

Protocol: Testing dead birds for West Nile virus using oral swab technique

California Department of Public Health

RNASound™ Cards

The West Nile virus (WNV) dead bird surveillance program uses nucleic acid preservation cards for WNV testing of dead birds. Testing dead birds using RNASound™ 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, jays) via RNASound™ cards for qRT-PCR testing at the UC Davis Arbovirus Research and Training (DART) Laboratory. Submission of swabs from other groups of birds (passerines and raptors) will also be accepted, with the exception of doves, quails, pigeons, and chickens.

While dead bird testing at DART will still be free of charge, local agencies will need to purchase disposable swabs and RNASound™ cards at a cost of about \$5.60 per bird.

The use of oral swabs negates classifying results into recent and chronic infections. Therefore all results with Ct values of < 40 will be considered WNV positive.

Procedure

The WNV hotline and online reporting system will screen birds and coordinate with local agencies for dead bird pick up of suitable carcasses. **Please keep us updated as to what species of birds you will accept.** For the materials list and buying links, see **Materials**.

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 N-95 mask. 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 should also be stored in the refrigerator

until use. RNASound™ cards have a shelf life of one year if stored in a refrigerator (check the expiration date printed on the label).

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 RNASound™ card (over the two perforated discs). The sample may be dry; this is normal. Make sure to label the RNASound™ card with the 6-digit dead bird number (##-####). If you do not have a dead bird number for the bird, contact the hotline or log in to Cal-Surv Gateway to obtain one.

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™ may also be used; a product that kills viruses without corroding stainless steel.

9. Allow cards to dry in back of cabinet or outside for 2 hours. Make sure the dead bird number corresponding to the dead bird is written at the bottom of each card. Seal RNASound™ cards back into the small individual bags in which they were shipped.

10. Place all cards into a regular business envelope and mail to DART (address below). Include in your shipment a print out of each dead bird report corresponding to each RNASound™ card.

Note: we now recommend overnight delivery (FedEx, GSO) of cards to expedite turnaround time of dead bird test results during the Spring/Summer West Nile virus season. RNASound™ cards can also be added to mosquito pool shipments to avoid extra shipping costs. (If adding cards to a mosquito pool shipment please put cards in a labeled envelope, within in a ziplock bag. The cold temperature is fine for RNASound™ cards.)

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 West Nile virus-positive birds as biohazard waste (autoclave or incinerate); negative birds can be discarded in the trash.

12. Ship cards to this address:

Dr. Ying Fang
University of California
One Shields Avenue
Vet Med: Pathology, Microbiology, & Immunology
3336 Vet Med 3A
Davis, CA 95616

For UPS shipments:

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

Shipping Methods:

- a. Ship RNASound™ cards by GSO, FedEx, or UPS (overnight or 2-day, signature required). To save on delivery costs, you may “batch” your cards and send them in one envelope each Monday. This would ensure the cards arrive to the lab safely and in time for same-week processing (same-week results are most likely to occur May through September).
OR
- b. Enclose RNASound™ cards in mosquito pool shipments to avoid extra shipping costs. (Please put cards in a labeled envelope, within in a ziplock bag, and then inside the box. The cold temperature is fine for RNASound™ cards.)

Mailing by regular U.S. Postal Service is not recommended due to recent lost envelopes and slow service.

Materials

- Biosafety cabinet or N95 respirator masks
- Disposable Nitrile or latex gloves
- Lab coat
- RNASound™ ReadyPunched Cards. Order the cards online at:
http://www.fortiusbio.com/RNA_Sampling_Card.html

There is no longer a discount code when ordering to receive these prices: cards are priced at \$140 for 25 cards or \$60.20 for 10 cards. **NOTE: The cards must be stored in the refrigerator and expire after one year. It is recommended that agencies purchase cards for one season at a time.**

- The RNASound™ cards come with individually-wrapped Dacron polyester swabs. If extras are needed, they are available at Fisher Scientific. Catalog no. 23-400-116
- Small metal spatula
- Sterilization solution(s)
- Ziplock bags
- Permanent marker or pencil
- Envelopes for shipping (manila or business size)

Questions?

If you have questions or would like further information, please contact Leslie Foss, CDPH West Nile virus Dead Bird Surveillance Program Coordinator, at leslie.foss@cdph.ca.gov or (510) 412-6255.

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