1. **How does VacciCheck compare with the titer tests done at Kansas State?**

Kansas State University's has a comprehensive Veterinary Diagnostic Laboratory. They do perform antibody titers for vaccine-preventable diseases but I am not aware of any studies in which they specifically compared their titer results with VacciCheck results, or other antibody testing platforms.

On this point, it is important to understand that, within laboratory medicine, there is wide consensus on titer testing for distemper antibody (using serum neutralization) and parvovirus antibody (using hemagglutination inhibition). The VacciCheck antibody test results have been widely corroborated with titer results in several laboratories in the US and internationally. There is excellent correlation for both positive and negative test results.

2. **Since there are scenarios where there exist different strains of antigens causing a particular disease, could it be possible to have test kits that can determine specific antibodies against a particular strain of antigens?**

We have all watched the concern over emerging strains of the SARS-CoV-2 virus (cause of COVID-19) and the effort to test for individual variants of that virus. Testing for unique strains and variants is possible in research and diagnostic laboratories equipped with specific instrumentation. The development of "test kits" for unique strains/variants, while possible, does involve complex testing, manufacturing, and marketing considerations. In the absence of widespread demand, test kit development for unique virus variants or strains is unlikely in veterinary medicine.

3. **Can the in-house Point-of-Care test kit have a "maybe" answer and not just positive or negative?**

Yes…and the same is true of laboratory-based antibody TITER testing platforms as well. All of these test results must be interpreted by a person who either makes an assessment of color change or the % of virus neutralized. Varied interpretation of test results is always possible. And that could certainly lead to a "maybe" posting of a test result.

When using the semi-quantitative VacciCheck, results are always compared to a "positive" control color. A result that is LIGHTER than the control color suggests that the amount of antibody level in that patient is less than adequate. In reality, some of these patients will actually be protected, while others may not be. In this case, the clinician can use the "less than adequate" test result to make the objective decision to re-vaccinate the patient.

4. **Maternal antibodies. Can I use titers to see if a puppy has responded to their puppy DAPP vaccine series? Or will it just be positive because of maternal antibodies?**

Maternally derived antibody is PASSIVE. The concentration of antibody is in steady, rapid decline over the first few months of life. This is why antibody testing 2 to 4 weeks AFTER completion of the initial DAPP series is recommended. In fact, this is the most

common use of VacciCheck in clinical practice. By 4 months of age, maternal antibody levels are not likely detectable AND antibody test results provide an accurate assessment of vaccine-associated (ACQUIRED) immunity.

5. **We say: regardless of the actual antibody titer, a "positive" test result on the VacciCheck test correlates with protection.**

Yes…this is correct.

Then, why is there a range of positive states and how can I, based on your experience, correlate this with a specific clinical case?

This is a good question…and likely represents the level of technology (semi-quantitative) afforded by the VacciCheck testing platform.

ANY test result (VacciCheck) that is a darker color than the "positive" control gives the clinician objective information that the patient is immunized and protected. The "range" of positive results (semi-quantitative) is somewhat similar to titer testing. The interpretation is the same…results that are within the "positive" range (or above the threshold for a "positive" titer) indicate protection. It does not indicate degrees of protection.

6. **What is the reference that supports the data that after the 16 week vaccination, there are still 15% of the puppies without protection.**

This study was conducted through the U of Wisconsin (Schultz and Larson) but has not been published…at least not yet. The AAHA Canine Vaccination Task Force was given access to the data and the methodology involved. Thousands of young dogs presented to practices within the US were involved in this very large study. 55% of pups were not immunized against Distemper or Parvovirus following vaccination at 12 weeks of age; 15% were not immunized following vaccination at 16 weeks of age. These findings are reflected in the current vaccination guidelines published through the American Animal Hospital Assoc. as well as the World Small Animal Veterinary Assoc. Vaccination Guidelines for the Dog and Cat.

