

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 carcass collection site.
3. **Refrigeration and freezing:** It is recommended to refrigerate carcasses until ready for swabbing in lieu of maintaining at room temperature. RNA preservation cards must also be stored in the refrigerator until use. Freezing is only recommended if you cannot swab the bird for several days after collection (more than 3 days), as it will require many hours for the carcass to thaw before it can be swabbed.
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 and may even be colored with some blood; this is fine. Make sure to label the card with the dead bird number assigned to the bird by the WNV hotline.

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7. **Disposal.** Discard the swab into the bag containing the dead bird, and tie or seal the bag. 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 carcasses as biohazardous waste (incinerate); negative birds can be discarded in the trash.
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. **Drying and storage.** 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. Once used, the cards do not need to be stored in the refrigerator but kept at room temperature. They should be tested within 10 days of sample taken.
10. **Ship.** Place cards into an envelope with an inventory list (template on page 5), and ship to DART (address on page 3).

Shipping options:

- a. Add to weekly mosquito pool shipment. Seal all cards with card inventory list (page 5) in a Ziplock bag and place in mosquito box. The cold temperature of the mosquito boxes is fine for the cards, but cards should be protected from moisture.

- Or -
 - b. Ship batches of cards via overnight delivery (FedEx, GSO). Ship on Monday for fastest turnaround times during the testing season.

- Or -
 - c. Regular U.S. Postal Service mail is accepted; however, paying additional for tracking or requiring a signature upon receipt is highly recommended to help avoid lost packages.
11. **Change status in Gateway.** Change the status of each dead bird in CalSurv Gateway (<http://www.gateway.calsurv.org>) to “submitted” for tracking purposes. Upon receipt, DART will update the status of the card in Gateway to “received.”

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12. **Notify resident.** Once your agency receives test results, telephone the resident who reported dead birds which tested WNV-positive to let them know the bird had WNV and deliver risk prevention information if needed. Or, if you have an agreement with the CDPH WNV Dead Bird and Mosquito Hotline that they will make the call, staff at the hotline will inform citizens whose birds tested **positive only** within about one week of final test result confirmation.

Ship cards using one of the addresses below:

ATTN: Ying Fang
University of California
One Shields Avenue
Vet Med: PMI Room 3336
Vet Med 3A
Davis, CA 95616

For UPS shipments only:

Ying Fang
VM://PMI 3336
Vet Med 3A
1285 Veterinary Medicine Mall
University of California, Davis
Davis, CA 95616

Materials:

- Biosafety cabinet or N95 respirator masks
- Refrigerator to store RNA preservation cards and carcasses
- **RNA preservation cards** (specifically, RNASound ReadyPunched™ cards). Order online at 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.
- Disposable nitrile or latex gloves
- Lab coat
- Small metal spatula
- Permanent ink pen or pencil
- Shipping envelopes (business size, FedEx, etc.)

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Note for agencies conducting in-house testing by 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).**

Questions can be directed to Leslie Foss at the California Department of Public Health, at leslie.foss@cdph.ca.gov or 510-412-6255.

Updated Feb. 2018

RNA Preservation card inventory list for WNV dead bird testing

Use this template to create an inventory list of the RNA preservation cards in your shipment.

Agency name: _____

Date of shipment: _____

Count	Dead bird number (begins with 18-)	District Packing Checklist	DART Receipt Checklist	Notes
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				