

How to interpret diagnostic tests in wildlife

Fact sheet

Introductory statement

This fact sheet aims to provide general advice on the interpretation of diagnostic testing for infectious disease in Australian wildlife species. The principles outlined in this fact sheet are common to all diagnostic test interpretation, including those tests used for humans and domestic animals. The fact sheet discusses the general limitations of types of available tests and the challenges with interpretation of test results for wildlife.

Definitions and abbreviations

Disease is any disturbance in the health or function of an animal or human.

Pathogens (sometimes called **agents of disease**) are any infectious agent capable of causing disease in a host, e.g. viruses, bacteria, fungi, protozoa, internal parasites such as worms and external parasites such as lice and mites.

Infection refers to the presence of a pathogen within an individual.

Infectious disease are those diseases caused by organisms (or pathogens) such as viruses, bacteria, fungi and parasites.

Host species is the species is affected by disease or in which a pathogen is living.

PCR polymerase chain reaction.

Reservoir hosts provide an environmental 'reservoir' for a pathogen and generally don't get sick from infection.

Infection versus disease

Infection of an animal (or human) with a pathogen, or infectious agent, does not necessarily equate to disease. An individual may be infected with a virus, bacterium, fungus or parasite without suffering any ill-health, and without showing any signs of disease. Many infectious agents are a normal part of the biology of animals and humans and may be present without causing disease (called asymptomatic infection).

There are complex factors that determine whether infection of an individual proceeds to disease. These include **host** factors (at both individual and host species level); **pathogen** factors and **environmental** factors.

A range of outcomes are possible when an animal becomes infected with a pathogen. Individuals may:

- carry or be infected with a pathogen, without developing disease (some animal species are recognised as **reservoir hosts** for a disease. These species become infected with a pathogen with little or no resulting disease and provide an environmental 'reservoir' for a pathogen.)
- develop mild or moderate illness from which they recover
- develop a persistent infection
- develop a severe illness resulting in death.

In cases of persistent infection, the animal recovers to good health, but remains as a 'carrier' of the infection; it carries and sheds the pathogen but no longer shows any signs of disease.

Testing for disease

There are a wide variety of methods used to test for infectious disease. In general, a specific pathogen will have a particular group of tests that are recommended for use in diagnosis or investigation of this agent. A number of different biological samples may be used to test for pathogens and disease. These include

- serum (from blood)
- mucosal swabs (from oral, urogenital cavities, conjunctiva or rectum/ anus)
- faeces or urine
- scrapings or samples of skin, fur or scales
- biopsies (small samples of skin or another organ collected surgically from a live animal)
- samples of pus or other discharges
- tissue and organ samples (generally collected during post mortem investigation)

Generally, each specific diagnostic test requires a specific type of sample (e.g. mucosal swab, serum or faeces). Some types of test can be performed on a wide range of samples.

Many of the available diagnostic tests for infectious disease have been developed for use in domestic animals or in humans. Although some diagnostic tests have been specifically developed for use in wildlife, in many cases tests used in wildlife were originally developed for use in domestic animals and humans.

Tests for infectious disease can be classified into two broad groups:

1. Tests which look for direct evidence of the pathogen in the animal
2. Tests which look for evidence that an animal has previously been exposed to a pathogen.

Tests for direct evidence of pathogens

Tests that look for direct evidence of pathogens (in a biological sample collected from the animal) involve processes to identify the agent of disease itself. These tests can only reflect the infection status of an individual at a given point in time (when the sample was collected). These tests cannot determine the prior history of exposure of the individual to that pathogen and cannot provide information on whether the individual has previously been infected but has now cleared the infection. They generally do not provide information on whether the animal is suffering disease as a result of the infection.

Types of direct tests include:

- **culturing** for growth of pathogens (generally used for bacteria or fungi; viral culture is a specialised and difficult field; these techniques are less commonly used for protozoa and macroparasites)
- **molecular techniques** such as PCR (polymerase chain reaction) to look for evidence of DNA or RNA from pathogens (these are used for many different types of pathogens, including bacteria, viruses, fungi, protozoa and other parasites)

- **direct observation** of pathogens (gross or microscopic examination, which may be used for bacteria, fungi, protozoa and macroparasites. Viruses are generally too small for observation, unless specialised techniques such as electron microscopy are used).

