Dispense 250  $\mu$ l of grinding solution from test tube into conical tube.**



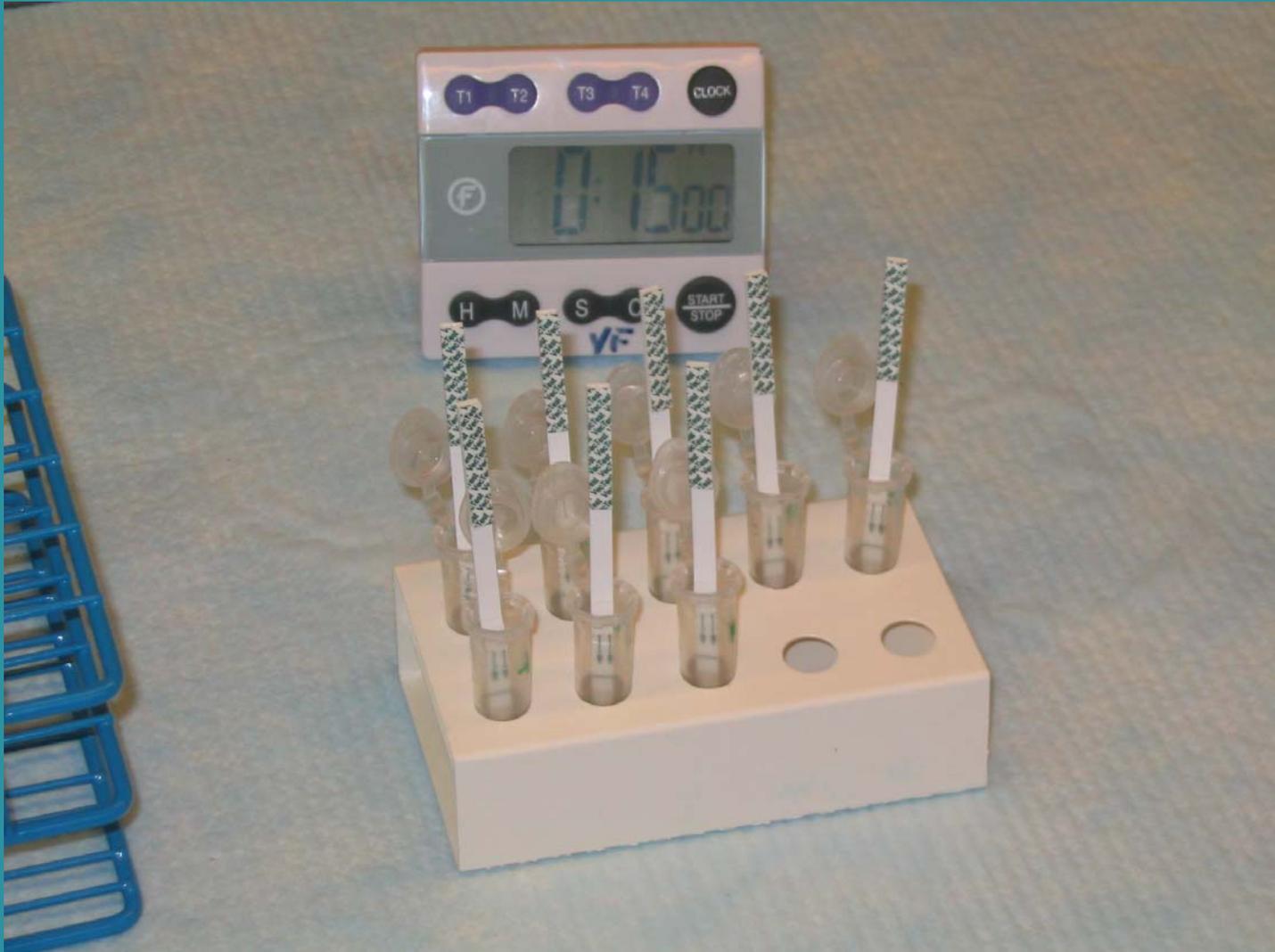
# 7. Place conical tubes into tube stand.



