



CANADIAN
WILDLIFE HEALTH
COOPERATIVE

WHITE NOSE SYNDROME

Specimen Submission Protocol

**CREATING A WORLD
THAT IS SAFE AND SUSTAINABLE
FOR WILDLIFE AND SOCIETY**



Preamble

This submission protocol is specifically for the diagnosis of the disease Bat White-nose Syndrome (BWNS) in bat specimens submitted for testing during the designated BWNS surveillance period of November 1 of a given year until May 31 of the following year.

Proper handling of bats prior to being sent to a Canadian Wildlife Health Cooperative (CWHC) Centre for diagnostic evaluation is essential. If there are multiple bats to submit from a mortality incident and a specific diagnosis is required for each bat, then each bat should be collected separately in its own plastic bag. Alternatively, if the goal is to diagnose BWNS at a species level, then all the bats of a given species can be collected in a single plastic bag as a pooled sample. However, in the cases of potential identification of BWNS in a new county, new hibernaculum or new species, it is preferable to submit bats from the mortality incident individually in their own plastic bag. Lastly, occasionally there are organizational requirements that dead bats collected in the field are sent to a central location for processing and data collection prior to shipment to a CWHC Centre for diagnostic evaluation. In these instances, it is **CRITICAL TO AVOID THE CROSS CONTAMINATION** of uninfected bats with *Pseudogymnoascus destructans* from infected bats.

Real time polymerase chain reaction (RT-PCR), the diagnostic test utilized to confirm the presence of *P. destructans*, is incredibly sensitive. To illustrate this point, there was an unfortunate accident at a CWHC Centre 3 years ago where a single bat with BWNS was handled for weighing prior to weighing 3 additional bats that did not have BWNS, and all of the bats were RT-PCR positive for *P. destructans*. In this case, a single weigh boat was used to weigh all of the bats and the person handling the bats did not change their gloves between handling each bat. A bat infected with *P. destructans* can have many fungal spores (conidia) and hyphae on its body, and it is very easy to transfer these to clothes, tables, floors, equipment, etc. while examining bats for data collection and identification purposes. Therefore, it is a good suggestion that collected bats remain within their individual plastic bags while being sexed, aged, weighed and identified prior to sending them for diagnostic examination. Otherwise, appropriate decontamination and biosafety measures for the room, clothing and equipment should be implemented to ensure the fungus is not spread around by the individuals handling the bats.

Again, it cannot be over-emphasized that it is only through careful handling of collected bats that an accurate diagnosis for individual specimens submitted for BWNS testing will be ensured.



Protocol for Documenting Bat Mortality Incidents and Submitting Specimens for the Purpose of BWNS Surveillance

Following the steps outlined below will prevent problems in the receiving, diagnosing and reporting on the BWNS status of bat specimens sent to a CWHC regional centre:

1. If the submitting organization has its own sample identification number, this should be legibly written on the CWHC submission form along with the printed name of submitting person and their email and mailing addresses so diagnostic reports can be returned to the correct person and office. Submission forms can be found by selecting the CWHC regional centre that serves your province at http://www.cwhc-rcsf.ca/report_submit.php.
2. Accurate geographic locations are required on the CWHC submission form: specifically latitude and longitude and/or provide address and postal code if found on the property of a member of the general public.
 - 2.1. **NOTE:** To protect site-specific sensitive information (e.g., locations of hibernacula) the following protocol is strictly followed by CWHC Information Services:
 - 2.1.1. Detailed geographical data are never shared with the general public.
 - 2.1.2. Summary BWNS surveillance data (e.g., monthly Canadian national surveillance updates) only include geographical information at the provincial or county level.
 - 2.1.3. Lastly, when data requests are received for research purposes, latitude and longitude are provided only to 2 decimal places which gives the site's location to an accuracy of approximately 1 square kilometer which is considered a 'town/village level'. If geographical information is required at a higher resolution, the submitting person and organization will be contacted for permission to share the data.
3. Clearly indicate on the submission form if the bats are found alive or dead in a hibernaculum or on the landscape. If found alive, please specify method of euthanasia if the bat did not die on its own. If the bat was kept in captivity for a period of time prior to death/euthanasia, provide the length of time in captivity. If the bat was kept in captivity for a period of time prior to death/euthanasia, provide the length of time in captivity.



4. Record the total number of bats found dead at the site on the CWHC submission form.
 - 4.1. **NOTE:** Not all of the bats collected from a mortality incident have to be sent to a CWHC Centre for diagnostic purposes. Diagnostic testing for BWNS only requires ~ 20% or 5 -10 of the FRESHEST dead bats, including a representative sample of all species involved in the event. It is completely understandable that all field staff might not necessarily be able to identify various bats to species. Therefore, specimens can simply be submitted as a “bat”, and they will be properly identified at the time of post mortem examination. The correct identification will be included in the diagnostic report sent back to the submitter upon completion of the case.
 - 4.2. If ≤ 5 bats are found dead, all of them should be submitted for diagnostic evaluation.
5. If possible, bats should be collected **INDIVIDUALLY** in a plastic bag with any identification numbers and date found written clearly on the bag. The CWHC submission form for each bat should be submitted in a separate plastic bag or an envelope within a plastic bag and shipped with the specimen.
 - 5.1. **NOTE:** Please **DO NOT** put the CWHC submission form in the bag with the dead bat because it cannot be removed prior to doing the post mortem examination of the bat and fluids can leak from the carcass during transit, ruining the form. These types of problems delay analyses and greatly increase the time and effort that has to be invested for each specimen.
6. In the case of a very large number of bats found dead at a single site, it might be deemed necessary to treat a particular sized group of bats as a pooled sample and place them in a single bag. **However**, please recognize this will prevent a specific diagnosis for each individual bat and may have implications when multiple species are found at a single site. Therefore, if samples must be pooled, if at all possible please collect the same species together and submit each species group in its own separate plastic bag. If field staff are not confident with species identification, it is better to submit each carcass as single specimen within its own individual bag.
7. In counties **NOT** previously identified as BWNS positive, any bats found dead in hibernacula or on the landscape during the BWNS Surveillance Season (November 1, of a given year – May 31, of the following year) should be sent for BWNS testing. In counties that were previously identified as Bat WNS positive, there should be emphasis placed on submitting bat species **NOT** previously identified as positive in the county and bats from hibernacula **NOT** previously identified as Bat WNS positive within the county. If you do not know this information, you can speak with the person responsible for BWNS Surveillance in your province and this contact list is available at http://www.cwhc-rcsf.ca/wns_resources.php.
8. Counties where BWNS has **NOT** been previously diagnosed should always be a priority geographical area to submit any dead bats from.



9. Lastly, the bats that are collected can be shipped as fresh or frozen specimens. Information on how to ship these specimens to your regional CWHC Centre for diagnostic evaluation can be found at http://www.cwhc-rcsf.ca/report_submit.php.

All bats submitted to a CWHC Centre are scanned for PIT tags and samples are collected for inclusion in other ongoing research projects (e.g., Dr. Hugh Broders' genetic studies and Christina Davy's genetic and stress studies). In addition, all bats submitted will be tested for rabies.

If any of the above instructions are not clear, please do not hesitate to seek clarification by contacting your regional CWHC Centre (see <http://www.cwhc-rcsf.ca/contact.php>).



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