All these tests require the pathogen to be present in the sample for a positive result. Microscopic examination of tissues or fluids may provide some evidence for a disease state (e.g. evidence of an inflammatory reaction from the host), as well as potential evidence of pathogens.

Inaccuracies in direct tests may result from:

- poor or incorrect sampling technique (e.g. contamination of samples)
- inappropriate samples being collected
- poor or incorrect sample handling or storage (e.g. incorrect transport medium or temperature)
- laboratory errors
- low concentration of pathogen in samples
- poor or incorrect laboratory technique
- poor specificity or sensitivity of the test for the pathogen
- lack of observational skills and experience in lab staff.

Inaccuracies may result in false positive and/ or false negative results.

Tests for indirect evidence of pathogens

Indirect tests look for evidence that the animal has experienced previous exposure to the infectious agent. These tests generally look for presence of antibodies in blood, a part of the host's immune system which develop in response to an individual's exposure to a specific infectious agent. These serological tests require serum (the liquid portion of blood, with red and white blood cells removed). Other indirect tests may look for evidence of different types of immune response from the animal, such as a Mantoux or skin test for Mycobacterial disease, or a gamma interferon test, which measures the host's immune reactivity, from a blood sample cultured in the laboratory setting.

Inaccuracies in serological tests can result from

- poor quality serum (e.g. haemolysed or contaminated serum)
- poor storage of serum (e.g. poor temperature control)
- poor specificity or sensitivity of the test for the host species
- cross reactivity of antibodies or immunological responses to similar but different pathogens.

Inaccuracies in other immune-response tests such as a Mantoux or gamma interferon test can result from

- poor testing technique
- poor sample collection or poor sample management
- poor specificity or sensitivity of the test for the host species
- cross reactivity of the immunological response to similar but different pathogens.

In most cases, antibody and other immunological response to infection by a pathogen wanes over time. Serological tests will only detect antibodies for as long as they continue to circulate in the host's system. In most cases in wildlife species, the duration of persistence of antibodies following infection is not known.

Some serological tests can be run on serum from any host mammal while other types of serological tests are specifically designed for the host species. In most cases, commercially available serological tests for pathogens in Australian labs have been developed for domestic animals. Few serological tests have been developed specifically for wildlife species, and most of the serological tests used for Australian wildlife fall into the category of those types of tests which are not host-species specific. Some tests have been validated

(tested for general accuracy) for wildlife species, but many of the tests used in wildlife have not been validated for the host species. A general overview of diagnostic test validation for wildlife is available at www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.6.07_WILDLIFE.pdf

Accuracy of tests

As a broad rule, **no diagnostic test can be considered 100% accurate** for a pathogen. Every test method has some inherent inaccuracy. Diagnostic tests may vary significantly in their accuracy. In any disease testing process, there are chances of false positive results (i.e. detection of disease when it isn't actually there) and false negative results (i.e. failure to detect disease when it is present). Some commonly used tests have low accuracy, but are used because they are convenient, or because there is no more accurate test available. The accuracy of the tests in wildlife species may not be known.

Diagnostic test accuracy is described by two different parameters:

Test sensitivity (Se) is the likelihood that a truly positive (infected) individual will return a positive test result.

Test specificity (Sp) is the likelihood that a truly negative (non-infected) individual will return a negative test result.

Some diagnostic tests may have relatively high sensitivity but low specificity (i.e. the test can accurately detect a positive (infected) individual, but negative (non-infected) animals also test positive (false-positive). This is most common for indirect tests.

Interpretation of test results

Interpretation of test results must be undertaken with care in wildlife studies, where baseline knowledge is often limited, and tests have not been validated for the species under investigation. It is rare for a test result not to require assessment and interpretation, which requires a level of understanding of the pathogen, disease process, host species and test methodology. Interpretation of test results and assessment of disease status of individuals and populations is often a complex process that relies on a multitude of inputs and sound clinical judgment. Sensitivity and specificity of tests are often unknown for wildlife species and must be inferred or assumed based on expert knowledge. Table 1 outlines common interpretations of test results.