# 8. Insert test strip into solution with arrows pointing DOWN.



**9. Wait 15 minutes for the test to be completed.**

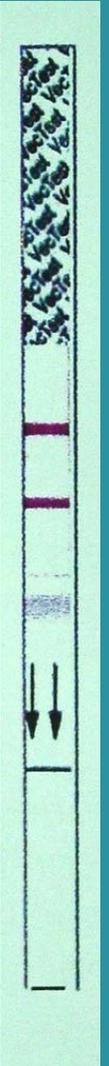


**10. Determine test results by removing test strip and checking for presence of two lines (positive control and test line).**

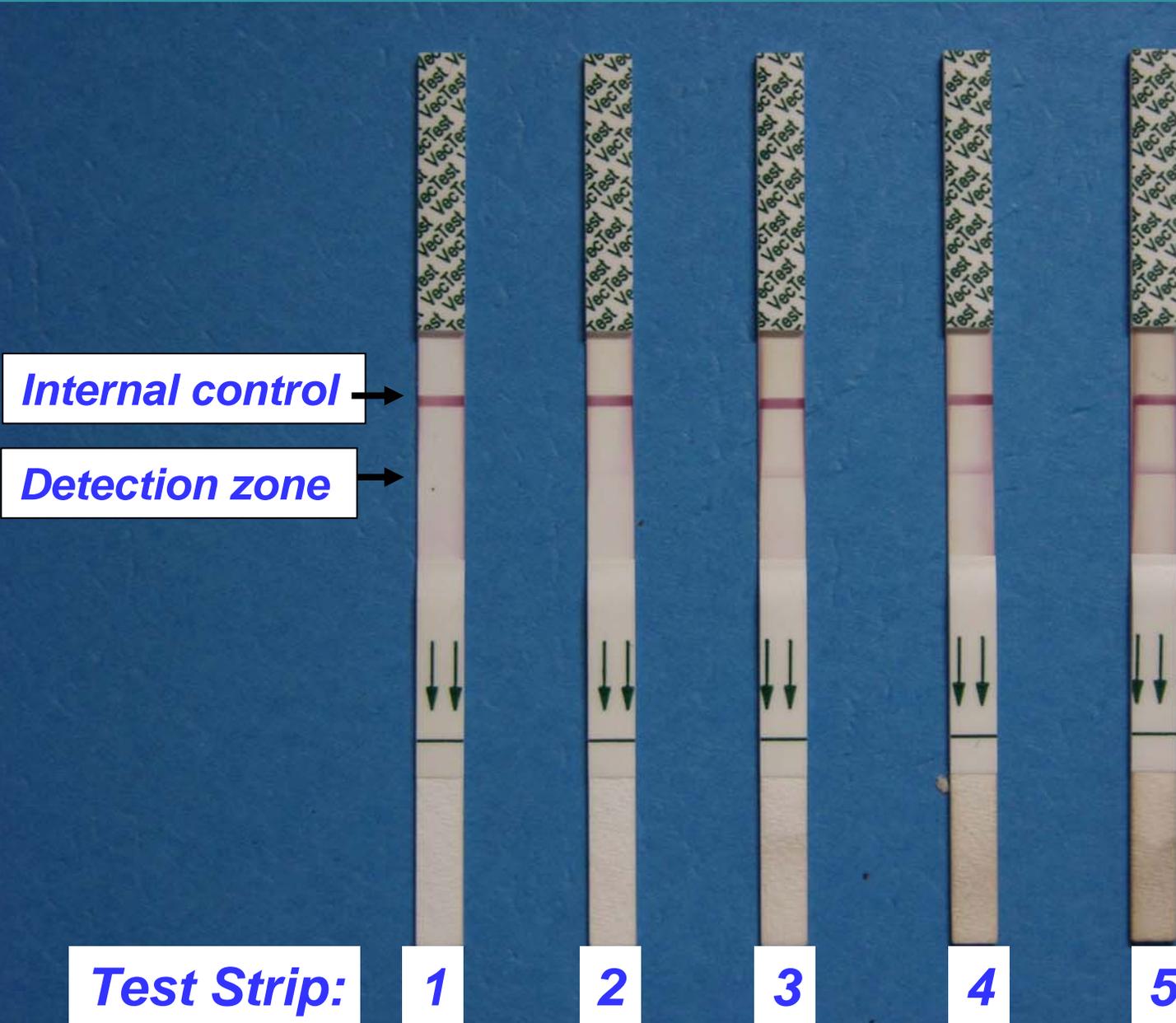


# Interpretation of Results

- 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	Visual Scoring
1	Negative
2	Low Positive/ Negative?
3	Low Positive
4	Positive
5	Positive