7. **Any specific kind of vaccine for the Distemper non-responder cases?**

This is an interesting point, and due to time limitations, was not specifically addressed during the webinar. Genetic non-responders to parvovirus, distemper, and adenovirus are known to occur throughout the world. It is estimated that 1 in 1000 dogs (globally) are not capable of responding to parvovirus vaccine; 1 in 5000 dogs are not capable of responding to distemper vaccine; and around 1 in 10,000 are not capable of responding to adenovirus vaccine. (These numbers, of course, are only estimates)

Point of fact…if a puppy is determined to be a non-responder to any of these vaccines (as determined by antibody testing following the initial puppy series), that dog is susceptible to infection if exposed and will remain that way. Vaccinating with more doses, or vaccinating more frequently, or using different vaccines will not alter the immune status of a genetic non-responder.

8. **How to start educating the client before I start a program testing antibodies?**

I think this is an important point for any clinician…

First, the client has be made aware that administering at least 3 doses of a combination vaccine, starting between 6 and 8 weeks of age, is best way to protect the young dog from becoming infected with a potentially fatal virus.

Second….vaccination never guarantees immunization.

Third, the clinician can emphasize that the only way to assure the pup is protected is to test for antibody 2 to 4 weeks after the last, most important vaccine dose, in the initial series.

Antibody testing is not equally relevant to all patients…but, for those dogs that will have an opportunity to interact with other dogs (dog parks, boarding, day-care, show dogs, etc.) knowledge that this young dog is, or is not, protected, can be a very important part of the health care equation.

9. **If a genetic Non-Responder dog gets parvovirus infection, and recovers from the infection, does it make immunity against it after disease is gone?**

Very interesting question. We have observed a small number of genetic non-responders (parvovirus) that became infected (which is NOT unusual) but then recovered from their infection (which is unusual). AND…even after recovering, they still had NOT produced parvovirus antibody.

So…HOW WERE THESE DOGS ABLE TO RECOVER???

It is likely these dogs enjoyed a robust cell-mediated immune response… (Remember…antibody <u>prevents</u> parvovirus infection…cell-mediated immunity is critical in <u>recovery</u> from infection).

And…it's always possible that the infecting parvovirus was a less virulent variant. That does happen.

10. **Perhaps this is beyond the scope of this talk....Is there a genetic test to look for non-responding dogs, or lines of dogs?**

Actually, this is a very relevant question to ask…based on a 2020 review paper addressing vaccination failure in dogs, the answer to your question is…NO…there is not a genetic test for vaccine non-responders. Leukocyte antigen (DLA) type II haplotype has been studied in dogs. Diversity in dogs varies widely between breeds, but not within breeds. Genetic diversity is restricted in the Rottweiler as compared to other breeds. This led to the suggestion that DLA type II might be a test of susceptibility to parvovirus infection. But, that has never been substantiated.

How do you identify a non-responder?

The antibody test result will be "negative" (parvovirus antibody negative dogs are most common) at 2-weeks following the last dose in the initial series…RE-TEST…2 weeks after re-vaccination, the dog is still antibody negative…

This genetic fault is discrete. For example, parvovirus non-responders still make protective levels of distemper and adenovirus antibody.

11. **What is the clinical application of the acronym PIE and how does this facilitate client understanding of the advantages to antibody testing.**

I use the acronym P-I-E to emphasize the fact that the clinician is ultimately responsible for interpreting a "positive" antibody test result and making objective clinical decisions regarding the individual patient. A "positive" antibody test result has different meanings depending on which antigen induces the antibody response.

Globally, there are over 30 antigens used in canine vaccines. Antibody responses can be measured against almost all of these vaccine antigens. In addition, there are many other infectious pathogens (for which vaccines are not available) that induce a measurable antibody response. HOWEVER, it is ultimately the clinician who must interpret what the "positive" test result actually means for the individual patient.

PROTECTED: positive antibody responses to the core vaccines (distemper-parvovirus-adenovirus) indicate the patient is immune and provides the clinician with objective information that the patient is protected if exposed. Abundant data supports this.

INFECTED: However, the antibody response to some antigens do NOT reflect protection, but are used to diagnose infection. A "positive" antibody test result for leptospirosis or feline immunodeficiency virus (FIV) are good examples.

