Laboratory Testing and Disease Surveillance in Wildlife: Understanding Test Validation and Laboratory Certification

Executive Summary: Accurately assessing the health and disease status of wildlife and wildlife populations to inform management decisions requires population demographic and disease surveillance data. Field staff and biologists are well-versed in the collection and interpretation of population demographic data including the variables that can affect analysis. However, field biologists may not have the background to understand and interpret disease and health data, such as disease test results. Interpretation of disease test results can be even more challenging when tests have not been properly developed and validated for wild species. The purpose of this document is to inform biologists and managers about recommended laboratory procedures for test development. In addition, we would like biologists and managers to be aware of certifications recommended of diagnostic and research laboratories. When tests are not properly validated and good laboratory procedures are not followed, population health and disease status will be impossible to determine with accuracy, leading to poor management decisions. Furthermore, unreliable wildlife health information can lead to confusion and distrust in stakeholder groups and ultimately a lack of support for agency decisions and management. The information provided here will assist biologists and managers when discussing surveillance and disease testing protocols with stakeholders. The recommendations provided are not meant to be prescriptive. When determining agency wildlife health testing needs, wildlife managers should consider their agency's research and management objectives as well as the laboratory resources available for diagnostic disease testing of wildlife.

The WAFWA Wildlife Health Committee strongly recommends:

- Advising hunters through direct communication that testing harvested animals should be conducted by accredited diagnostic laboratories using validated tests.
- Encouraging hunters to participate in agency disease surveillance by collecting samples as directed by the agency and submitting specimens according to agency protocol.
- Where possible, testing should be conducted as part of an agency's wildlife disease surveillance program.
- Agencies continue to develop science-based surveillance programs and include validated disease tests as they become available.
- Contacting companies offering direct-to-hunter disease testing to establish a method for coordination with wildlife management agencies so agency surveillance and disease management can be maintained.
- If agency-provided testing is unavailable, make recommendations to hunters wishing to test their animal about which labs offer validated direct-to-hunter testing.

Background – diagnostic testing in human and domestic animal health

Testing for diseases is performed for several reasons. For livestock, the World Organization for Animal Health (WOAH) and the USDA have identified diseases of high concern and established prevention and management programs. Included in these programs are testing requirements for interstate and international movement and the approved protocols for conducting the tests. In

human and animal medicine, reliable, validated tests are essential to the accurate diagnosis and treatment of diseases. In wildlife, we need validated tests to ascertain the disease status of populations and inform management decisions.

The detection of disease in an animal population can be accomplished using multiple methods. Testing for the presence of antibodies, which are developed after surviving an infection, in individuals will identify pathogen exposure. Examples of antibody tests are Enzyme-Linked Immunosorbent Assays (ELISA) and serum neutralization. Monitoring antibody levels (titers) and seroprevalence (proportion of animals with antibodies) can inform population-level impacts of disease. For diseases that do not produce antibodies because all infected animals die or because the pathogen does not stimulate the immune system, as in Chronic Wasting Disease (CWD), a test that targets the pathogen is more appropriate. Examples of direct agent tests are culture, immunohistochemistry, and Real-time Quaking-Induced Conversion (Rt-QuIC). Additionally, specific agency goals may necessitate the use of pathogen-targeted tests, such as to determine pathogen strains, or comply with testing regulations for exportation or importation. The advantage of direct agent tests is that they can be used across host species. Collection of the appropriate sample is necessary so we also need to understand the distribution of the pathogen within the infected animal (where to look for the disease agent). With tests that target the agent it is important to know how few organisms or infectious proteins can be detected because in early stages of infection, the number of organisms may be too small to detect.

In human and domestic animal medicine, there are established standards, licenses, and accreditations (e.g., certifications) for laboratories conducting health and disease testing. Where no certification process exists, such as for private veterinary laboratories, most still follow standard procedures and use validated testing methods for quality assurance. Wildlife health specialists and veterinarians seeking to conduct disease testing on wildlife species also have several resources available including private and state/provincial veterinary diagnostic laboratories and university research laboratories. However, with few exceptions, tests have not been validated for specific wildlife species. For some diseases, e.g., CWD in cervids and *Mycoplasma ovipneumoniae* (associated with pneumonia in bighorn sheep), university research laboratories developed wildlife specific tests which were then validated for use in accredited diagnostic laboratories.