Sample size has a major influence on interpretation of tests results at a population level. Studies to look at presence and prevalence of a disease in a population will be influenced, among other factors, by the number of individuals sampled. Small sample sizes will lower the level of detection in the population and may make interpretation of results more challenging.

A lack of evidence for a pathogen (i.e. a negative test) within a population does not prove that pathogens are absent from that population. If the sample size is small, a negative result may mean the disease is present, but the prevalence is below that able to be detected with that sample size, and given the test accuracy. Tests for direct evidence of a pathogen will only be positive if the individual is shedding the pathogen at the time of testing, and the pathogen is present and detectable in the sample collected.

Many pathogens are shed intermittently (e.g. *Salmonella*) and some are shed only during times of stress (e.g. herpesviruses). In some diseases, infection is transient (e.g. influenza virus) and the individual will only test positive during the period of active infection. Infected individuals may die before an immune response can be manifested, and if all infected individuals die, no animals in the population will show a serological response to the pathogen.

A positive test result in an individual should also be interpreted with caution, in particular if the test has not been validated for the host species in question. In many cases, positive tests may give a strong indication of

the presence of an infection, or a pathogen. Best practice recommends that additional testing (using alternative methodologies), or independent testing by a reference laboratory is undertaken to confirm positive test results. This may be particularly important if test results indicate evidence of a novel infection, presence of a pathogen in a previously unrecognised host species or country, or if tests reveal a marked higher prevalence than expected.

Table 1: Common interpretations of test results

Test	Comments	Positive means...	Negative means...
Direct tests			
Pathogen culture or isolation	Infected individuals may only excrete pathogen intermittently, in low amounts, or only in specific tissues/ secretions. Must know which samples to collect, and how to handle and store appropriately, for the pathogen in question	The animal is currently infected	The animal might be uninfected or infected but not shedding or insufficient pathogen in sample for detection (false negative)
Direct observation	As above	The animal is currently infected	As above
PCR and other molecular techniques	As above. PCR detects pathogen genome OR a genome fragment. DNA/ RNA extraction from tissue or biological samples can be problematic.	The individual is currently infected (may also have recently cleared the infection but it is lingering in tissues or secretions).	As above. No genome/ fragment was detected in that particular sample at that particular time.
Indirect (immunological) tests			
Serology	A single serology result gives limited information about current infection status. Immune responses in wildlife are incompletely understood and may differ between host species. Antibodies may take time to be produced in response to infection.	The individual could be: currently infected and infectious (shedding pathogen); previously infected, immune, and not infectious.	The individual could be: uninfected; currently infected and shedding pathogen without having seroconverted (in the period of time before antibodies are produced by the immune system and detectable in the blood stream).
Other immunological tests (cell-mediated immunity) e.g. gamma interferon test	Only useful for some pathogens, where this immune response is significant in the host. Gamma interferon blood tests require careful handling of samples and highly specialised laboratory techniques.	The individual is currently infected or has previously been exposed to infection	The animal's immune response is not showing a reaction. The animal could be: infected but not reacting; not infected; or there could errors in the way the test was administered)

Conclusion

Diagnostic testing in wildlife follows the same general principles used in domestic species and humans. However, many tests used in wildlife were originally developed for use in non-wildlife species, and the validity of the tests, including the sensitivity and specificity, may not be known for the wildlife species in question. No

diagnostic test can be considered 100% accurate, so false positive and false negative results will always occur. When assessing wildlife populations, the impact of false results is likely to be greater, as sample sizes are often small. A lack of baseline information on wildlife disease may also limit our ability to accurately interpret the results of disease testing in wildlife. Expert input, for example from a wildlife veterinarian, pathologist, pathogen expert, or epidemiologist, is recommended whenever disease testing is undertaken in wildlife species.

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To provide feedback on this fact sheet

We are interested in hearing from anyone with information on this subject. If you can help, please contact us at admin@wildlifehealthaustralia.com.au.

Wildlife Health Australia would be very grateful for any feedback on this fact sheet. Please provide detailed comments or suggestions to admin@wildlifehealthaustralia.com.au. We would also like to hear from you if you have a particular area of expertise and would like to produce a fact sheet (or sheets) for the network (or update current sheets). A small amount of funding is available to facilitate this.

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