

Antibody Testing vs. Vaccination

Applications in Clinical Practice

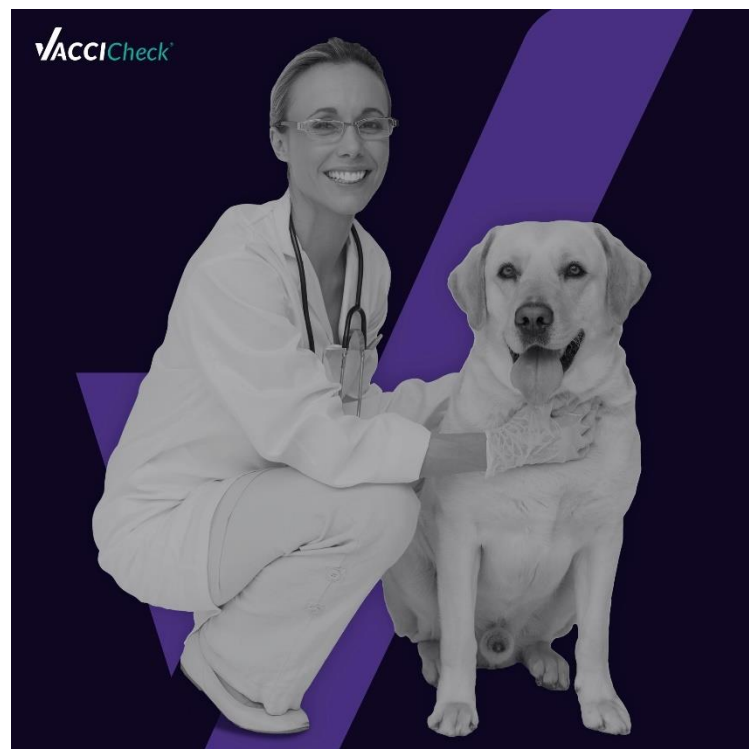
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In companion animal practice, serological testing continues to gain acceptance as a reliable means of assessing either: *the results of vaccination...or the need for vaccination*. Two key factors are likely behind the decision by clinicians to recommend or perform antibody testing on individual patients for the core vaccine-preventable diseases¹:

Current Vaccination Guidelines: vaccination recommendations, as outlined in current textbooks, taught in veterinary schools, and published in Vaccination Guidelines, highlight triennial booster intervals in dogs and cats for the core vaccines rather than a conventional “annual booster”. Despite supporting data demonstrating durations of immunity lasting 5 years and longer for core vaccines, veterinarians continue to challenge triennial booster recommendations. Clinician concerns have prompted requests for serological testing in individual patients as a means of validating the fact that administration of core vaccines elicits levels of antibody that are sustained beyond 1 year...and...

Owner concerns about injury associated with over-vaccination of pets.

Owner concerns with over-vaccination are likely attributable to the anti-vaccination movement and the purported link between Measles-Mumps-Rubella (MMR) vaccination and development of autism-spectrum disorders (ASD)² in children.



Today, it is not uncommon for a pet owner to specifically request antibody testing of a dog or cat rather than subject their pet to a scheduled ‘booster’.

And, so it is. With the demand for patient-centered antibody testing continuing to emerge in companion animal practice, veterinarians are faced with a few fundamental, yet highly relevant questions...three of which are addressed in this review:

- Does **antibody correlate with protection**?
- What are the **indications** for antibody testing?
- What is **actionable**? For each *indication*, how should the patient be managed if the test result is “positive” vs. “negative”?

ANTIBODY vs. PROTECTION

What does an antibody test result really mean in the individual patient? Making rational clinical decisions based on serological (antibody) test results depends on understanding a few “must know” facts. Consider the following 5 points:

1. The only **true test of protective immunity involves exposure (challenge) to a virulent pathogen** in which non-vaccinates (controls) are infected and manifest clinical illness while vaccinated animals remain healthy. The licensing of animal vaccines is based on this premise. On the other hand, “positive” antibody test results

can, for select (core) diseases, correlate very well with protective immunity.

