In-Clinic Canine IgG Antibody Titer Test Comparative Study: Results from Five Clinics

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ABSTRACT

This study was carried out in order to study the performance of three in-clinic test kits for the detection of protective plasma IgG antibody titers against canine Adenovirus (CAV), canine Parvovirus type 2 CPV-2) and canine Distemper Virus (CDV). Plasma samples from 46 adult vaccinated dogs were tested for the presence of protective IgG antibodies using three commercial in-clinic test kits; Canine VacciCheck (Biogal, Israel), RapidStatus Titer Test (Biotech Laboratories USA) and FASTest CDV-CPV Ab (Megacor, Austria). The accuracy of the three in-clinic test kits was evaluated against two respective gold standards tests, serum Virus Neutralization (for CAV and CDV) and Hemagglutination Inhibition (for CPV) assays, performed at the Animal Health Diagnostic Center in Cornell University, New York. The gold standard tests determined that all 46 dogs had protective serum IgG antibodies against CPV-2, 45 against CDV and 44 against CAV. Sensitivity calculations revealed that both VacciCheck and FASTest demonstrated equally good results while RapidStatus generated less accurate results when compared to the gold standards. CPV-2 sensitivity evaluations of VacciCheck, FASTest and RapidSTATUS were 98%, 98% and 78% respectively. The CDV sensitivities of VacciCheck, FASTest and RapidSTATUS were 100%, 100% and 60% respectively and the CAV sensitivities of VacciCheck and RapidSTATUS were 98% and 57% respectively. The FASTest does not test for CAV.

Keywords: In-Clinic Titer Testing; Accuracy; Canine Core Vaccination.

INTRODUCTION

According to the World Small Animal Veterinary Association (WSAVA) and the American Animal Hospital Association (AAHA) Vaccination Guidelines, canine core vaccines are highly effective in protecting the majority of dogs against infection by the canine distemper virus (CDV), canine adenovirus (CAV) and canine parvovirus type 2 (CPV-2) (1, 2, 3). The guidelines recommend that adult dogs be revaccinated with these core vaccines no more than every 3 years. Although this interval is recommended, many studies have shown that the modified attenuated virus vaccines confer protection for a period considerably longer than 3 years. The presence of plasma IgG antibody against CDV, CAV and CPV and the associated conferred protection against infection have been confirmed by numerous studies (4, 5, 6, 7, 8). These studies concluded that, when properly administrated, core vaccine protocols lead to a long-lived persistence of vaccine-induced protective antibodies and consequently, immune protection for more than 3 years after the last administration.

Positive titer serological tests can generally be attributed to an undetected subclinical exposure, maternally derived antibodies (MDA), or vaccination generated antibodies.

Table 1. Veterinary clinics

Clinic ID	Investigator	Clinic name
A	Karin Salomon, DVM	Dierenkliniek Lunetten, Netherlands
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Core vaccine IgG antibodies circulating in the blood are commonly quantified in reference laboratories while semi-quantitative evaluations can be performed with a few commercial in-clinic assay kits (9). Quantitative IgG assays can be useful in determining the presence of MDAs, providing information that may be useful to determine the best time to begin core vaccination. Lack of seropositivity 4 weeks after completion of the puppy series at approximately 16 weeks of age, usually indicates the absence of protective immunity and justifies revaccination (10, 11, 12). Failure to mount an immune response following additional vaccination is a strong indicator that the puppy may be incapable of developing protective immunity. Infection following immunization can be associated with vaccine factors resulting in inadequate antigenic stimulation or host factors, owing to an inadequate immune response to an adequate antigen challenge. Vaccine factors may arise from fault in manufacturing, failure of the end user to adhere to expiry of the shelf-life, inadequate cold chain maintenance during transport and storage or incorrect vaccination technique. Host factors include, in addition to interference by MDAs, genetic mutations leading to immune deficiency syndromes (10). Antibody tests can be used to demonstrate the duration of immunity (DOI) following vaccination with core vaccines and spare the dogs unnecessary and possibly detrimental revaccination (12, 13, 14).