# 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.**



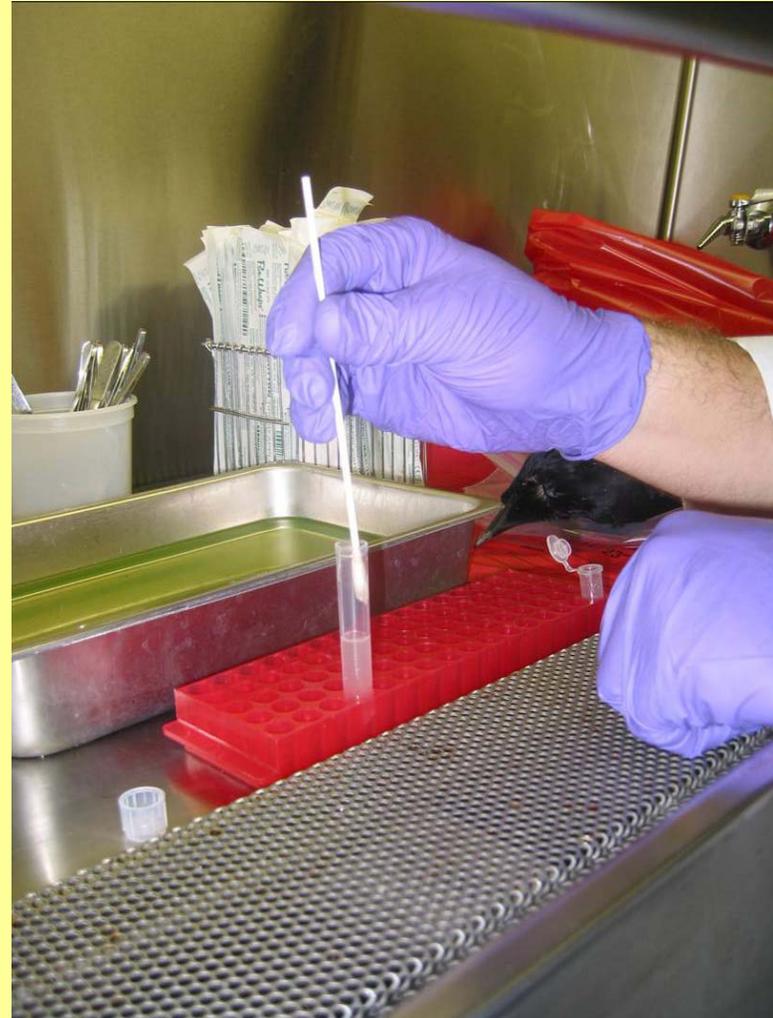
## 2. Using absorbent end of swab, swab bird's oral cavity.



