

Testing dead birds for West Nile virus using oral swab technique

RNA Preservation Cards

The California West Nile virus (WNV) dead bird surveillance program uses nucleic acid preservation cards (RNA Sound ReadyPunched™ cards, 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 collect oral swab samples from dead birds via RNA preservation cards and send them to the DART laboratory for free RT-qPCR testing.

Methods

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. **Note on storage via 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 dead birds 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.
3. Clean and disinfect biosafety cabinet or prepare for outdoor sampling and gather needed supplies. Dead birds should be handled in a Class II biosafety cabinet within a laboratory. If it is not possible to work in a biosafety cabinet, work should be conducted outside while wearing an N95 respirator.

Materials needed:

- Biosafety cabinet or N95 respirator masks
- Refrigerator to store RNA preservation cards
- **RNA preservation cards** (specifically, RNA Sound ReadyPunched™ cards). Order online at the Fortius Bio LLC website: <https://www.fortiusbio.com/products/perforated-rna-sampling-cards>
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 such as Fisher brand catalog no. 22-029-682.
- Disposable nitrile or latex gloves
- Lab coat
- Small metal spatula
- Permanent ink pen or pencil
- Shipping envelopes (business size, FedEx, or other.)

4. **Sampling.** Partially unwrap the disposable swab. Open the bag containing the bird to expose the head. With gloved hands, pry open the beak (a metal spatula may help with this) and put swab into the mouth. Aggressively swab the mouth and oropharyngeal cavity (throat).
5. Press and roll 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 Call Center or CalSurv.
6. **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 (residents usually appreciate the removal of the bird). Agencies conducting in-house testing must dispose of any WNV-positive carcasses as biohazardous waste (incinerate); WNV-negative birds may be discarded in the trash.
7. 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™ is a product which kills viruses without corroding stainless steel and may also be used.
8. **Drying and storage.** Allow cards to dry in back of cabinet or in a cool place for 2 hours. Make sure the dead bird number corresponding to the dead bird is written on the front flap of each card. Seal RNA preservation cards back into their small individual bags. Once used, the cards do not need to be stored in the refrigerator but kept at room temperature. However, they should be tested within 10 days of sample taken.
9. **Ship.** Place cards into an envelope with an inventory list (page 14), and ship to DART (address on next page).

Shipping options:

- a. Add to weekly mosquito pool shipment. Seal all cards with card inventory list 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, GLS). 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 shipping in a larger, more conspicuous envelope is recommended to help avoid lost packages.

10. **Change status in CalSurv Gateway.** To be notified when the cards have arrived at the lab, change the status of each dead bird in CalSurv (<https://gateway.vectorsurv.org>) to “submitted”. Upon receipt, DART will update the status of the card in CalSurv to “received.”
11. **Notify resident.** Once your agency receives test results, telephone the resident who reported the WNV-positive bird, to let them know the bird had WNV and deliver risk prevention information if needed. Or, if you have an agreement with CDPH that they will make the call, staff at the Call Center will inform residents whose birds tested positive each Monday.

Ship cards using the address below:

when shipping via GLS:

ATTN: Anil Singapuri
UC Davis, Dept. of PMI
1285 Veterinary Medicine Mall
Building: Vet Med 3A, Room 3336
Davis, CA. 95617

when shipping via FedEx or UPS

ATTN: Anil Singapuri
Vet Med 3A, Room 3336
1285 Veterinary Medicine Mall
University of California, Davis
Davis, CA 95616

Note for agencies conducting in-house testing by RT-qPCR 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: all positive birds must be disposed of as biomedical waste (incineration).**

References that may be of interest:

Foss L, WK Reisen, Y Fang, V Kramer, and K Padgett. 2016. Evaluation of nucleic acid preservation cards for West Nile virus testing in dead birds. PLoS ONE 11(6): e0157555. Doi: 10.1371/journal.pone.0157555.

Foss L, and K Padgett. 2016. Public usage of the West Nile virus dead bird hotline and website in 2015. Proc. Mosq. Vector Control Assoc Calif. 84:37-42.

RNA Preservation card inventory list

Use when shipping RNASound ReadyPunched™ cards to the UC Davis DART lab for WNV testing. (The lab requires documentation of the cards to ensure there is a correct matching bird number for each sample tested.)

Agency name: _____

Date of shipment: _____

Count	Dead bird number (begins with 25-)	District Packing Checklist	DART Receipt Checklist	Notes
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				