EXPOSED: A "positive" antibody response to some infections, and some vaccines, indicate the patient has only been exposed to the antigen…and is NOT protected or infected. *Ehrlichia* and *Anaplasma* antibody response are examples. IMPORTANT:

exportation of dogs/cat to rabies free countries and areas of the world often requires a "positive" antibody (rabies virus neutralizing antibody or RVNA) test result. Internationally, a "positive" rabies antibody test result is interpreted to mean the patient has been adequately vaccinated (ie, exposed to the vaccine)…a "positive" rabies antibody titer is NOT a legal index of protection.

12. **What would be your advice for a cat hotel (boarding facility) that offers both individual and common housing. The managers want to only admit cats that have a "positive" antibody test response to herpesvirus and calicivirus.**

Many commercial laboratories currently offer antibody titers for feline herpesvirus and calicivirus. It is important to understand that "positive" test results do NOT correlate well with protection. There are 2 points that all veterinarians should be aware of regarding vaccine-associated immunity to feline herpesvirus and calicivirus.

First: immunity to herpesvirus is predominantly through cell mediated immunity…NOT antibody. Immunity to feline calicivirus is somewhat complex and involves both cell-mediated immunity and local (IgA) immunity. Vaccines induce systemic levels of antibody (IgG) which plays a minor role in reducing the severity of disease.

Second: all commercial vaccines for feline herpesvirus and calicivirus induce "non-sterilizing" immunity. This means that the vaccines only reduce the severity of clinical disease if a cat is exposed. Neither vaccine will prevent infection, neither vaccine will prevent development of a chronic carrier state, and neither vaccine will prevent shedding after a vaccinated cat becomes infected.

Administering a booster dose 2-weeks prior to boarding is not likely to provide substantial benefit to any cat that has been vaccinated within the past 3 years.

13. **What is the most likely cause of vaccine adverse (allergic) reactions?**

While this is a topic that needs additional study, most authors agree that vaccine-associated acute adverse reactions in both dogs and cats are most likely associated with the individual animal becoming sensitized (Type I hypersensitivity) to one or more excipients (these are chemicals, proteins, adjuvants added during the manufacturing process). Interestingly, the vaccine antigen, eg, parvovirus, leptospira bacteria, etc, is much less likely to be the direct cause of hypersensitivity reactions following vaccination.

14. **Do you recommend that older cats get the intranasal vaccine vs the injectable vaccine?**

No…I've used both vaccines extensively and have studied systemic immune responses following intranasal vaccination against herpesvirus and calicivirus in kittens and adult

cats. There is no observable clinical advantage to the intranasal vs. injectable vaccines currently available.

About one-third (in my experience) of kittens that receive an intranasal vaccine will develop post-vaccinal sneezing associated with replication of vaccine virus within nasal mucosa. Kittens may appear to have developed the clinical infection resulting from the intranasal vaccine.

15. **Is it true that antibody titers can be "falsely low" if the patient has not been recently exposed to the antigen…and…**
How can you be sure that if a dog tests positive one day it will be protected in a month. How long does that "memory" last, how can you know they are safe after achieving a "positive" antibody test result.

Actually…the antibody titers are NOT "falsely low"…over time, the concentration of antibody does become low, or even undetectable by, as measured by conventional laboratory methods. Antibody is a protein…and, without repeated antigen exposure (naturally or by vaccination), the concentration of antibody will usually decline over time. But it depends on the antigen that stimulates production of antibody. Some antibody levels (eg, parvovirus and distemper) can remain high (protective) for many years after the initial vaccination series. Other antibody levels (eg, leptospira) will decline below detectable levels within a few months following vaccination.

IMPORTANT: a "positive" antibody test result to any of the core vaccines (distemper-parvovirus-adenovirus) indicates protection. However, a "negative" antibody test result does NOT always indicate susceptibility. In the adult, previously vaccinated dog…a dog with a history of having a "positive" antibody test result means the patient has developed "B-cell memory" (if you make antibody, you've made "memory") and will serve to protect the patient if exposed for many years, even if antibody levels become very low. That's the value of testing a patient at least one time.