There is a positive correlation between CDV, CAV and CPV seropositivity and immune protection against infection with these diseases. Both guidelines mentioned previously advocate the use of serological testing in place of automatic yearly revaccination. The WSAVA guidelines specify that the presence of IgG antibody (regardless of the titer) indicates protective immunity and immunological memory (1, 2). Negative test results in fully vaccinated dogs, on the other hand, could indicate that the titers are below the limit of detection for the test or that there is a complete lack of protective antibodies in the sample tested. In rare cases, dogs that have been tested to have a low titer or be seronegative may be in fact immune and are still capable of generating a significant anamnestic response to vaccination (14). Such animals are not easily identified, therefore the guidelines recommend that an animal with a negative result, regardless of the test used, should be considered as having no protective antibody and potentially susceptible to infection.

The gold standard serological tests used to determine IgG antibody titers generated from core vaccine antigens were the Virus Neutralization (VN) test for CDV and CAV and the Haemagglutination Inhibition (HI) test for CPV (15, 16, 17). These tests can be performed only in specialized diagnostic laboratories. Accurate and affordable in-clinic test kits are marketed in several different configurations ranging from lateral flow to ELISA and Solid Phase Dot Blot. Some of these point-of-care (POC) kits have been validated against the gold standard tests and are defined as semi-quantitative since they generate results in numerical values which correlate positively with the gold standard tests (9). These test kits can rapidly determine the presence of serum IgG antibody against canine core vaccine antigens within 10-23 minutes, providing valuable in-clinic information.

The aim of the present study was to evaluate and compare the performance of 3 different titer kits in an in-clinic setting and to compare the results with the gold standard tests performed at the Animal Health Diagnostic Center in Cornell University, New York. Five different veterinary clinics tested three different POC titer test kits using samples collected from vaccinated adult client-owned dogs received in their clinics.

MATERIALS AND METHODS

The study took place at five veterinary clinics, four in the Netherlands and one in Belgium (Table 1). The five clinics received instructions regarding how to collect the samples

and perform the individual tests. Each clinic worked independently and did not have contact with each other during the trial. The samples consisted of plasma separated from blood collected in EDTA from healthy adult dogs with known vaccination histories (Table 2). The median age of the dogs was 6 years (range 1-13 years) and the median time which elapsed between administration of the last vaccination and sample collection was 3.7 years (range 0.3-10.2 years). In total, 46 plasma samples were collected. Each clinic tested between 7-10 samples with the three in-clinic test kits, Canine VacciCheck® (Biogal, Israel), RapidStatus Titer Test (Biotech laboratories USA LLC) and FASTest CDV-CPV Ab (Megacor, Austria). The 3 kits were used sequentially in accordance with the manufacturers' instructions.

An aliquot (0.5 ml) of the same plasma samples was labeled with the clinic code (A-E) and sample number 1-10. The samples were kept frozen prior to processing. The plasma samples were transported frozen to the AHDC at Cornell University for gold standard titer testing. The identity of the clinics performing the in-clinic tests and their results were masked from the reference laboratory and were revealed only at the conclusion of the trial.

Gold Standards.

The Gold Standard tests were performed at the Animal Health Diagnostic Center at Cornell University, New York according to standard protocols. Antibody titers were determined using Virus Antibody Neutralization Tests (VN) for CAV and CDV and Haemagglutinatin Inhibition (HI) for CPV-2 (15, 16). The antibody titers for the HI test were reported as the reciprocal of the highest dilution of plasma that prevents agglutination of red blood cells by parvovirus (endpoint dilution) while titers for the SN tests were registered as the reciprocal of the highest dilution of plasma that neutralizes the infectivity of the target virus. Cut off values for positive and negative titers were previously defined by the virology laboratory at AHDC. Dilution values of less than 1:10 (<10) were considered negative for CPV (HI) while the cut off value of less than 1:8 (<8) were considered negative for both CAV and CDV (VN). Results for the lowest final dilutions (1:8 and 1:10) were rated as positive or negative based on whether virus neutralization or haemagglutination inhibition was observed. In these cases, the lowest dilutions could be classified as either positive or negative in the final report.

Point of Care In-Clinic Test Kits

The three kits used in the study consisted of Canine VacciCheck® (Biogal, Israel), RapidStatus Titer Test (Biotech laboratories USA LLC) and FASTest CDV-CPV Ab (Megacor, Austria). RapidStatus and FASTest were both based on lateral flow technology while VacciCheck has been described as a solid phase dot blot ELISA. All three kits contained all of the components necessary to perform the tests. The RapidStatus platform was composed of three separate reaction strips and sample wells (one for each disease) connected into a single cassette, while FASTest had a similar cassette structure but with two reaction areas – one for CPV-2 and one for CDV. In both of these test kits the same sample volume was applied to each individual well according to instructions. The VacciCheck platform was composed of 12 connected but individual reaction forms (teeth). Each tooth contained all three tests, therefore only one sample volume was used for each test.

