

**Protocol: Testing dead birds for West Nile virus using oral swab technique**  
California Department of Public Health

### **RNA Preservation Cards**

The California West Nile virus (WNV) dead bird surveillance program uses nucleic acid preservation cards (RNASound™ card brand, hereafter referred to as RNA preservation cards) for WNV testing of dead birds. Testing dead birds using RNA preservation cards is a proven reliable alternative procedure that does not require necropsy. Local agencies are encouraged to collect oral swab samples from dead corvids (crows, ravens, magpies, and jays), other perching birds, and raptors via RNA preservation cards for qRT-PCR testing at the UC Davis Arbovirus Research and Training (DART) Laboratory. Most bird species may be tested; however, doves, quails, pigeons, and chickens are not accepted for testing as they are not known to die of WNV.

### **Procedure**

1. Avoid direct contact with the dead bird by using disposable gloves and/or handle the carcass only with plastic bags as described below.
2. Dead birds should be handled in a Class II biosafety cabinet within a laboratory. If it is not possible to work with the bird carcass in a biosafety cabinet, work should be conducted outside while wearing an N95 respirator. One option is to collect the oral swab sample at the dead bird collection site.
3. **Refrigeration:** It is recommended to refrigerate carcasses until ready for swabbing in lieu of maintaining at room temperature. RNASound™ cards must also be stored in the refrigerator.
4. Partially unwrap the disposable swab.
5. Open the bag containing the bird to expose the head. With gloved hands, pry open the beak with a metal spatula, and put swab into the mouth. Aggressively swab the mouth and oropharyngeal cavity (throat).
6. Wipe, press, and roll the contents of the swab onto the target area of the RNA preservation card (over the two perforated discs). The sample may be dry; this is normal. Make sure to label the card with the dead bird number assigned to the bird by the WNV hotline.
7. Discard the swab into the bag containing the dead bird.

8. Wipe the inside of cabinet and metal spatula used for opening the beak with a fresh solution of 10% bleach, followed by 70 to 100% ethanol or isopropyl alcohol and change gloves after each bird. Cavicide™, a product that kills viruses without corroding stainless steel, may also be used.
9. Allow cards to dry in back of cabinet or outside in the shade for 2 hours. Make sure the dead bird number corresponding to the dead bird is written at the bottom of each card. Seal RNA preservation cards back into the small individual bags in which they were shipped.
10. Place all cards into a Ziplock bag and ship in an envelope to DART (address below). **IMPORTANT:** Include an inventory list of bird numbers corresponding to RNA preservation card samples in each shipment.

**Shipping options:**

- a. Seal all cards with card inventory list in another Ziplock bag and add to weekly mosquito pool shipments. The cold temperature of the mosquito boxes is fine for the cards, but cards should be protected from moisture.
  - b. Ship batches of cards via overnight delivery (FedEx, GSO). Ship on Monday for fastest turnaround times during the testing season.
  - c. Regular U.S. Postal Service mail is accepted; adding shipment tracking and requiring a signature upon receipt is highly recommended to help avoid lost packages.
11. Dead bird carcasses and used polyester swabs which are double-bagged can be discarded in the trash. If you sample birds at the place of collection, the resident may dispose of the carcass in an outdoor trash bin, or you may do it for them. Agencies conducting in-house testing must dispose of any WNV-positive birds as biohazardous waste (incinerate); negative birds can be discarded in the trash.
  12. Once your agency receives test results, telephone the citizens who reported dead birds which tested WNV-positive to let them know the bird had WNV and deliver risk prevention information if needed.

**Ship cards using one of the addresses below:**

ATTN: Ying Fang  
University of California  
One Shields Avenue  
Vet Med: PMI Room 4206  
Vet Med 3A  
Davis, CA 95616

**For UPS shipments only:**

Ying Fang  
VM://PMI 4206  
Vet Med 3A  
1285 Veterinary Medicine Mall  
University of California, Davis  
Davis, CA 95616

**Materials you will need:**

- Biosafety cabinet or N95 respirator masks
- Refrigerator to store RNA preservation cards and carcasses
- Disposable nitrile or latex gloves
- Lab coat
- **RNA preservation cards** (specifically, RNASound ReadyPunched™ cards). Order online at [http://www.fortiusbio.com/RNA\\_Sampling\\_Card.html](http://www.fortiusbio.com/RNA_Sampling_Card.html). Quantities of 25 (\$140) or 10 (\$60.20) are available. (Once cards arrive, store in the refrigerator and note the expiration date on the silver pouch. These cards are fine to use up to 18 months past the expiration date. Order as needed annually.)
- Individually-wrapped polyester swabs are included with the cards. If more are needed: Fisher brand cat. no. 23-400-116.
- Envelope or sandwich-size Ziplock bags
- Small metal spatula
- Permanent ink pen or sharp pencil
- Shipping envelopes (business size, FedEx, etc.)

Note for agencies conducting in-house testing by RAMP™ or qRT-PCR of tissues:

Once agencies pass the yearly proficiency panel, agencies may conduct in-house testing. Results may be entered directly into the CalSurv Gateway. **Note: any positive bird must be disposed of as biomedical waste (incineration).**

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