2. Antibody testing methods vary (see TABLE 1):

Although numerous serological technologies are in use today (beyond the scope of this discussion), it is helpful to distinguish two categories of testing, each of which is applicable to clinical practice:

a. Quantitative Testing...aka 'titers'...refers to laboratory-based, end-point testing methods these are sometimes referred to as "gold standard tests" because all other testing methods must be correlated with titer results) used to determine the amount of antibody that has been produced against a specific antigen (epitope). Results may take days and are typically reported as an inverse of the greatest dilution that gives a "positive" result (eg, hemagglutination or virus neutralization). AND...results that are reported may include a laboratory statement such as: "POSITIVE" (ie, the titer meets or exceeds the reference threshold for this laboratory) or "NEGATIVE" (ie, the concentration of antibody is below the reference threshold for this laboratory...it could also mean there was no detectable antibody). See TABLE 2.

b. Qualitative Testing...refers to commercial "point-of-care" testing methods (or, test kits) practical for use within a veterinary practice. Results can be obtained in as little as 10 to 30 minutes, depending on the test. Results indicate "POSITIVE" (ie, there is sufficient antibody present to meet or exceed a defined "positive" control) or "NEGATIVE" (antibody was either not present or was present in levels below a defined "positive" control).

The VacciCheckⁱⁱⁱ point-of-care test kit provides "semi-quantitative" results.

Although results are not read as end-point titers, the test kit utilizes a graduated (graypurple) color scale to determine the relative amount of antibody present compared to a "positive" reference (control) color for each antigen (virus) tested. The color scale is scored from zero (0) to six (6). Scores ranging from 2 to 6 represent a protective level of antibody.

In the clinical setting, documentation of a protective antibody response following vaccination (canine distemper, parvovirus, adenovirus) serves as a reference point, or baseline result, in the event a patient has a "negative" test result at some point in the future. Documenting a "positive" response indicates the patient has, in fact, developed sustained, long-term immunity (B-cell "memory") and is expected to be protected for several years (life?) even though circulating levels of antibody may decline to "negative" levels. Establishing a baseline result can impact patient management decisions (risk vs. benefit) in the event that vaccine administration should ever become contraindicated (eg, vaccine adverse event or immune-mediated disease).

3. "POSITIVE" vs "NEGATIVE": This is important...

➤ A "positive" test result, whether quantitative or qualitative, means only that the patient sample has sufficient antibody to meet or exceed a defined "positive" reference threshold or control established by the laboratory or the manufacturer of the test kit. The clinician is responsible for interpreting the test result, whether "positive" or "negative", and making subsequent recommendations regarding the management of the patient.

To be very clear...

...consider a patient sample returned from a commercial laboratory with a titer result for canine parvovirus antibody: 1:1600 "POSITIVE"

By using the term "positive", the laboratory is *only* stating that the level of antibody detected in that sample met or exceeded their reference threshold for positivity. The laboratory does not, and will not, make an interpretation of what the "positive" test result means in the individual patient.

That's the clinician's responsibility.

➤ A "negative" test result only indicates that the patient's sample either did not have a detectable level of antibody or that the level present was below the defined "positive" threshold or control. Note...a "negative" test result *doesn't necessarily correlate with "susceptibility"* (see INDICATIONS & INTERPRETATION below)

4. When “POSITIVE” ...think PIE:

“Positive” antibody test results have a significantly different meaning depending on the pathogen (or vaccine) that induced the antibody. Consider the **PIE** acronym below when interpreting a “positive” test result:

Protection, or...Infection, or...Exposure

For example, a “positive” antibody test result for canine parvovirus correlates exceptionally well with protection.

On the other hand, a “positive” *Leptospira* antibody test result, as used in the clinical setting, correlates with *infection*. *Leptospira* antibody does NOT correlate well with protection following either vaccination or natural infection.