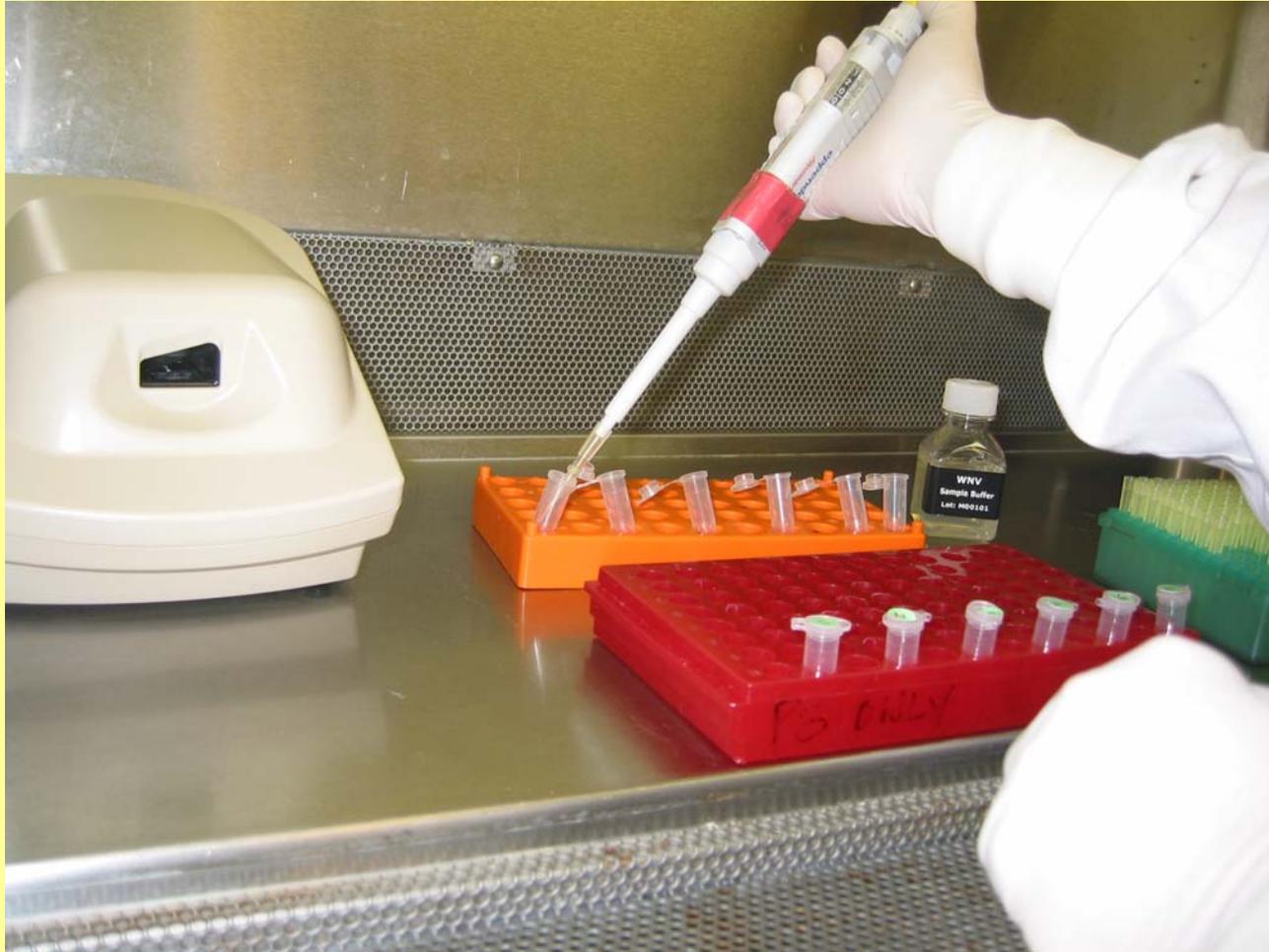
**3. Place corvid swab into sample buffer and rotate for at least 10 seconds.**



