Vaccination of companion animals has proven to be effective and generally safe for the prevention or control of common and potentially life-threatening diseases. The benefits reach past the individual animal and contribute to herd health, establishing an immune population that helps prevent spread of disease and/or outbreaks. However, the potential for adverse events (ranging from mild to life-threatening) and vaccine failure exists, and development of a vaccination protocol should be an informed medical decision established between the client and the veterinarian and tailored to patients on an individual basis.

As part of this decision-making process, public attention has shifted toward the utility of vaccine titer testing. Vaccine titers are measures of the levels of serum antibodies against pathogens, which clinicians can use to determine whether a vaccination is required. The 2017 American Animal Hospital Association (AAHA) canine vaccination guidelines and the 2015 World Small Animal Veterinary Association (WSAVA) vaccination guidelines incorporate recommendations for this diagnostic testing in their protocols; in addition, vaccine titer testing is advocated by the American Holistic Veterinary Medical Association. This article provides a literature-based review of the limitations and benefits of this type of testing and guidance for interpreting results.

**WHAT VIRUSES DO VACCINE TITER TESTS APPLY TO?**

In the companion animal sector, core vaccine titer testing is well established; diagnostic tests measure antibodies against the common viruses covered in a canine core vaccine (canine distemper virus [CDV]; canine parvovirus [CPV]; and canine adenovirus type-2 [CAV-2], which also provides cross-protection against canine adenovirus type-1) and feline panleukopenia virus (FPV). Diagnostic laboratories can also determine levels of antibody against feline herpesvirus (FHV) and feline calicivirus (FCV).
WHAT TITER TESTS ARE AVAILABLE?

The gold standard for titer testing for antibodies against the core vaccine viruses CDV, CAV-2, FHV, and FCV is virus neutralization (VN).4 For detecting antibodies against CPV and FPV, the preferred method is hemagglutination inhibition (HI).4 These diagnostic tests are available through reference laboratories and have the benefit of detecting functional antibodies and providing an end-point titer, which helps quantify the antibody concentration. Enzyme-linked immunosorbent assay (ELISA) point-of-care tests provide rapid, qualitative (positive/negative) results.

**VN Testing**

VN detects neutralizing antibodies, which may also be described as antibodies that inhibit virus replication. In the laboratory, serial dilutions of the patient’s serum (e.g., 1:8, 1:16, 1:32) are incubated with live virus and subsequently exposed to cells in culture.5 If the virus is appropriately neutralized, the cells survive and the resulting dilution is considered that patient’s antibody titer. However, if the virus is not neutralized, it will kill the cells. Cell death is detected by microscopic visual inspection, use of dye, or fluorescence, depending on the laboratory.

![Diagram of titer testing](image)

**FIGURE 1.** Titer testing by virus neutralization (VN) and hemagglutination inhibition (HI). Both tests require serial dilutions of the patient’s serum that are subsequently incubated with cells from culture (VN) or red blood cells (HI). In VN testing, cell survival indicates that the patient’s antibodies neutralized the virus. In HI testing, lack of agglutination indicates that the patient’s antibodies bound the agglutinating virus. The greatest dilution with cell survival (VN) or lack of agglutination (HI) is the patient’s titer.
HI Testing

HI is useful for assaying antibodies against viruses that cause hemagglutination, or the formation of a lattice between red blood cells. In this type of testing, live virus is incubated with serial dilutions of the patient’s serum and exposed to red blood cells. If levels of antibody are adequate, the virus is complexed (bound to antibodies), leaving a pellet of red blood cells, and the patient is considered protected at the level of that dilution. However, if levels of antibodies are inadequate, the virus will cause agglutination of the red blood cells, which is detected on the microwell plate as a mesh of cells. Therefore, the patient’s titer is the greatest dilution at which there are sufficient antibodies to inhibit virus agglutination of red blood cells.

Understanding the methods used for VN and HI testing is helpful for interpretation of results (FIGURE 1). Quantitative titers are reported as the dilution value (i.e., 1:160) or the inverse whole number (i.e., 160). Generally, the higher the number (in this example 160), the greater the amount of antibodies present at greater dilutions and the higher the likelihood that the patient is protected from that virus. The reference ranges established by a given laboratory are valuable because the method used can affect these values. For example, if a laboratory’s reference range for response to vaccination is >1:80, a result of 1:160 is considered positive. However, a patient with even more antibodies and a result of 1:320 is not necessarily more protected than the patient with the result of 1:160; both are positive results. Numerical titers are beneficial for tracking patients’ detectable immune responses over time. To reduce laboratory error, VN and HI typically are performed in triplicate.

ELISA

In-clinic ELISA tests yield positive or negative results, which cannot differentiate between neutralizing and non-neutralizing antibodies. Despite this limitation, in a study that compared ELISA to gold standard VN/HI testing for 431 shelter dogs, overall accuracy of ELISA testing was 95% for CDV and 98% for CPV antibodies.

