Antibody Testing in Clinical Practice
Indications for Point-of-Care Testing

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In companion animal practice, testing serum/blood for the presence of antibody (serology) to vaccine-preventable diseases (see TABLE 1) continues to gain acceptance as a means of assessing the immune status of individual patients.

It is likely that two key factors have predominantly served to influence the decision by clinicians to perform antibody testing on individual patients:

- **Triennial Vaccination Recommendations:** vaccination recommendations, as outlined in current textbooks, taught in veterinary schools, and published in Vaccination Guidelines, highlight 3-year booster intervals for the core vaccines rather than the conventional "annual booster" recommendation. Despite supporting data demonstrating durations of immunity lasting 5 years and longer for core vaccines, veterinarians continue to challenge triennial booster recommendations. Such concerns have prompted greater demand 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 Request. Random surveys of over 3000 veterinarians in the US and Canada suggest that "pet-owner requested" testing is the most common reason for performing serology on an individual cat or (especially) dog. Emerging owner concerns about over-vaccination and vaccine-associated injury to pets have prompted some owners to challenge the need for revaccination. Interestingly, such concerns may be attributable to the anti-vaccination movement in human medicine and the (now refuted) link between Measles-Mumps-Rubella (MMR) vaccination and development of autism-spectrum disorders (ASD)[1] in children.

It is important to note that as the demand for patient-centered antibody testing continues to emerge in companion animal practice, veterinarians are faced with fundamental, highly relevant questions related to antibody testing in clinical practice…three of these questions are addressed in the following review:

- **DOES ANTIBODY TESTING CORRELATE WITH "PROTECTION" vs "SUSCEPTIBILITY"?**
- **WHAT ARE THE INDICATIONS FOR ANTIBODY TESTING?**
- **TEST INTERPRETATION: HOW DO TEST RESULTS IMPACT PATIENT MANAGEMENT?**

[1] 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.

DOES ANTIBODY TESTING CORRELATE WITH "PROTECTION" vs "SUSCEPTIBILITY"?

Can a "positive" antibody test result reliably identify a dog/cat that is protected from infection if exposed? In fact, there is a strong correlation between a "positive" antibody test result and protection *against canine distemper virus, canine parvovirus, canine adenovirus and feline panleukopenia virus (feline parvovirus).*

However, making rational clinical interpretation and patient management decisions based on serological (antibody) test results depends on understanding a few "must know" facts. NOTE: in the context of this manuscript, the points outlined below apply ONLY to *canine distemper virus, canine parvovirus, canine adenovirus and feline panleukopenia virus (feline parvovirus).*

1. The only *true* test of protective immunity involves exposure (challenge) to a virulent pathogen in which non-vaccinates (controls) become infected and manifest clinical illness while vaccinated animals remain healthy. Licensing of all animal vaccines is based on well-controlled challenge studies (efficacy).

2. Antibody testing methods vary: Although numerous serological methodologies are in use today (beyond the scope of this discussion), it is important to distinguish two key terms relevant to antibody testing:

a. ***Quantitative* Testing**…aka 'titers'…refers to laboratory-based, end-point testing methods (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. 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).

b. ***Qualitative* Testing**…refers to commercial "point-of-care" testing methods (or, test kits) practical for use within a veterinary practice (see TABLE 2). 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). Again, it is the clinician who must interpret the meaning of the "positive" or "negative" test result.

The VacciCheck[2] point-of-care test kit provides "semi-quantitative" results. Although results are not read as end-point titers, the test kit utilizes a graduated (gray-purple) 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 can be interpreted to represent a protective level of antibody.

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[2] VacciCheck Antibody Titer Test™, Biogal-Galed Laboratories, Gal'ed, Israel

3. IMMUNE "MEMORY": In the clinical setting, documenting a "positive" antibody response following vaccination (eg, against canine distemper, parvovirus, or adenovirus) serves as an immunological reference point for protection (possibly for life) in most patients. For example…over time, it is possible that a dog with a "positive" antibody test result would become "negative" (due to lack of exposure or failure to re-vaccinate). However, the fact that the dog previously had a "positive" test result indicates that immune "memory" exists…if challenged, a rapid, anamnestic response is expected and will serve to protect against natural challenge. Therefore, a "negative" test result does not always imply *susceptibility*, especially in adult, previously vaccinated patients.

