

Protocol: Testing dead birds for West Nile virus (WNV) using an oral swab technique
California Department of Public Health
August 28, 2013

Introduction

As of September 1, 2013, the Dead Bird Surveillance Program will no longer ship and perform necropsies on dead birds. As an alternative, 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 is discouraged because of the low frequency of positive oral swabs from kidney positive birds in previous comparisons. Use of oral swabs also negates classifying results into recent and chronic infections; all results should be considered a recent infection because most corvids still die when infected. While the testing of birds at CVEC will still be free of charge, local agencies will need to purchase disposable swabs and RNase cards at a cost of approximately \$6 per bird.

Procedure

The WNV hotline will continue to screen birds this season and will assign dead bird numbers as before. Field-collected birds should remain cold until ready for swabbing.

1. Avoid direct contact with the dead bird by using disposable gloves or handle the carcass only with plastic bags as described below.
2. For carcasses to be handled in BL-2 level laboratories, it is recommended to refrigerate carcasses until ready for swabbing (bird must be swabbed within 24 hours).
3. For obtaining the oral swab specimen in a laboratory, carcasses should be placed within a Class II biosafety cabinet. If it is not possible to work with the bird carcass in a biological safety 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.
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 inside of cabinet and metal spatula used for opening beak with 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. Seal RNase cards back into the small individual bags in which they were shipped.
10. Place all cards into a plastic sandwich bag and mail/ship in regular business or manila envelope to CVEC (address below). Include an inventory list of bird numbers corresponding to RNase card samples in shipment. It is acceptable to send via regular mail (USPS).

11. Dead bird carcasses and used polyester swabs which are double-bagged can be disposed of 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.
 - a. Agencies conducting in-house testing must dispose of any positive birds as biohazard waste (autoclave or incinerate); negative birds can be disposed of in the trash.

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Materials

- Biosafety cabinet or N95 respirator masks
- Nitrile or latex gloves
- Lab coat
- Individually-wrapped Polyester Swabs (Example: Fisherbrand cat no. 23-400-116) Link: <http://www.fishersci.com/ecom/servlet/itemdetail?storeId=10652&langId=-1&catalogId=29104&productId=5560571&distype=0&highlightProductsItemsFlag=Y&fromSearch=1&searchType=PROD>
- RNase cards. Call Fortiusbio, LLC to order at (818) 651-3838. Use code "CDPH" for discount price of \$140 for 25 cards and free shipping.
- 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.