OTHER TESTS

Aside from canine and feline core vaccine titer testing, veterinarians may be familiar with titer testing for rabies and Leptospira. One must consider the legal and diagnostic nuances of these options when considering their use.

Readily available laboratory microscopic agglutination testing (MAT) for Leptospira measures primarily IgM and some IgG levels and is more accurate for assessing exposure or infection rather than protection. Rabies titer testing. Several options for rabies titer testing exist. Fluorescent antibody virus neutralization (FAVN) testing is most commonly used for animal export purposes and is performed at specialized laboratories and yields an end-point titer that is converted to IU/mL. This option is associated with a moderate cost and turnaround time. The rapid fluorescent focus inhibition test (RFFIT) is used for rabies titer testing in humans and animals; it is available with end-point (quantitative) and screening (greater than or less than a reference value associated with protective immunity) options. Because of the legal implications of forgoing rabies vaccination, this type of titer testing has limited usefulness with regard to informing vaccination decisions.

Leptospira titer testing. Readily available laboratory microscopic agglutination testing (MAT) for Leptospira measures primarily IgM and some IgG levels and is more accurate for assessing exposure or infection rather than protection. MAT titers against Leptospira do not correlate well with protective immunity. In addition, MAT does not differentiate between natural infection and vaccination. Typically, MAT titers against Leptospira decline within 4 to 6 months after vaccination, despite manufacturers’ recommendations for annual vaccination, which are based on challenge studies.

ARE TITERS A MEASURE OF IMMUNITY?

The goal of vaccination is to create protection through immunologic memory by stimulating both humoral and cell-mediated immunity. Titer testing measures humoral immunity through levels of blood antibodies against a particular pathogen. Antibodies are formed by
the body in response to protection (i.e., vaccination) or infection/exposure. Titer testing alone does not differentiate between these mechanisms. Detection of functional antibodies (e.g., with VN and HI titer testing) at appropriate levels indicates that a dog or cat should be protected when exposed to a given virus. The amount of circulating antibody is upregulated or down-regulated, according to exposure. The immune system also responds to attack with cell-mediated immunity through memory B and T cells, which cannot be measured reliably. Full assessment of vaccine efficacy would require a challenge study, which is not feasible for client-owned animals. Therefore, a patient with lower titer results may be able to mount an appropriate immune response, but succumbing to the given disease is unlikely for a patient with positive VN/HI results.

The type of disease produced by an infection can help determine whether circulating antibodies (primarily IgG, some IgM) should be indicative of immune status. Infections most susceptible to protection by circulating antibodies are systemic infections. According to comparison with challenge studies, antibody titers against viruses such as CDV, CPV, CAV, and FPV have a well-established correlation with protective immunity (BOX 1). In a study of 10 specific pathogen-free (SPF) puppies vaccinated against CDV, CPV, and CAV, serologic testing indicated that all vaccinated puppies maintained immunity and all resisted challenge at 4.5 years, except for 1 dog that did not respond to CDV vaccination. In another study, researchers vaccinated SPF cats against the feline core vaccine antigens and monitored serologic response and response to challenge at 30 to 36 months. The positive predictive value of HI serology for resistance to challenge with FPV in this study was 100%.

Protection against mucosal infections, such as FHV and FCV infections, is often provided by mucosal immunity (secretory IgA), and therefore titers of circulating antibodies against the agents of mucosal infections provide only a fair-to-good correlation with protective immunity. Similarly, the expectation of prevention associated with parenteral FHV and FCV vaccination should be to mitigate clinical signs of disease and reduce virus shedding, not necessarily to prevent infection entirely.

**TO TITER OR NOT TO TITER?**

As previously stated, the most established predictors of immunity are titers against CDV, CPV, CAV (the common viruses covered in a canine core vaccine), and FPV (1 of the 3 components of the feline core vaccine). A variety of circumstances affect whether titer testing is indicated or elected for a patient (BOX 2), and include immunocompromised status, vaccine reaction history, and genetic nonresponsiveness.

