WNS Case Definitions – revised January 2020

Note: Diagnostic categories described in this document apply to individual specimens submitted for diagnostic evaluation and may differ from site-level designations used for official reporting purposes based on pending guidance for interpreting the weight of evidence.

Diagnostic Criteria for Reporting Cases of Bat White-Nose Syndrome (WNS) at the individual specimen level

1. **Positive for WNS** – Characteristic histologic lesions of WNS¹ are present on an individual bat **AND** the bat is positive for *Pseudogymnoascus destructans* (*Pd*) either by qPCR² or by fungal culture.

2. **Suspect for WNS** – (one of the following criteria must be met)
   a) Characteristic histologic lesions of WNS¹ are present on an individual bat but *Pd* is not detected, the test result for *Pd* is inconclusive (either by qPCR² or by fungal culture), or testing for *Pd* is not performed.
   b) One or more field signs* are observed in a bat colony **AND** *Pd* is detected (either by qPCR², fungal culture, or a tapelift performed directly on visible fungal growth on bat skin) on at least one individual of the same species **AND** histopathology is negative or is not performed.
   c) **MULTIPLE** field signs* are observed among species known to be susceptible to WNS **AND** are within the currently recognized range of WNS but no samples are collected for diagnostic evaluation.
   d) Individual bats that are part of a confirmed WNS morbidity/mortality event are submitted to, but not tested by, a diagnostician. This criterion is for instances in which multiple samples from the same site are submitted, but only a subset of those samples is tested. The untested samples may be classified as suspect for WNS if the subset of tested samples is **Positive for WNS** and consists of the same species as the untested samples. Representatives of all species involved in the disease event should be tested.

3. **Negative for WNS** – Characteristic histologic lesions of WNS¹ are not present **AND** bat is negative for *Pd* (either by qPCR² or fungal culture).

Diagnostic Criteria for Reporting the Detection of *Pseudogymnoascus destructans* (*Pd*) at the individual specimen level in the absence of field signs of WNS

1. **Pd Positive** – *Pd* detected by qPCR² or by fungal culture in accordance with criteria (Appendix A) accepted by the WNS Diagnostic Laboratory Network in an environmental sample or on an individual bat with no other field signs of WNS* observed within the surveyed population. Bat carcasses submitted for diagnostic testing are placed in this category if *Pd* is detected on the carcass but there were no field signs of WNS observed on the individual **AND** histopathology is negative or is not performed. Repeat testing and/or independent secondary confirmation of *Pd* Positive results is warranted before this designation is applied to bat species of unknown susceptibility to WNS or areas outside the known geographic distribution of *Pd*.

2. **Inconclusive for *Pd*** – Non-negative results by qPCR² that are outside the range of accepted, standardized laboratory-defined criteria for *Pd* Positive (Appendix A). This designation is made independent of other epidemiological evidence. The real-time PCR assay for *Pd* provides a cycle threshold (Ct) value that is inversely related to the amount of target DNA in that sample (i.e. lower Ct values indicate higher amounts of DNA). Interlaboratory testing indicates that across a range of techniques and platforms, results of Ct ≤ 37 exhibit reliable reproducibility. Results with a Ct > 37 but ≤ 40 are categorized as inconclusive. While
these results may indicate the presence of very small amounts of target DNA, they are less likely to be reproducible, which makes them more difficult to differentiate from laboratory contamination. Thus, no determination of Pd status can be made about the specimen based on an inconclusive result. Caution is urged in using inconclusive results for making inference about the presence of Pd in a broader population, hibernaculum, or geographic area. Additional sampling is warranted before broader inferences can be made about the true presence or absence of Pd at the survey site for official reporting purposes.

