Antibody Testing in Clinical Practice

Indications for Point-of-Care Testing

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In veterinary medicine, the concept of measuring antibody as a means of assessing the need for, or the response to, vaccination is still relatively new. Initial interest in patient-centered testing seems to have started over concerns that the 3-year booster recommendations for core vaccines had not been validated. More recently, clientele concerned about risks associated with pet over-vaccination have prompted veterinarians to offer serological testing in lieu of routine vaccination. As demand for antibody testing of individual patients increased, more laboratories began offering serological testing for vaccine-preventable diseases. In addition to laboratory-based testing, point-of-care antibody test kits are now available that offer results within 25 minutes at reasonable cost.

As the demand for patient-centered antibody testing continues to emerge in companion animal practice, more *indications* for patient testing have also emerged, along with questions regarding testing interpretation and patient management.

It is the purpose of this webinar to address 3 of the most fundamental questions pertaining to the use of antibody testing in clinical practice:

- **ARE ANTIBODY TEST RESULTS VALID?** Does a "positive" test result actually correlate with protection?
- **WHAT ARE THE INDICATIONS FOR TESTING?** The AAHA Canine Vaccination Guidelines have identified 12 indications…representative examples will be described.
- **INTERPRETATION of results?** When testing is indicated, how should the individual patient be managed if the test result is "positive" vs. "negative"?

ARE ANTIBODY TEST RESULTS VALID?

Making rational, patient-centered clinical decisions based on serological (antibody) test results depends on understanding a few "must know" facts. Consider the following 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 after a significant challenge dose. Licensing of animal vaccines is based on this premise.

Serological studies conducted in conjunction with "challenge" studies have consistently demonstrated exceptional correlation between antibody level and protection *for canine distemper (CDV), canine parvovirus (CPV), canine adenovirus (CAV), and feline parvovirus (FPV, or panleukopenia).* See references: 3,4,5,6,8,10,11, and 12.

2. "POSITIVE" vs "NEGATIVE" Test Result…*this is important.*

- A "positive" antibody test result means that the patient's 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.

To be clear…

…consider a patient sample submitted to a commercial laboratory is returned with results 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….

- A "negative" test result only indicates the patient's sample either did not have a detectable level of antibody or that the level present was below a defined threshold. Note…a *"negative" test result does not necessarily define "susceptibility"* (see INDICATIONS & INTERPRETATION below).

3. Some antibody test results correlate with PROTECTION…some do not…

Commercial laboratories offer serological testing for several bacterial, fungal, and viral pathogens.

"Positive" antibody test results have a significantly different meaning depending on the pathogen (or vaccine) that induced the antibody. THINK **PIE** when interpreting a "**positive**" test result:

PROTECTION or…**I**NFECTION or…**E**XPOSURE

For example, as described above, a "positive" antibody test result for CDV, CPV, CAV, and FPV correlates exceptionally well with *PROTECTION*…that fact is well established.

In practice, a "positive" Leptospira antibody testing is used as a diagnostic tool to identify *INFECTION.* Leptospira antibody does NOT correlate well with protection following either vaccination or natural infection. Other factors, such as cell-mediated immunity, are primarily involved. The same is true of Feline Immunodeficiency Virus (FIV)…a cat having a "positive" FIV antibody test is deemed infected…not protected.

On the other hand, a "positive" antibody test result for *Ehrlichia spp.* or *Anaplasma spp.* only correlates with prior *EXPOSURE*…results do NOT correlate with either protection or infection. The same is true for canine influenza virus…a "positive" CIV antibody test denotes prior exposure. CIV antibody levels become positive *after* the short-lived viremia (2-4 weeks) is over. Furthermore, a CIV antibody-positive patient is still susceptible to infection if re-exposed …antibody does not correlate with protection.

4. The Testing "Platform"…a technical point. Antibody testing can be performed *quantitatively* (laboratory-based titer) or *qualitatively* (point-of-care, in-clinic test kit):

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 relative concentration of antibody, expressed as a ratio, that has been produced in response to a specific antigen. Patient samples must be sent to a laboratory and results are usually available within days. NOTE: the amplitude of the titer is not a correlate of the degree of immunity. A dog with a high "positive" parvovirus antibody titer is not more immune, or better protected, than a dog with a low "positive" parvovirus antibody titer.

b. ***Qualitative* Testing**…refers to "point-of-care" testing methods (or, test kits) practical for use within a veterinary practice. Depending on the test, results can be obtained in as little as 10 to 30 minutes. Test kits licensed for use in dogs and cats reliably distinguish "POSITIVE" results from "NEGATIVE" results.