Diagnostic test validation

Validation is a process within assay (disease test) development that ensures that the assay to detect or quantify the disease agent in a biological sample is appropriate for the stated purpose (e.g., early detection of disease, determine disease status of an individual, determine disease status of a population; WOAH 2024). The first step is to clearly define the purpose for the assay and to describe the conditions under which it will be applied (Figure 1). For example, a food safety test for pathogenic *E. coli* needs to be able to detect very small amounts of bacteria within a sample. The next steps in the validation process are optimization and standardization, including defining the species to be tested, sample type, method of collection, sample handling and preservation, and the minimum amount of agent detectable in a sample (WOAH 2024, Hewitt et al. 2025). Optimization and standardization of a test requires specimens with known amounts of the disease agent. These samples need to match the conditions under which the test will be applied. For

example, if a disease occurs in the intestinal tract of birds and the sample that will be collected is a cloacal swab, then the test needs to be developed using samples with the pathogen contained in avian feces.

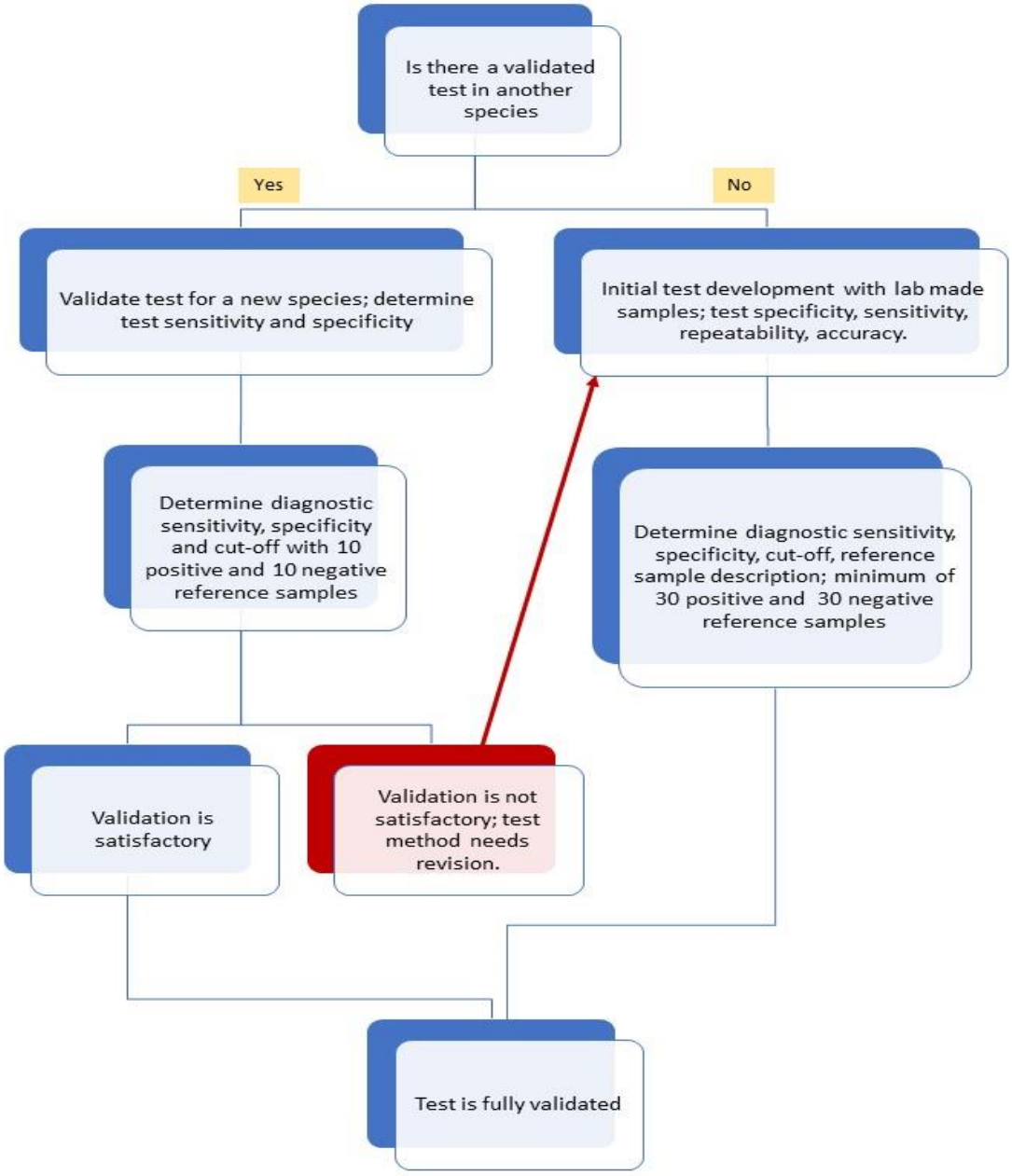

Figure 1: Steps in validation process for new tests and tests validated in another species.