Does a "positive" antibody test result look forward?

…or…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 sense…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 levels 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. "POSITIVE" vs "NEGATIVE" TEST RESULT: *This is important*…

Laboratory test results (whether quantitative or qualitative) must be interpreted by the clinician…results are *not* interpreted (clinically speaking) by the laboratory. While a laboratory may report an antibody titer as "POSITIVE", the clinician must understand that the laboratory is merely stating that the titer (concentration of antibody) meets or exceeds the reference threshold for this laboratory. Likewise, results reported as "NEGATIVE" mean that the concentration of antibody is below the reference threshold for this laboratory. The clinician must determine whether or not the specific antibody tested and the results correlate with susceptibility vs. protection. See TABLE 2.

To be very clear…

…consider a patient sample returned from a commercial laboratory with a titer result for canine parvovirus antibody reported as: **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 a *clinical* interpretation of what the "positive" test result means in the individual patient.

That's the clinician's responsibility….

5. When results are "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…**I**nfection, or…**E**xposure

For example, a "positive" antibody test results (whether quantitative or qualitative) for canine distemper virus, canine adenovirus, and parvovirus (canine and feline) correlate exceptionally well with protection.

On the other hand, a "positive" Leptospira antibody test result does not correlate well with protection. In the clinical setting, Leptospira antibody denotes infection and, therefore, is used as a diagnostic test.

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.

6. "Positive" antibody test results, whether using quantitative or qualitative testing, correlate strongly with protective immunity for canine parvovirus, canine distemper virus, canine adenovirus, and feline panleukopenia virus (feline parvovirus).

WHAT ARE THE INDICATIONS FOR ANTIBODY TESTING? and HOW DO RESULTS IMPACT PATIENT MANAGEMENT?

The online version of the AAHA Canine Vaccination Guidelines[3] includes a menu option entitled "Antibody Testing for Vaccine-Preventable Diseases". 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. Six common indications for testing of individual patients in clinical practice are described below.

The examples included below are representative indications for assessing serological responses in patients vaccinated against canine distemper virus, canine adenovirus, canine parvovirus, and feline panleukopenia virus (feline parvovirus).

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[3] Available as an open, on-line educational resource for veterinary medicine: *Search*: AAHA Canine Vaccination Guidelines

1. Assess antibody response following administration of the *initial* core vaccine series in young animals.

For example, young, recently vaccinated dogs/cats that will travel (showing or sale) prior to reaching adulthood are justifiably tested to verify a protective immune response following completion of the initial series. 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).

Patient Management: 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 single dose of combined core vaccines ("booster") is recommended 1 year later.

If results are "negative" for antibody against any virus, the patient is considered *susceptible.* A booster dose of a combined core 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. To assess the immune status of an adult dog that is DUE (or OVERDUE) for re-vaccination.

For example, antibody testing would be indicated in a dog/cat owned by a client who is particularly anxious or concerned over the potential for vaccine-associated injury. Testing offers the client an alternative to routine administration of the core vaccines.

Patient Management: If the results are "positive" the patient has mounted a sustained, protective immune response. Re-vaccination would not be necessary. If "negative", it is possible that the patient is susceptible (especially in young animals) or has B-cell "memory" and is, in fact, protected (especially in adult, previously vaccinated animals). The recommendation to re-vaccinate the individual patient with a "negative" test result is indicated.

3. To assess the immune status in an adult dog with an unknown vaccination history.

For example, antibody testing is useful in determining whether or not a recently adopted adult (stray) dog requires vaccination. As in the example above, some owners may be opposed to arbitrarily administering doses of vaccine if, in fact, the patient has been previously vaccinated and is protected.

Patient Management: Similar to the recommendations outlined in the previous scenario, if the results are "positive" the patient is likely to have been previously vaccinated (or exposed and recovered) and is considered to be protected. Re-vaccination would not be necessary. If "negative", it is possible that the patient is susceptible (especially in young animals) or has B-cell "memory" and is, in fact, protected (especially in adult, previously vaccinated animals). The recommendation to re-vaccinate the individual patient with a "negative" test result is indicated.

4. To assess the need for re-vaccination of the geriatric patient.

For example, if the clinician (or owner) is questioning the need to continue re-vaccinating older adult pets with core vaccines, antibody testing would provide objective data in making the decision to discontinue routine vaccination (rabies vaccination is an important exception) in the geriatric dog or cat..