In immune-suppressed patients, such as those undergoing chemotherapy, the need for or response to vaccination can be determined through titer testing and used to assess health status. Titer testing also offers the advantage of assessing humoral immunity for patients with immune-mediated diseases that could be triggered

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**BOX 1 Viruses with Excellent Correlation Between VN/HI Titers and Protective Immunity**

- Canine distemper virus
- Canine parvovirus
- Canine adenovirus
- Feline panleukopenia virus

**BOX 2 To Titer or Not to Titer?**

**Advantages**

- Identify genetic nonresponders
- Evaluate health status of immune-suppressed animals
- Provide basis for vaccination decisions for animals with history of vaccine reactions
- Provide basis for vaccination decisions for animals with history of immune-mediated disease
- Address client concerns about vaccinations

**Contraindications**

- Young puppies
- Patients with unknown vaccination history
- Animals in dense populations or at high risk for exposure
- Legal implications of forgoing rabies vaccination
Vaccine reactions can range from mild to severe, and titer testing can provide guidance for tailoring a preventive care plan for patients. The incidence of genetic nonresponders, or animals with the inability to mount an appropriate immune response after vaccination, ranges from 1 in 1000 dogs for CPV to 1 in 5000 dogs for CDV. The 2016 WSAVA vaccination guidelines suggest that screening puppies after they have received their initial series of vaccinations can identify these nonresponders. Veterinarians may also recommend titer testing when clients are concerned about “overvaccinating.”

Titer testing is not appropriate for all situations, and decisions to measure titers or vaccinate should be discussed and agreed upon within the context of a valid veterinarian/client/patient relationship. In accordance with the American Veterinary Medical Association vaccination principles, the duration of immunity after vaccination depends on patient health status, type of vaccination used (e.g., modified live, killed), patient age, and patient exposure risk. These factors must all be considered when making decisions about vaccination and whether titer testing is appropriate. Titer testing results should be interpreted cautiously for young puppies or kittens because of the inability of testing to differentiate between maternal antibodies and antibodies present in response to vaccination. Because core vaccine titer testing cannot differentiate between immunity resulting from vaccination and immunity resulting from exposure or infection, it may not be appropriate for guiding vaccination decisions in dense population situations. However, ELISA titer testing may help inform population management decisions in the event of an outbreak of CPV or CDV in a shelter. Titer testing does not reflect future immunity and provides only a snapshot of the immune system at the time that the sample was taken; therefore, animals exposed to a high burden of infectious agents or animals with concurrent illness may not demonstrate protective immunity despite historically positive titers. Neither vaccination nor titer testing results absolutely ensure immunity; however, these methods can be used in synergy to provide the best preventive care plan for each pet.

HOW DO I INCORPORATE TITER TESTING INTO MY PRACTICE?

Some practices use and advocate for vaccine titer testing for most of their patients, and some base this testing on client request or recommend it because of an established protocol or specific medical need. Titer testing has become more affordable and accessible than it was in the past. However, when assessing the financial burden for the client, consider the potential need for further visits and vaccination costs as the result of negative titers. Several veterinary diagnostic laboratories make this diagnostic option more budget-friendly by offering discounted shipping and bundled deals for testing for the various pathogens (BOX 3). The 2017 AAHA canine vaccination guidelines suggest incorporating titer testing into preventive care protocols at veterinarians’ discretion. The AAHA guidelines state that vaccine titers for CPV, CDV, and CAV-2 provide a “reasonable assessment” of protective immunity and can be used to determine the need for booster vaccinations of adult dogs. If using this approach, the veterinarian should perform titer testing when the canine core vaccine is due. If positive, titer testing would be repeated in 1 year. If negative, the booster vaccine should be administered and a postvaccination titer test considered. The 2016 WSAVA vaccination guidelines recommend obtaining canine core vaccine titers at least 4 weeks after completion of the puppy series to assess for genetic nonresponders. When reporting results to clients, consider verbiage such as “consistent with appropriate response to vaccination” rather than “positive,” explaining the potential for error with any diagnostic test.

The 2013 American Association of Feline Practitioners feline vaccination guidelines do not advocate titer testing to determine the need for vaccination of cats due

BOX 3 Veterinary Diagnostic Laboratories Offering VN/HI Titer Testing

- Colorado State University Veterinary Diagnostic Laboratories
  csu-cvmbs.colostate.edu
- Cornell University Animal Health Diagnostic Center
  vet.cornell.edu/animal-health-diagnostic-center
- Kansas State Veterinary Diagnostic Laboratory
  ksvdl.org
- Michigan State University Veterinary Diagnostic Laboratory
  cvm.msu.edu/vdl
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Core vaccine titer testing to assess protective immunity is recommended for dogs only.

■ Core vaccine titer testing to assess protective immunity is recommended for dogs only.
■ Positive titer results are highly correlated with protective immunity for CPV, CDV, CAV, and FPV, although patients with negative titers may still mount an effective immune response through cell-mediated immunity.
■ Vaccine titers have become more available and financially feasible than they were in the past.
■ Titer testing is not something that should be approached broadly and may be best considered on the basis of risk factors, clinic protocols, and client preferences. TVP

REFERENCES

12. Lappin MR, Andrews J, Simpson D, Jensen WA. Use of serologic tests to predict resistance to feline herpesvirus 1, feline calcivirus, and feline parovirus infection in cats. JAVMA 2002;220(1):38-42.