

USGS National Wildlife Health Center

Diagnostic Case Submission Guidelines for Avian Botulism

(addendum to NWHC's standard [Diagnostic Case Submission Guidelines](#))

Avian Botulism is a disease in birds caused by a naturally occurring toxin produced by the bacterium *Clostridium botulinum*. Outbreaks may involve multiple species and occur on waterbodies of various sizes often during warm weather. For more information about this disease and management actions, please see [Avian Botulism in the Field Manual of Wildlife Diseases](#).

This document specifically addresses diagnostic testing services for avian botulism and guidelines for submitting specimens for botulism testing.

Diagnosing botulism and limitations of testing:

Botulism is a difficult disease to diagnosis because botulinum toxin leaves little evidence of its presence. Since it does not cause visible tissue damage, botulism cannot be diagnosed solely by examining a bird's tissues (even microscopically). Further complicating matters, the bacterium *Clostridium botulinum*, including types that produce the botulinum toxin, naturally occurs in the intestinal tract of healthy birds and in the environment. This means that detection of the bacterium does not prove that the bird died of botulism. At NWHC, we test for the presence of the activated botulinum toxin in the blood of a dead bird. Other laboratories may use bacterial culture or PCR to test for the presence of *Clostridium botulinum* and the genes that produce the toxin (not the toxin itself); however, these latter testing methods cannot confirm a botulism diagnosis. They can only confirm presence of the bacterium.

The accuracy of a diagnostic test for detecting botulinum toxin is highly dependent on the quality and size of the specimen that is collected in the field and submitted for diagnostic evaluation. The test to detect botulinum toxin requires approximately 2 mL of blood from an affected bird. At NWHC, blood is collected from the bird's heart during necropsy. Even when the laboratory test fails to confirm the presence of botulinum toxin in the blood sample, a final diagnosis of "suspected botulism" may still be made if the event history and clinical signs observed in the field are consistent with botulism and if other diagnostic analyses do not support a different disease as the cause of the mortality event.

Suitable specimens to submit for botulism testing:

For an accurate diagnosis of botulism, it is very important to collect only fresh carcasses (dead for a few hours) or to nonchemically euthanize birds that are near death and subsequently to preserve and submit these carcasses to the laboratory as instructed by NWHC epidemiologists (see also [Wildlife Specimen Collection, Preservation, and Shipment](#)). A sufficient volume of blood is most likely to be obtained from freshly dead specimens that are minimally the approximate size of a small duck (e.g., a teal). The blood sample that can be recovered from the carcass of a small bird, such as a shorebird, is often insufficient and therefore unsuitable for botulism testing (however shorebirds may still be submitted for diagnostic evaluation). Botulism intoxication often occurs in a variety of species at a botulism outbreak site. Even though avian botulism events may involve multiple species of birds, it can be challenging to detect the toxin in all specimens. Confirmation of botulism in all species affected is typically unnecessary; diagnosing botulism in one (or a few) species provides good evidence that mortality in other species presenting with similar clinical signs (including species that may be too small to provide adequate samples for testing), in the same geographic location, and at same time period may also be attributed to botulism.

In the field, birds may die from botulism or be euthanized while having amounts of toxin in their blood that is too low for the test to detect. Birds showing only mild clinical signs (i.e., are difficult to capture by hand) are more likely to return a false-negative test result because the botulinum toxin in their blood may not yet be at high enough levels for the test to detect it. Conversely, birds that have been dead for some time are more likely to return a false-positive test result. This is because *Clostridium botulinum* living in the birds' intestine and the surrounding environment may begin to produce botulinum toxin as the bird decomposes. Thus, a bird that died of another cause may have detectable botulinum toxin in its blood if the carcass has begun to decompose.

Recommendations:

In summary, carcasses should meet the following criteria to maximize potential to obtain a confirmed botulism diagnosis:

- 1) Species tested for botulism should be teal-sized or larger. Other species such as shorebirds should still be collected and submitted for diagnostic evaluation but do not make good specimens for botulism testing.
- 2) Specimens collected should be freshly dead, died in hand (not euthanized), or euthanized only if near death and by a non-chemical means.
- 3) To facilitate thorough analysis for presence of botulinum toxin, if warranted, at least three appropriate carcasses should be submitted from a mortality event. Submitting more than three carcasses is highly recommended, as upon examination some carcasses may prove to be unsuitable for testing.
- 4) Do not submit carcasses from birds that have been treated with botulism anti-toxin.

Submissions not meeting the above criteria may still be accepted by NWHC for diagnostic purposes, but botulism testing may not be performed. Please contact the NWHC epidemiologist on duty (608) 270-2480, NWHC-epi@usgs.gov for more information.

Test turn-around time:

A tentative diagnosis of suspected botulism can often be made within a few days of receiving appropriate carcasses based on field information and necropsy findings. Turn-around time for confirmatory botulism tests generally takes 2-3 weeks during the peak botulism season of July to September, or > 2 months during other times of the year. In cases where testing for botulinum toxin is not possible (e.g. due to inadequate blood volume) or botulinum toxin is not detected, a final diagnosis of suspected botulism is based on consideration of multiple factors including history, gross and microscopic assessment, and the results of diagnostic tests that rule-out other disease processes.

Actions for “suspected botulism” diagnoses:

Botulism outbreaks can worsen without intervention. Taking action to respond and possibly reduce additional wildlife mortality suspected to be caused by botulinum toxin should not be delayed while awaiting confirmatory test results. Instead, action can be taken immediately or as soon as a preliminary diagnosis of suspected botulism is designated. When NWHC is contacted about a mortality event, Center epidemiologists can provide assistance to determine appropriate disease response actions.

Please contact the NWHC epidemiologist on duty (608) 270-2480, NWHC-epi@usgs.gov. For events in Hawaii or the Pacific Islands, contact the NWHC Honolulu Field Station (808) 792-9520, thierry_work@usgs.gov. More information can be found on the USGS NWHC website- [Disease Investigation Services](#).