A “positive” antibody test result for *Ehrlichia canis* should be interpreted as prior exposure only, and is not indicative (alone) of infection or protection. Establishing a diagnosis (infection) of *E. canis* demands that the clinician perform additional laboratory tests as well as a thorough physical examination.

5. “Positive” antibody test results, whether using quantitative or qualitative testing, correlate strongly with protective immunity for canine and feline parvovirus, canine distemper virus, and canine adenovirus.

TESTING INDICATIONS & INTERPRETATION of RESULTS

The online version of the AAHA Canine Vaccination Guidelines^{4iv} includes a menu option entitled “Antibody Testing vs. Vaccination”. The purpose of the section is to provide veterinarians with several scenarios for which serological testing of an individual dog would be indicated. For each indication cited, recommendations on patient management are provided for both a “positive” and a “negative” test result. The reader is referred to the AAHA Canine Vaccination Guidelines for access to the complete list of indications and management recommendations.

The examples included below are representative indications for assessing serological responses in

patients vaccinated against canine distemper virus, canine adenovirus, and canine/feline parvovirus.

1. Assess antibody response following administration of the *initial Core Vaccine series*.

For various reasons, clients of young dogs/cats may request antibody testing *in response to vaccination*. A common example being the client who desires to transport puppies/kittens for sale or show purposes following completion of the initial series of core vaccines. Antibody testing can be conducted as early as 2 weeks following administration of the last dose in the initial series (2 to 4 weeks following the last is commonly recommended).

Interpretation: If the last dose is administered at 16 weeks of age, blood can be collected as early as 18 weeks of age and tested for the presence of antibody. If results are “positive” for antibody, the patient is protected...a booster dose of core vaccines is recommended 1 year later.

If results are “negative” for antibody against any virus, the patient is considered *susceptible*. A booster dose of vaccine should be administered as soon as practical. A combination vaccine can safely be administered even if the antibody level against one virus is “negative” while other results are “positive”.

Sustained, interfering levels of maternally derived antibody are the most likely reason vaccination fails to immunize a young dog or cat. Therefore it would be reasonable to recommend an additional test, 2 weeks following administration of the additional dose, to verify the patient has seroconverted and is protected.

2. Identification of genetic “non-responders” to canine parvovirus

With the introduction of canine-origin parvovirus vaccines in the early 1980s, veterinarians soon recognized that well vaccinated dogs, particularly among certain lines of Doberman pinschers and Rottweilers, became infected and died following exposure to canine parvovirus. Ultimately, vaccination failure in these dogs was attributed to a highly specific genetic mutation that resulted in a low, or no, antibody response following administration of modified-live parvovirus vaccine. Interestingly, antibody responses to canine

distemper and adenovirus were protective. The term “genetic non-responder”, or “genetic low-responder”, is used to describe the affected animal.

Today, genetic non-responders (and low-responders) have been recognized throughout the world and are *not* limited to Doberman pinschers and Rottweilers. (In the author’s recent experience, confirmed genetic non-responders have all been pure-bred dogs...little ones and big ones). It is presumed that feline genetic non-responders (to feline panleukopenia virus [feline parvovirus]) also exist.

Interpretation: Testing for canine or feline parvovirus antibody is the only means of identifying a genetic non-responder.

If the results for parvovirus antibody are “negative” 2 or more weeks following administration of the last dose in the initial vaccination series, the patient should be revaccinated against parvovirus as soon as practical and scheduled for a follow-up antibody test. Another “negative” test result, obtained 2 to 4 weeks after administering an additional vaccine dose indicates that the patient is likely a genetic non-responder.

Administration of additional doses of parvovirus vaccine are not expected to immunize. The seronegative patient must be considered *susceptible* if exposed to parvovirus.

3. Antibody testing of adults in lieu of administering a booster.

In the previous 2 examples, antibody testing was performed to assess a patient’s response to vaccination. In the next 2 examples, antibody testing is performed to determine *the need for re-vaccination*. Clients who are concerned about risks associated with ‘over-vaccination’ may request antibody testing in lieu of re-vaccination.