3. **Pd Negative** – Pd is not detected (either by qPCR\(^2\) [Appendix A] or fungal culture) in an environmental sample or on an individual bat. [Note: Although a negative qPCR or fungal culture result indicates that Pd was not detected in the tested sample, this does not guarantee the roost site or bat colony from which the sample was collected is free of Pd. A lack of observed field signs* in the resident bat population is also not sufficient for assuming that a roost is Pd-free. Consistently negative results from a statistically robust sample size can, however, increase confidence that Pd is absent from the sampled population or environment.]

*Field Signs Associated with WNS in Bats

**Winter/Spring** – excessive or unexplained mortality at or near a hibernaculum; visible fungus on flight membranes, muzzle, or ears of live or fresh dead bats; abnormal behaviors including daytime activity, premature egression from the hibernaculum, or unexpected population shift to entrance of the hibernaculum; moderate to severe wing damage\(^3\) in nontorpid bats or thin body condition (each considered a nonspecific field sign when observed by itself); yellow-orange fluorescent pattern of non-haired skin under UVA light.\(^4\)

**Summer/Fall** – There are not consistent field signs associated with WNS during summer/fall.

**Additional Comments**

When screening for the presence of Pd, qPCR is preferred over fungal culture or tapelift due to the greater sensitivity of the qPCR assay.

Results obtained using testing methodologies other than those referenced here will be considered preliminary and further testing with accepted standard methodologies is necessary for official reporting purposes.

For management purposes, hibernacula should be considered contaminated with Pd if they contain at least one sample (bat or environmental) that is determined to be **Positive for WNS, Suspect for WNS, or Pd Positive** as described above. A contaminated hibernaculum retains this designation indefinitely. The ability of Pd to persist long-term outside of hibernacula is not currently well understood however, appropriate biosecurity measures are advised to minimize the risk of human-mediated spread.

**Citations**

For Pd qPCR diagnostic interpretation:

Ct ≤ 37 = Pd positive
Ct > 37 but ≤ 40 = Pd inconclusive
Ct > 40, or no amplification = Pd negative

When testing replicates*, readily interpretable categories include:

Ct ≤ 37 for all replicates = Pd positive
Ct > 37 but ≤ 40 in all replicates = Pd inconclusive
Ct > 40 or no amplification in all replicates = Pd negative

Running multiple replicates increases the probability of detecting small amounts of target DNA in the extracted material. However, replicate results that fall into multiple diagnostic categories present a challenge for interpretation. Divergence between results can generally be categorized as:

1. Extreme: Differences for which there is no biological or statistical explanation. Example: A mixture of positive (Ct ≤ 37) and negative (above 40 or no amplification) replicates; or a mixture of inconclusive replicates near or equal to 40 together with a strong (i.e., low Ct) positive.
2. Minimal to moderate: Differences for which there is a biological or statistical explanation. Example: A mix of positive and inconclusive replicates with Ct values near the cut-off threshold of 37, or a mix of inconclusive and negative replicates.

Whenever discordant results arise, all possible explanations, including user error or contamination, should be explored. Discordant replicates determined by the laboratory to be extreme, must be reconciled prior to reporting. If extremely discordant results cannot be reconciled, the result should be reported as invalid.

Discordant replicates determined by the laboratory to be minimal or moderate, should be reported as follows:

<table>
<thead>
<tr>
<th>Any mixture of:</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd positive and Inconclusive</td>
<td>Pd Positive</td>
</tr>
<tr>
<td>Pd negative and Inconclusive</td>
<td>Pd Inconclusive</td>
</tr>
</tbody>
</table>

Laboratories or submitters may elect to have samples with an original Ct value ≤ 37 retested in a different laboratory if the result represents a novel documentation of Pd (by species or geographic area). Final sample categorization should be agreed upon by both laboratories. It is not likely productive to send samples with original Ct values greater than 37 to other laboratories for confirmatory testing because the low amount of target DNA in these samples makes replication less probable. If independent verification is desired, re-sampling should occur, with duplicate samples taken and submitted directly to the separate laboratories for parallel testing.

* Replicates are defined as any number of qPCR reactions amplified from the same sample extract