**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).*



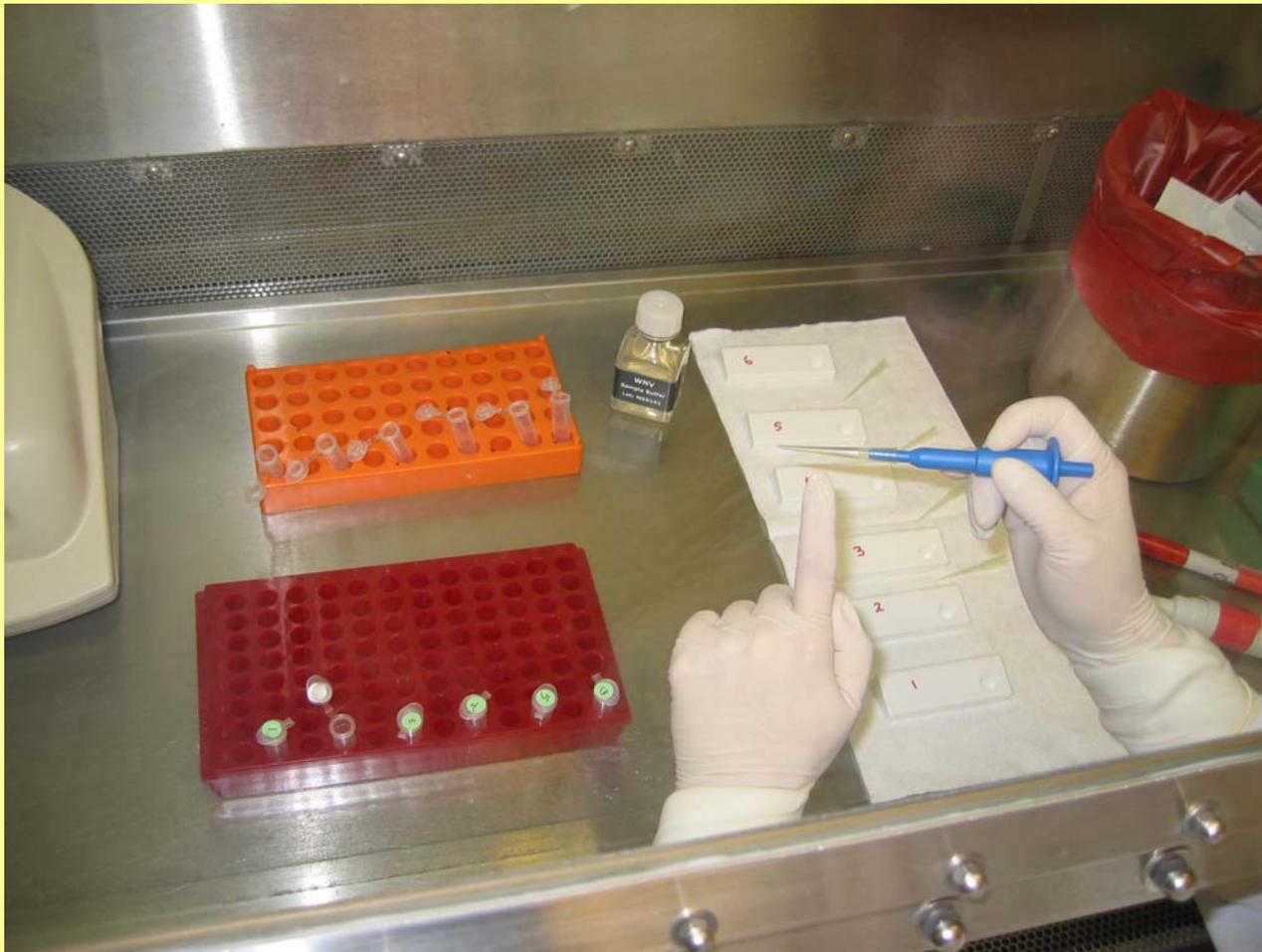
**5. Remove 120  $\mu$ L supernatant and transfer to empty 0.6mL sample vial\***  
*\*(recommended to use 2mL tube as shown below).*



**6. Open foil pouch containing assay tip and test cartridge. Place on clean, dry surface.**



**7. Firmly place assay tip on Minipet or 70 $\mu$ L 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 70 $\mu$ L 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> and should be submitted to [arbovirus@dhs.ca.gov](mailto: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@dhs.ca.gov](mailto:arbovirus@dhs.ca.gov)

**REPORTING AGENCY NAME:** \_\_\_\_\_

**AGENCY CODE:** \_\_\_\_\_

**AGENCY CONTACT:** \_\_\_\_\_

**WEEK NUMBER:** \_\_\_\_\_

**SERVICE DATES:**      **FROM:** \_\_\_\_\_      **TO:** \_\_\_\_\_

Date	Bird Number*	Species	Address (street, city, zip code)	VecTest Results	RAMP Results	RAMP Units	Buffer to CVEC?	Carcass to CAHFS?

\* 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 inch<sup>2</sup> 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](http://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

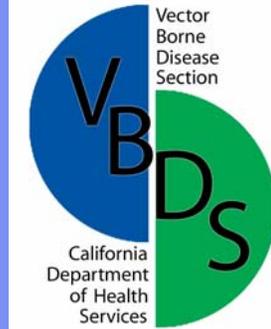
(Dr. Barbara Cahoon-Young)

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)**

**Jackie Parker and Ian Holser (CAHFS)**

**Anne Kjemtrup (VBDS)**

**Min-Lee Cheng (West Valley MVCD)**