Once the test protocol has been developed, the minimum numbers of organisms that can be detected, test sensitivity (odds that a sample containing the target of the assay tests positive), and test specificity (odds that a negative specimen will test negative) must be determined to complete validation. Accuracy (closeness of the test result to the expected value) and precision (degree of variance for repeated tests) must also be determined, usually by repeatedly testing samples with

known numbers of organisms (WOAH 2024). Many tests provide numeric results on a continuous scale (e.g., percent inhibition). In order to interpret the results as detected or not detected, the laboratory identifies the range of values for positive (detected) and negative (not detected) samples (Figure 2, WOAH 2024). Often there is a gap between the upper and lower limits of these ranges, and the result is "indeterminant" (WOAH 2024).

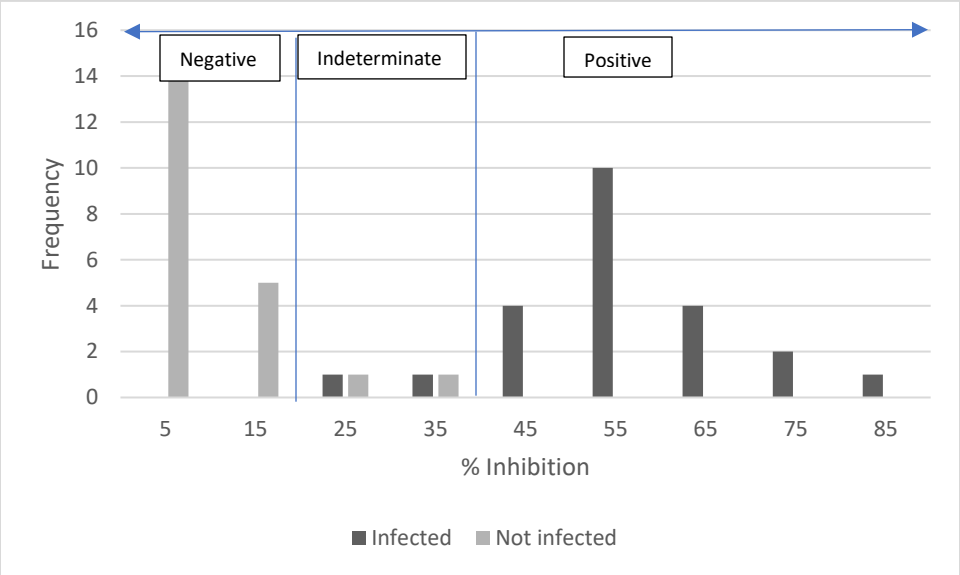

Figure 2: An example of cut-off values

Once an assay has completed initial validation, its performance under field conditions is evaluated to determine realistic test sensitivity (probability that an infected individual will test positive) and specificity (probability that an uninfected individual will test negative). This often involves testing samples with an established method in addition to the assay being validated. When an infected animal tests negative this is called a false negative and the result is a decreased sensitivity. When an uninfected animal tests positive this is called a false positive and the result is a decreased specificity. There are animal (recent exposure, intermittent shedding), sample (poor handling, contamination), and test (poorly designed, cross-reaction with other diseases) related factors that can contribute to a decreased sensitivity and specificity. Ideally, sensitivity should be high, especially when the consequences of not detecting a diseased individual has public or population health implications, as with a zoonotic or disease with epidemic potential.

Challenges for diagnostic test validation for wildlife

Test validation in wildlife species presents a number of challenges: acquisition of a sufficient number of samples, ensuring that samples used for development are representative of the population in which the test will be used, and ensuring that the test is appropriate for the intended purpose (e.g., early detection of disease, estimating prevalence, food safety), (Jia et al. 2020, WOAH 2024). Of these challenges, obtaining samples from animals with a known disease status is the greatest. For example, animals in the early stages of CWD infection will not have a positive test result nor exhibit clinical signs, making the understanding of their disease status difficult. For other diseases, such as bighorn sheep pneumonia where intermittent shedding of organisms occurs,

multiple tests may be required to determine the status of an individual. Although completing assay validation is challenging, it should not be overlooked or bypassed (Jia et al. 2020, WOAH 2024).

Some tests that have been validated for disease agents that occur in domestic animals and wildlife are suited for testing of wildlife. The most applicable tests are those that directly detect the pathogen, such as PCR and immunohistochemistry. However, distribution and proliferation of the infectious agent may vary (Jia et al. 2020, WOAH 2024). Some antibody tests that are also less sensitive to species differences because they don't rely on the direct detection of a species-specific antibody. This does not mean that one can assume that such tests will have the same reliability, sensitivity, and specificity as for the validated species (WOAH 2024).