Tests were performed according to the manufacturers' instructions using the same plasma samples. Results were recorded according to visual reading and compared to the positive and negative results generated by the gold standard tests.

Statistical Methods

Sensitivity values were calculated using MedCalc statistical software for Windows version 19.4 (MedCalc Software, Ostend, Belgium) with a confidence interval (CI) of 95%. The positive and negative results of each test kit were rated against the gold standard tests performed at AHDC. The study group did not contain naïve-unvaccinated subjects therefore the specificity of the test kits could not be evaluated.

RESULTS

Five veterinary clinics (Table 1) collected plasma from vaccinated adult dogs and tested the samples employing 3 commercial POC rapid test kits. In addition the samples (N=46) were tested at AHDC using the gold standard VN and HI tests.

Gold Standard

According to the gold standard tests performed at the AHDC, 44 samples had detectable titers for CAV-1, 45 for

Table 2. Results generated at the Animal Hospital Diagnostic Clinic, Cornell. The titer values were tabulated for each gold standard test along with the clinic sample code, age of the animal tested, the brand of the last vaccine administrated and the lapse of time in years since the last vaccination.

Sample code	Age (yrs)	Vaccine manufacturer of last Vaccination	Lapse of time since the last vaccination (yrs)	CAV SN (Titer)	CPV HI (Titer)	CDV SN (Titer)
A1	2.5	Nobivac DHP	2.6	128	2560	4096
A2	6	Nobivac DHP	4.8	2048	1280	512
A3	7	Nobivac DHPPi	8.1	512	2560	512
A4	3.5	Nobivac DHP	3.7	4096	2560	32
A5	2	Nobivac DP	2.2	128	5120	1024
A6	5	Eurican DHPPi	5.1	32	320	32
A7	9	Nobivac DHP	2.1	512	640	128
A8	3.5	Nobivac DHP	3.7	2048	5120	16
A9	6	Nobivac DHP	4.9	64	1280	64
A10	8	Nobivac DHP	4.9	256	1280	32
B1	2.5	Nobivac DHP+L4	1.3	128	640	256
B2	8	Nobivac DHP+L4	3.7	64	640	128
B3	3	Nobivac DHP+L4	2.6	64	2560	256
B4	3	Nobivac DHP+L4	1.7	32	2560	256
B5	6	Nobivac DHP+L4	2.1	256	2560	32
B6	10	Nobivac DHP+L4	3.4	32	80	16
B7	6	Nobivac DHP+L4	0.7	1024	1280	256
B8	9	Nobivac DHP+L4	2.6	64	1280	128
B9	7.5	Nobivac DHP+L4	7.8	64	2560	128
C1	9	Nobivac DHPPi	7.9	8	40	32
C2	9	Nobivac DHPPi	8.0	128	640	32
C3	4	Vanguard 7	4.3	32	80	256
C4	7	Veriscan DHPPi	4.2	32	640	32
C5	4	Nobivac DHP	4.1	128	1280	128
C6	1	Veriscan DHPPi+Nobivac P	1.1	1024	1280	128
C7	1	Veriscan DHPPi	1.0	32	640	64
D1	12	Nobivac DHP	10.2	32	1280	Neg (16)
D2	12.5	Nobivac DHP	6.0	32	80	16
D3	3	Nobivac DHP	3.2	256	640	256
D4	12	Nobivac DHP	9.7	32	640	64
D5	5.5	Nobivac DHP	5.1	2048	640	256
D6	13	Nobivac DHP	1.4	Neg (8)	160	16
D7	10	Nobivac DHP	5.6	256	640	256
D8	7.5	Nobivac DHP	5.6	64	320	64
D9	3	Nobivac DHP	2.1	1024	1280	1024
D10	3.3	Nobivac DHP+PC	3.2	Neg (8)	2560	4096
E1	5	Nobivac DHP	4.5	256	2560	2048
E2	1	Versican DHP	0.3	64	1280	128
E3	9	Nobivac DHP	5.6	512	2560	64
E4	11	Nobivac DHP	5.2	128	640	128
E5	7	Nobivac DHP	5.2	256	2560	128
E6	3	Nobivac DHP	3.0	256	2560	256
E7	6	Nobivac DHPPi	2.3	256	640	16
E8	10	Nobivac DHP	3.8	1024	5120	256
E9	4	Nobivac DHP	0.8	512	1280	512
E10	1	Virbac CAN DHPPi/L	0.9	64	1280	128

Table 3. Positive and negative results of all three point-of-care kits and the gold standard. Discrepancies between the gold standard results and those of the POC kits are accented in grey.