The **VacciCheck**[1] 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 (CDV-CPV-CAV) tested. The color scale is scored from zero (0) to six (6). Scores ranging from 2 to 6 represent a protective level of antibody.

A "positive" antibody test result, whether performed by quantitative testing, "semi-quantitative" testing, or qualitative testing, is interpreted the same way…a "positive" is a "positive".

WHAT ARE THE INDICATIONS FOR TESTING?

The online version of the AAHA Canine Vaccination Guidelines[1] includes a menu option entitled "Antibody Testing for Vaccine-Preventable Diseases". The purpose of the section is to provide veterinarians with various scenarios (12 are listed) for which serological testing (CDV, CPV, and CAV) of an individual dog would be indicated. Several of these indications are also applicable to the cat.

For each *indication*, recommendations on patient management are offered for a "positive" test result as well as a "negative" test result.

Listed below are representative indications for assessing serological responses in patients vaccinated against canine distemper virus, canine adenovirus, and canine (and feline) parvovirus. Refer to the AAHA Canine Vaccination Guidelines (online) for additional testing indications.

[1] 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.

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 (although 2 to 4 weeks following the last dose 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 immunized (protected)…a booster dose of the 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 not be unreasonable to recommend an additional test, 2 weeks following administration of the additional booster 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 series, the patient should be re-vaccinated against parvovirus as soon as practical and scheduled for a follow-up antibody test. A second "negative" test result, obtained 2 to 4 weeks after administering an additional (booster) dose of vaccine, 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 re-vaccination.

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 result for one (or more) of the viruses for which vaccine was previously administered. It happens… antibody is a protein and blood levels will 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 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 CDV, CPV, CAV, and FPV 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 (within days) develops a "secondary" (anamnestic) antibody response, even if the antibody level has declined to a level below the "positive" threshold on a test. In effect…the patient's immunity is "boosted" by that exposure.

That's why a "negative" antibody test result in a dog that has previously been vaccinated against distemper, parvovirus, or 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 becomes an important alternative to re-vaccination.

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.

LIMITATIONS to ANTIBODY TESTING for CORE VACCINES

Seroconversion, the antibody response, from a seronegative state to a seropositive state, 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 performed as a clinical service to veterinary practices.

- The so-called "gold standard test" (quantitative titer) used to measure FCV antibody has been judged only as *fair to good*. For this reason, antibody testing for FCV antibody is generally not recommended.

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 been "adequately vaccinated"…*that's it!* Do not submit serum for RVNA titers as a means of confirming protective immunity against rabies.

WELLNESS and ANTIBODY 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 a physical examination & history (lifestyle assessment), 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 practical.

Concluding Comments

Point-of-care testing for antibody to CDV, CPV, CAV, and FPV represents a relatively new clinical resource in veterinary medicine that enables the clinician to assess patient risk against the most serious (core) infections. The application of this technology in practice requires an awareness of the indications for testing *and* the knowledge to properly interpret test results. Given the high degree of correlation between a “positive” antibody test result (whether using quantitative, semi-quantitative, or qualitative testing platform) and protection, serological testing offers veterinarians a relevant, reliable tool for making informed decisions relevant to managing individual patients in the clinical setting.

Additional Reading

1. AAHA Canine Vaccination Guidelines: 2017 (updated February 2018): available online at: www.aaha.org (120+ references).
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Updated: November 2019

TABLE 1. Serological Testing for Vaccine-Preventable Core Diseases[2]

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

[2] 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 (zoetisus.com)	Biogal Galed Laboratories (biogal.co.il)
Canine Antibody	CDV and CPV only	CAV, CDV, and CPV
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-Quantitative: 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; RVNA = rabies virus neutralizing antibody; FPV = feline parvovirus (panleukopenia); Ig = immunoglobulin; MDA = maternally-derived antibody; VN = virus neutralization test; HI = hemagglutination inhibition test.