In addition, veterinarians concerned about the need to administer routine booster doses of vaccine in geriatric patients may elect to recommend antibody testing in lieu of revaccination.

Interpretation: The adult dog/cat that has a history of prior vaccination, “positive” antibody test results are expected for each of the viruses, even in patients that are significantly overdue for a

scheduled booster. “Positive” test results indicate that the patient does have protective immunity and that re-vaccination is not necessary.

On the other hand, antibody testing of previously vaccinated, adults will occasionally yield a “negative” antibody test results for one (or more) of the viruses. It happens... antibody is a protein and blood levels can diminish over time in the absence of exposure (or re-vaccination).

In contrast to the previous two examples, in which a “negative” test result indicates *susceptibility*...a “negative” test result in the adult, previously vaccinated dog or cat likely does NOT correlate with *susceptibility*...see the BOX below.

Does a “positive” antibody test result look forward?

Consider this: *Does a “positive” antibody test result today assure the patient will be protected tomorrow? ... or a year from now? ...or 3 years from now?*

In a way...YES...it does. “Positive” antibody test results for the core diseases not only correlate with protection, but indicate that the patient has produced long-term immune (B-cell) “memory”.

This “memory” (clones of B-lymphocytes residing in germinal centers of lymphoid tissue) enables the patient to “remember” specific antigenic epitopes (binding sites) on the virulent virus.... For years...depending on the antigen. If the patient is exposed to virulent virus, the patient rapidly (days) develops a “secondary” (or, anamnestic) antibody response, even if the antibody level has declined to a level below the “positive” threshold on a test.

FOR THIS REASON: a “negative” antibody test result in a dog that has previously been vaccinated against distemper, parvovirus, and adenovirus, does NOT necessarily correlate with *susceptibility*.

4. Assessment of patients having a history of a vaccine adverse reaction or immune-mediated disease.

Serious adverse reactions following vaccination are uncommon in both dogs and cats. Among the contingent of patients with a history of having recovered from a known, or suspected, vaccine

adverse event (reaction)...or, are known to have been treated for and recovered from an immune-mediated disease (eg, hemolytic anemia or thrombocytopenia), evaluating the level of antibody is a reasonable alternative to revaccination.

Interpretation: Patients having a “positive” test result can avoid re-vaccination and the potential risk for eliciting an acute-onset reaction or re-activating an immune-mediated disease.

If, on the other hand, a patient has one or more *negative* test results, the decision whether or not to administer vaccine becomes more complicated, because:

...among previously vaccinated adults, immune “memory” is likely sustained and is expected to provide a rapid, protective response if exposure to virulent virus occurs even in the absence of detectable levels of antibody.

...among young animals, especially if having experienced an adverse reaction prior to completing the initial 3 or 4 dose vaccination series, a “negative” antibody test likely correlates with susceptibility. The decision to vaccinate, or not, becomes a clinical decision that must take into consideration not only the owner’s concerns, but the potential risks associated with administering a dose of vaccine vs. the risk of not immunizing the animal.

... then there’s rabies vaccination...depending on State or local law, the decision NOT to administer a dose of rabies vaccine *may not be an option*. Today, most States in the US do not grant rabies vaccination exemption authority to veterinarians. From the veterinarian’s perspective, rabies vaccination may not be an option regardless of the patient’s medical history (...of course the client can always decline a vaccine but accepts the consequences if the dog/cat is subsequently exposed to a known or suspect rabid animal). Testing for rabies antibody (appropriately referred to as: RVNA or rabies virus neutralizing antibody) in lieu of re-vaccination is not an option. In the US, RVNA results cannot be used as documentation of protective immunity (see LIMITATIONS below).

LIMITATIONS to ANTIBODY TESTING for CORE VACCINES

Seroconversion, the antibody response that follows vaccination, can be determined for each of the core vaccines administered to dogs and cats. However, the development of antibody does not always equate to **protective immunity**.