In effect…a "positive" antibody test result today…does look forward many years (for the core vaccines). Published studies have shown protective immunity (again, for the core vaccines) at least 7 to 9 years after the last vaccine was administered (and, likely for life… infection with parvovirus or distemper virus is exceptionally rare in adult dogs.) On the other hand, immune memory following administration of a bacterin (eg, any of leptospira vaccines) is much shorter; immunization has only been shown to last from 12 to 16 months, depending on the serovar.

16. **Regarding geriatrics, what about immune senescence that has been discussed elsewhere recently?**

Good question…there is a paucity of information on the biology of immune senescence in animals. It certainly must occur, but to what extent and at what age(s) does it begin, we really can't offer precise guidance. Limited, and unpublished, studies support the

fact that immune memory (at least in most dogs) is likely to last for many years, if not life following initial vaccination with core vaccines. A 3-Year labeled rabies vaccine probably offers protection for 5, or so, years…but, re-vaccination is determined by regulation or law, not immunology. Therefore, rabies should be administered throughout life. For non-core vaccines, annual vaccination is still appropriate as long as the risk of exposure exists.

17. **In Brazil, we have a lot of leukemia virus infected cats. Vaccination against FeLV is recommended as core. Unfortunately, we don t have an antibody test to confirm immunization for cats (except for panleukopenia). What should I do in the case of a cat that showed an adverse vaccination reaction when the last booster dose was administered?**

The immune response to FeLV vaccines will always remain a challenging issue. The reason: antibody can be detected and measured, BUT…FeLV antibody is NOT a reliable indication of protection, infection, or exposure. That's why routine antibody testing is not performed. Infection is confirmed by testing for the p27 antigen, and that is very reliable.

Here's something important to consider regarding FeLV. Primary infection typically occurs in kittens (6 to 8 months of age or younger). It is quite likely that every cat (even adults) you have diagnosed with FeLV were infected as kittens.

Adult cats (over about 8 months of age, enjoy "age-related resistance" to FeLV. It is very difficult to infect an adult cat, even when challenging cats with virulent virus injected into the abdominal cavity. Consider limiting vaccination to young cats (2 doses; 3 to 4 weeks apart beginning not earlier than 8 weeks of age. If available in Brazil, use the recombinant FeLV vaccine…it does not contain adjuvant and is much less reactive.

18. **How often is it recommended to measure antibodies to be able to postpone vaccination?**

There is no defined time-line that can be applied in clinical practice for using antibody test results as a substitute for routine vaccination. It's my personal opinion that doing so is not a particularly valuable application of the testing technology. The reason is based on the fact that once it is established that a dog/cat has developed protective levels of antibody following vaccination, that dog/cat will have developed lasting immunity (memory) that is sustained for several years…likely for life when considering parvovirus antibody.

Testing a patient every 3 years sounds reasonable, and correlates with the recommended vaccination interval for core vaccines, but the majority of patients will have a "positive" test result for most, or all, of their life. And, even if the results are "negative"…a dog/cat that has had a "positive" test result in the past will still enjoy protection (ie, memory).

19. **What about the S2 status of VacciCheck: you re-test the next year and if it still S2 you can sign of for 3 years.**

While an S2 color result on VacciCheck is slightly lighter than the "positive" control and is interpreted as less that adequate antibody response, a dog that consistently has an antibody S2 result may, in fact, be a "genetic low-responder" (vs. a genetic non-responder). The opinion among most immunologists I've talked with about this suggest that although the antibody level may be lower that the "positive" control, it is likely that this dog is still immune.

In the clinical setting, it would be appropriate to administer a dose of vaccine annually, but the fact is, this dog may never develop a robust, strongly positive antibody response.

20. **At what point should we say a dog is a genetic non-responder?**

In practice, the best, most reliable way to identify the genetic non-responder is to test for distemper, parvovirus, and adenovirus antibody at some point between 2 and 4 weeks following the last dose in the initial (puppy) vaccination series. If "negative" results are obtained for any of the 3 viruses, immediately re-vaccinate with one dose of a combination core vaccine. Re-test for antibody 2 to 4 weeks later. IF STILL "negative" for antibody to that same virus, the dog should be considered a non-responder and will continue to be susceptible if exposed.

