

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

California Department of Public Health

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RNAse Cards

The West Nile virus (WNV) dead bird surveillance program is no longer supporting necropsy of birds for WNV testing. Testing birds using RNAse cards is a proven reliable alternative procedure that does not require bird necropsy. Local agencies are encouraged to collect oral swab samples from dead corvids (crows, ravens, magpies, jays) via RNAse cards for qRT-PCR testing at the UC Davis Center for Vectorborne Diseases (CVEC). Submission of swabs from other groups of birds (passerines and raptors) will also be accepted.

While dead bird testing at CVEC will still be free of charge, local agencies will need to purchase disposable swabs and RNAse 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. The RNAse cards should also be stored in the refrigerator until use. RNAse cards have a shelf life of one year if stored in a 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 RNase card (over the two perforated discs). The sample may be dry; this is normal. Make sure to label the RNase card with the dead bird number.

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.

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 RNase cards back into the small individual bags in which they were shipped.

10. Place all cards into a regular business envelope and mail to CVEC (address below). Include an inventory list of bird numbers corresponding to RNase card samples in shipment. **New note:** we now recommend overnight delivery (FedEx, GSO) of cards to expedite turnaround time of dead bird test results. RNase 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 RNase 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 can, or you may do it for them. Agencies conducting in-house testing must dispose of any positive birds as biohazard waste (autoclave or incinerate); negative birds can be discarded in the trash.

12. Ship cards to this address:

Center for Vectorborne Diseases
Dept. of Pathology, Microbiology and Immunology
Rm 3336, VetMed3a
University of California
One Shields Ave.
Davis, CA 95616

Shipping Methods:

- a. Ship RNase cards by GSO, FedEx, or UPS (overnight or 2-day, signature required). To save on delivery costs, you might “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 RNase 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 RNase 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
- Individually-wrapped Polyester Swabs (Example: Fisherbrand cat no. 23-400-116) Link: <http://www.fishersci.com/ecommerce/servlet/itemdetail?storeId=10652&langId=-1&catalogId=29104&productId=5560571&distype=0&highlightProductsItemsFlag=Y&fromSearch=1&searchType=PROD>
- **RNase Cards** (specifically, RNASound ReadyPunched™ cards). Order by calling Fortiusbio at (818) 651-3838. Or buy the cards online at: http://www.fortiusbio.com/RNA_Sampling_Card.html
Use the code “CDPH” when ordering on the phone or online to receive the \$140 price for 25 cards and free shipping. (Please Note: the kit also contains tubes and other supplies that you will not use.)
- Sandwich-size ziplock bags
- Small metal spatula
- Permanent markers
- Envelopes for shipping (manila or business size)

Questions?

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