Feline Calicivirus (FCV) & Feline Herpesvirus (FHV)

- “Positive” antibody test results for feline herpesvirus (FHV) and feline calicivirus (FCV) vaccination *do not correlate well with protective immunity*. For this reason, serology is not generally recommended to assess protection following vaccination or to determine the need for re-vaccination.
- Assessment of **cell-mediated immunity (CMI)** is a better correlate of protection against FHV-1 than serology. However, CMI tests are complex and not routinely available performed as a clinical service to veterinary practices.
- The so-called “gold standard test” (quantitative) used to measure FCV antibody has been judged only as *fair to good*. For this reason, qualitative test kits for FCV antibody are not available.

Rabies Virus

- Rabies virus neutralizing antibody (RVNA) testing is available through a limited number of certified laboratories only. Point-of-Care test kits are not available. One point all veterinarians should note: a “positive” RVNA titer result is NOT *a legal index of immunity in lieu of revaccination*.
- The interpretation of an RVNA, as would be performed on dogs or cats being exported to a rabies-free country or region of the world, is that the “positive” animal has recently been vaccinated...*that’s it!* Do not sell RVNA titers to clients as a means of confirming protective immunity in a pet. *That’s law!*

WELLNESS TESTING

In clinical practice today, the concept of “wellness” and “wellness testing” continues to evolve in a variety of ways that provide measurable, long-term health benefits to the individual dog and cat. It’s not surprising that “wellness” programs are being integrated into the curriculum at veterinary schools and individual State Veterinary Medical Associations continue to promote wellness exams and testing to the pet-owning public. With the increased acceptance and practice of “wellness exams” in human medicine, increasing numbers of pet owners accept this approach to preventive health care offered by individual veterinary practices.

Parameters for pet wellness testing have not been strictly defined, but reasonably include heartworm testing, complete blood count, biochemistry profile (especially in geriatric patients), urinalysis, etc. As the emphasis on intervals for administering core vaccines continues to shift from “annual boosters” to triennial boosters, or longer, the concept incorporating antibody testing as part of a pet wellness program becomes increasingly feasible.

Concluding Comments

The purpose of this review is to provide veterinarians with key facts and information relevant to serological testing of individual dogs and cats in the clinical setting. Specifically, this paper addresses the role of antibody testing for the core, vaccine-preventable diseases *canine distemper virus*, *canine and feline parvovirus*, and *canine adenovirus*.

Understanding when serological testing is indicated, and, for each indication, knowledge of what actions should be taken if the test results are “positive” vs. “negative” is fundamental. Given the high degree of correlation between a “positive” antibody test result (whether a quantitative or qualitative testing platform is utilized) and protection, serological testing offers veterinarians a relevant, reliable tool in managing individual patients in the clinical setting.

Additional Reading

1. AAHA Canine Vaccination Guidelines: 2017 (updated February 3, 2018): available online at: www.aaha.org (123 references).
2. *Compendium of Animal Rabies Prevention and Control, 2016*. National Association of State Public Health Veterinarians. Released March 1, 2016. Available online at: <http://nasphv.org/Documents/NASPHVRabiesCompendium.pdf>
3. Day MJ, Horzinek MC, Schultz RD and Squires RA: Guidelines for the vaccination of dogs and cats. WSAVA Vaccination Guidelines Group. *J Sm Anim Practice* 2016, 57: E1-E45: available online at www.wsava.org
4. Greene CE, Levy J. Immunoprophylaxis. In Greene CE (ed): *Infectious Diseases of the Dog and Cat*, 4th ed. St. Louis: Elsevier-Saunders, 2012, pp 1163-1205.
5. Gill M, Srinivas J, Morozov I, et al. Three-year duration of immunity for canine distemper, adenovirus, and parvovirus after vaccination with a multivalent canine vaccine. *Intern J Appl Res Vet Med* 2004; 2(4):227-234.
6. Kinch M. *Between Hope and Fear: A history of vaccines and human immunity*. Pegasus:New York, 2018.
7. Schultz RD, Ford RB, Olsen J, Scott F. Titer testing and vaccination: A new look at traditional practices. *Roundtable Discussion*. Lenexa, Kansas: Veterinary Healthcare Communications, 2002, pp 1-16.
8. Twark L, Dodds WJ. Clinical use of serum parvovirus and distemper virus antibody titers for determining revaccination strategies in healthy dogs. *JAVMA* 2000; 217:1021-1024.
9. Waner T, Mazar S, Keren-Kornblatt E. Application of a dot enzyme-linked immunosorbent assay for evaluation of the immune status to canine parvovirus and distemper virus in adult dogs before revaccination. *J Vet Diagn Invest* 2006; 18(3):267-270.