21. **If a dog is determined to be a genetic non-responder to a particular vaccine (eg, a parvovirus non-responder Rottweiler) what are the management options?**

The non-responder is susceptible…and, if exposed to distemper, parvovirus, or (rarely) adenovirus, infection is highly likely. Death may be the ultimate consequence. The objective management options include: not using the dog for breeding purposes, and avoiding interaction with other dogs. Problem is, infective levels of parvovirus can live in the environment for months (years?) and exposure/infection can occur without the need for direct dog-to-dog contact.

22. **In case of allergic patients; can we recommend Antibody testing to determine a good response to vaccine.**

No… a correlation between vaccine immunogenicity and hypersensitivity has never been established. It's important to remember that the immune system is remarkably complex. Abnormal or altered immune responses are very discrete and can occur without impacting the ability of the patient to respond to a dose of vaccine.

23. **I understand that IMT and HAI-related vaccines are those with adjuvants, mainly the rabies vaccine. Is this true?**

A few papers have, in fact, associated adjuvant-containing vaccines with (and possibly the cause of) immune mediated disease. Cause-and-effect has not been definitively established. Furthermore, there is no clear evidence implicating vaccination (with or without adjuvant) as the cause of immune-mediated thrombocytopenia or hemolytic anemia.

24. **How often does the testing have to be repeated again after a positive response?**

This really is determined by the specific indication for testing. If testing a patient to determine whether or not they still have protective levels of antibody one or more years following administration of vaccine, the patient may only need to be tested once. If the results indicate "positive" for Distemper, Parvovirus, and Adenovirus …they have made lasting (lifetime?) "memory".

25. **While I recognize that what is applicable in one species cannot necessarily completely applied to another, I think it is important to remember that human healthcare providers who care for people with chronic illnesses like heart disease, diabetes, cancer, routinely recommend that their patients get vaccinated (at least with killed products) because they are afraid that their patients are immunosuppressed and, by extension, far more vulnerable to a severe outcome if infected with vaccine preventable disease like flu or Hepatitis B. When I talk with veterinarians about vaccinating in the face of a chronic disease like various forms of cancer or renal failure, I am told that they are afraid of "stimulating the immune system" or of "pushing the patient over the edge" immunologically and I cannot find much, if any, basis in the literature for this. it would be helpful to have further information about why the trend in vet med is so different from human medicine.**

I hear this as well…in talking with immunologists, the concept of 'over stimulation" or "overwhelming" the immune response by over-vaccinating or vaccinating in the face of chronic disease is not an issue, immunologically speaking. At issue is administering vaccine to a patient that has a serious acute febrile, or chronic, illness. The only risk in these cases lies in the fact that the patient may NOT respond adequately to the vaccine. Hence, the statement associated with all vaccines: "for administration to healthy dogs/cats".

26. **What do you think of administering diphenhydramine (Benadryl) or a corticosteroid (eg, dexamethasone) immediately prior to vaccinating a dog or cat that has a history of an adverse reaction associated with vaccination?**

Administration (orally or by injection) of diphenhydramine about 20 to 30 minutes prior to vaccination is a reasonable consideration for any patient that has experienced acute angioedema (swollen face, ears) following vaccination.

Some veterinarians will administer both corticosteroid and an antihistamine. But, there are no studies documenting the fact that either drug, or a combination of drugs, will benefit the patient. Pre-treating prior to vaccination is not considered harmful or a factor in diminishing the immune response to a vaccine. It is done as precaution, but there's really no way to determine effectiveness.

27. **What tests do you think are best when checking for titers? Brands?**

Testing for antibody "titers" is actually a laboratory testing platform that is not likely to be performed inside the practice. Qualitative and Semi-quantitative (VacciCheck) test kits, however are available for use in practice and offer the clinician the opportunity to assess an individual patient's response to prior vaccination…or, to assess the lack of response.

The other factor, of course, is that all test kits that are licensed in the US, are NOT available in all countries. The answer to your question really depends on where you are practicing and product availability.

Among the point-of-care antibody testing platforms available to veterinarians today, the VacciCheck Antibody Test Kit is the most advance technology available, is rapid (~25 minutes), easy to perform, results are consistently reliable, and this testing platform is very well suited to testing either individual patients or efficiently "batch-testing" multiple patients at the same time.