Patient Management: If antibody test results are "positive" in a 10 year old dog or cat, it would be reasonable to discontinue routine vaccination. In the event one or more antibody test results are "negative" it reasonable to recommend a booster dose (despite the fact immune "memory" is likely to exist).

5. To assess the immune status of an individual dog/cat undergoing treatment for a chronic, systemic illness.

For example, it is unknown how well an adult dog or cat with chronic, compensated kidney disease will respond following re-vaccination. Considering that all vaccines are labeled for administration to "healthy animals" it would be reasonable to perform antibody testing in any chronically ill patient to determine the need for re-vaccination.

Patient Management: A chronically ill patient with a "positive" antibody test result is still protected; re-vaccination is not indicated. On the other hand, if the antibody test results are "negative", the clinician must assess the risk for exposure as well as the likelihood an individual patient is truly susceptible (juvenile) vs. protected (adult).

6. To assess the immune status of a patient having a history of suspected or known adverse reaction associated with vaccination.

For example, antibody testing of a patient with history of having experienced a known, or suspected, adverse reaction following vaccination administration is a reasonable alternative to merely administering a scheduled "booster" dose of vaccine then planning to medically treat any adverse consequences that might develop.

Patient Management: If antibody test results are "positive", there is no need to re-vaccinate the patient at this point. However, if the results are "negative", the decision to re-vaccinate is more complicated and should take into consideration the age of the patient, overall health status, the type and severity of the adverse reaction that previously occurred, the number of adverse reactions associated with vaccination, and the amount of time that has lapsed since the previous reaction occurred.

The reader is referred to the AAHA Canine Vaccination Guidelines for access to the complete list of indications and management recommendations.

LIMITATIONS to ANTIBODY TESTING

Seroconversion, the antibody response that follows vaccination, can be determined for most of the vaccines licensed for administration to dogs and cats. However, the development of antibody may not always equate to **protective immunity,** or the ability to prevent infection/shedding... *for example:*

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.

Canine Influenza Virus (CIV)

- Canine influenza virus (CIV H3N8 and H3N2) antibody develops toward the end of clinical infection (3-4 weeks following infection). Antibody testing during the acute phase of infection is likely yield "negative" results. Therefore, CIV antibody is more indicative of exposure than infection.

- Following CIV vaccination, antibody to the H3N8 and H3N2 strains can be measured. However, CIV vaccine-induced antibody does not prevent infection or virus shedding; CIV vaccination mitigates the severity of disease and may shorten the duration of virus shedding following exposure.

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.

WELLNESS TESTING IN PRACTICE

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 of 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, parvovirus, adenovirus*, and *feline panleukopenia virus (feline parvovirus).*

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 June 2019): available online at: www.aaha.org (120+ 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.

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TABLE 1. Serological Testing for Vaccine-Preventable Core Diseases[4]

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 *well* with quantitative test results (VN).
Distemper virus	In-clinic titer test results correlate *well* with quantitative test results (VN).
Parvovirus	In-clinic titer test results correlate *well* with quantitative test results (HI).
FELINE	
Calicivirus	The correlation between a "positive" antibody titer (VN) and protection is only *fair*.
Herpesvirus	The correlation between a "positive" antibody titer (VN) and protection is only *fair*; cell-mediated immunity is a *better* correlate of protection.
Parvovirus (Panleukopenia)	In-clinic titer test results correlate *well* quantitative test results (HI).

[4] 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.

TABLE 2. In-Clinic Antibody Titer Test Kits

	TiterCHEK CDV/CPV	VacciCheck Antibody Test Kit
Manufacturer	Zoetis (zoetus.com)	Biogal Galed Laboratories (biogal.co.il)
Canine Antibody	CDV + CPV	CDV + CPV + CAV
Feline Antibody	Not Available	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-Quanitative: utilizes a graduated color scale to determine the relative amount of antibody present compared to a “positive” reference (control) color.

Abbreviations used throughout this article:

CAV = canine adenovirus; CDV = canine distemper virus; CPV = canine parvovirus; CIV = canine influenza virus; DOI = duration of immunity; FPV = feline parvovirus (panleukopenia); HI = hemagluttination inhibition; Ig = immunoglobulin; RVNA = rabies virus neutralizing antibody; VN = virus neutralization (VN)