Updated: November 2018

Table 1. Serological Testing for Vaccine-Preventable Core Diseases^v

Virus	Interpretation of Test Results
Rabies Virus	Rabies virus neutralizing antibody (RVNA) levels are available through certified laboratories only. “Positive” test results are only indicative of prior (recent) vaccination and are not to be interpreted as an index of protection.
Canine	
Adenovirus	In-clinic titer test results correlate <i>well</i> with gold standard testing (VN).
Distemper virus	In-clinic titer test results correlate <i>well</i> with gold standard testing (VN).
Parvovirus	In-clinic titer test results correlate <i>well</i> with gold standard testing (HI).
Feline	
Calicivirus	The correlation between gold standard testing (VN) and protection is only <i>fair</i> .
Herpesvirus	The correlation between gold standard testing (VN) and protection is only <i>fair</i> ; cell-mediated immunity is a <i>better</i> correlate of protection.
Parvovirus (Panleukopenia)	In-clinic titer test results correlate <i>well</i> with gold standard testing (HI).

Table 2. In-Clinic Antibody Titer Test Kits (QUALITATIVE TESTING)

	TiterCHEK CDV/CPV	VacciCheck Antibody Test Kit
Manufacturer	Zoetis (zoetisus.com)	Biogal Galed Laboratories (www.biogal.com)
Canine Antibody	CDV and CPV only	CAV, CDV, and CPV
Feline Antibody	Not Available	FCV, FHV, and FPV
Sample	Serum or plasma (can use hemolyzed sample)	Serum, plasma, or whole blood (can use hemolyzed sample)
Test Time	15–20 min (minimum)	21 min (minimum)
Results	Qualitative: Positive or Negative	Semi-Qualitative: utilizes a graduated color scale to determine the relative amount of antibody present compared to a “positive” reference (control) color.

CAV = canine adenovirus; CDV = canine distemper; CPV = canine parvovirus; DOI = duration of immunity; FCV = feline calicivirus; FHV = feline herpesvirus; FAVN = fluorescent antibody virus neutralization; FPV = feline parvovirus (panleukopenia); HI = hemagglutination inhibition; Ig = immunoglobulin; MDA = maternally-derived antibody; VN = virus neutralization (VN)

ⁱ Specially, booster intervals have been recommended every 3 years for the CORE canine (distemper virus-parvovirus-adenovirus) and feline (panleukopenia virus-herpesvirus-calicivirus) vaccines. Although rabies vaccine is considered CORE in most countries today, rabies vaccine booster intervals are generally established by State, Provincial, or local statutes.

ⁱⁱ Any purported link between childhood vaccines and the development of ASD has been categorically disproven in several multi-institutional and international studies involving hundreds of thousands of children

ⁱⁱⁱ VacciCheck Antibody Titer Test™, Biogal-Galed Laboratories, Gal’ed, Israel

^{iv} Available as an open, on-line educational resource for veterinary medicine: Search: AAHA Canine Vaccination Guidelines

^v NOTE: laboratory results reported as “positive” or “negative” only imply that the antibody being measured was either present (“positive”) or was not present (“negative”) relative to a threshold defined by that laboratory. Commercial laboratories typically do not make a clinical interpretation of the results. That’s the clinician’s responsibility. Furthermore, the reference range for titer results reported by one laboratory should not be compared with the reference range for titer results from a different laboratory as testing methods used by different laboratories can, and do, vary.