A current example of test validation for a wildlife disease is the work being done at several laboratories for Rt-QuIC for CWD detection in cervids. Originally developed as a research tool, Rt-QuIC is currently being evaluated as a diagnostic test (Rowden et al. 2022, Darish et al. 2024). Similar to PCR which detects minute quantities of nucleotides, Rt-QuIC uses an amplification process to detect minute quantities of abnormal protein. Currently, laboratories are evaluating how to interpret the results, assessing interlaboratory agreement, and comparing the test to previously validated tests (Picasso-Riso et al. 2022, Rowden et al. 2023, Darish et al. 2024). One of the challenges in this process is comparing the tests under field situations where some individuals are infected but not showing signs of disease. The current tests, IHC and ELISA are not as able to detect animals that are in the earlier stages of infection in comparison to Rt-QuIC (Picasso-Riso et al. 2022). When Rt-QuIC is validated, agencies will need to determine how to incorporate this more sensitive test into their CWD surveillance. Because of the increased sensitivity, the apparent CWD prevalence will increase when in reality the test is identifying infected animals that were being missed. This will necessitate developing a communication plan to address stakeholder concerns.

When tests are not appropriately validated, results will be unreliable and decisions made on those results could have serious repercussions. An extreme example of the impact of diagnostic tests that have not been validated is the Theranos failure. Before it was determined that the tests were widely inaccurate (an estimated one in ten results were wrong), thousands of patients experienced unnecessary procedures and trauma (Das and Drolet 2022). Another example that affected disease management was when the FDA allowed Emergency Use Authorization (EUA) for diagnostic tests for SARS-CoV-2. Of 125 EUA tests, 82 were later found to have design or validation problems and were not approved for use until the tests had been appropriately validated (Shuren and Stenzel 2020). This situation was further compounded by physicians misunderstanding the implications of sensitivity, specificity, and inconsistent viral shedding when thousands of tests were conducted (Shuren and Stenzel 2020). These failures negatively affected public health communication about and management of the SARS-CoV-2 epidemic causing a failure in public trust which is still evident today. As a result of the increased public mistrust, vaccine hesitancy has surged in recent years and has likely contributed to the recent measles outbreak (Gambrel et al. 2022, Pandey and Galvani 2023).

Background – surveillance methods

State, provincial, and territorial wildlife management agencies are tasked with conserving wildlife populations as a public resource. This includes evaluating health and disease status, as a public resource. Agencies develop and implement robust science-based disease surveillance programs on which management decisions can be based utilizing validated diagnostic tests fit for the purpose and certified laboratories or research laboratories following international standards for quality control. Ideally, diagnostic samples are collected from a statistically significant number of randomly selected individuals given the expected rate of disease in the population. However, samples are often collected opportunistically from hunter-harvested animals or individuals captured for research (nonrandom sampling), and obtaining a sufficient number of samples to properly infer disease status is often challenging (Jia et al. 2020, National Academies of Sciences, Engineering, and Medicine 2024). All results, positive and negative, the sensitivity and specificity of tests, and population demographic and geospatial data of tested animals are used to determine the disease status of the population. For diseases where the epidemiology and ecology are not fully understood and for which management actions are complicated, it is vital that agencies be able to include the results of all samples collected and tested (National Academies of Sciences, Engineering, and Medicine 2024).

Disease testing of wildlife by other entities

Recently, private companies have developed assays for diseases (e.g., CWD) in wildlife that are unvalidated and jeopardize state, provincial, and territorial management of wildlife populations. These tests are being marketed as food safety tests to hunters looking for reassurance that their harvested animal is safe to consume as the Centers for Disease Control and Prevention advises against consuming a CWD-positive animal. However, these CWD tests have not been validated for determining food safety. For foodborne disease agents, the FDA establishes acceptable limits of detection and certifies laboratories for testing (FDA 2021). For example, Grade A pasteurized milk shall not have more than 20,000 bacteria per ml and less than 10 coliforms per ml (FDA 2019). The companies offering direct-to-hunter testing do not provide information on the detection limits, accuracy, laboratory practices or laboratory certifications for these diagnostic tests. Additionally, to determine the food safety limits for the infectious prion agent of CWD, another sensitive assay, such as a bioassay, would be required (National Academies of Sciences, Engineering, and Medicine 2024). Companies producing and selling diagnostic tests should clearly define how the test results should be interpreted (e.g., does a negative test mean that the animal is safe to eat or that it is not infected?).

Because these tests are sent directly to hunters who purchase them and test results are not required to be shared with the wildlife management agency where the animal was harvested, this information is not included in surveillance data collected by or available to wildlife management agencies. Disease management relies on accurate estimations of prevalence and incidence, which are based on an analysis of all test results and the sensitivity and specificity of the assay. Therefore, all results, positive and negative, and the associated wildlife demographic and geospatial information should be provided to wildlife management agencies. Reduced and inaccurate data resulting from the use and misuse of unvalidated tests can result in inaccurate measures of disease,

negatively affecting public and wildlife health and representing a significant risk to public trust and wildlife disease management.

For this reason, the WAFWA Wildlife Health Committee strongly recommends:

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- Encouraging hunters to participate in agency disease surveillance by collecting samples as directed by the agency and submitting specimens according to agency protocol.
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