Sample ID	VacciCheck			RapidStatus			FASTest		Gold Standard		
	CAV	CPV	CDV	CAV	CPV	CDV	CPV	CDV	CAV	CPV	CDV
A1	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Pos
A2	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
A3	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Pos
A4	Pos	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos
A5	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
A6	Pos	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos
A7	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Pos
A8	Pos	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos
A9	Pos	Pos	Pos	Neg	Pos	Neg	Pos	Pos	Pos	Pos	Pos
A10	Pos	Pos	Pos	Neg	Pos	Neg	Pos	Pos	Pos	Pos	Pos
B1	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Pos
B2	Pos	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Pos	Pos	Pos
B3	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos
B4	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
B5	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Pos
B6	Pos	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Pos	Pos	Pos
B7	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos
B8	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Pos
B9	Pos	Pos	Pos	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos
C1	Neg	Neg	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Pos	Pos
C2	Pos	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos
C3	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos
C4	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
C5	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
C6	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
C7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
D1	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Neg
D2	Pos	Pos	Pos	Neg	Pos	Neg	Pos	Pos	Pos	Pos	Pos
D3	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Pos
D4	Pos	Pos	Pos	Neg	Pos	Neg	Pos	Pos	Pos	Pos	Pos
D5	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
D6	Pos	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Neg	Pos	Pos
D7	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos
D8	Pos	Pos	Pos	Pos	Neg	Neg	Pos	Pos	Pos	Pos	Pos
D9	Pos	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos
D10	Pos	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Neg	Pos	Pos
E1	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
E2	Pos	Pos	Pos	Neg	Pos	Neg	Pos	Pos	Pos	Pos	Pos
E3	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Pos
E4	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
E5	Pos	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos
E6	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Pos
E7	Pos	Pos	Pos	Neg	Pos	Neg	Pos	Pos	Pos	Pos	Pos
E8	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
E9	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Pos
E10	Pos	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos

Table 4a. Canine Distemper Virus (N=46)

	VacciCheck	FASTest	RapidStatus
True Positive	45	45	26
True Negative	0	0	0
False Positive	1`	1	1
False Negative	0	0	19
Sensitivity (CI) %	**100.0 (CI 92.1-100)**	**100.0 (CI 92.1-100)**	**58.0 (CI 42.2-72.3)**

Table 4b. Canine Parvovirus (N=46)

	VacciCheck	FASTest	RapidStatus
True Positive	45	45	36
True Negative	0	0	0
False Positive	0	0	0
False Negative	1	1	10
Sensitivity (CI) %	**97.8 (CI 88.5-99.9)**	**97.8 (CI 88.5-99.9)**	**78.3 (CI 63.6-89.1)**

Table 4c. Canine Adenovirus (N=46)

	VacciCheck	RapidStatus
True Positive	43	24
True Negative	0	1
False Positive	2	1
False Negative	1	20
Sensitivity (CI) %	**97.7 (CI 88.0-99.7)**	**54.6 (CI 38.9-69.6)**

CDV and 46 for CPV-2. Two samples (D6 and D10) registered negative titers for CAV but were positive for both CDV (titers 1:16 and 1:4096 respectively) and CPV-2 (titers 1:160 and 1:2560 respectively). Sample D1 did not return a positive titer for CDV but had a positive titer for CAV (Titer 1:32) and CPV-2 (Titer 1:1280). All three these samples came from the same clinic. Table 2 contains the titer results of all the tests performed at AHDC according to clinic. Included in the table are the age of the dog, vaccine manufacturer and time that elapsed since the last vaccination.

Point of Care Test Kits

Three commercial POC titer-testing kits were compared with the gold standard tests performed at the AHDA. The results were translated to a positive or negative response based on predefined parameters specified by the manufacturers or the diagnostic laboratory. The results generated by all three commercial POC kits are shown in Table 3.

Statistical Analysis

The sensitivity of each individual POC kit was calculated using the reference laboratory gold standard tests, Serum Neutralization and Haemaglutination Inhibition as reference tests. Sensitivity values were calculated with a CI of 95%. Tables 4a, 4b and 4c contain the summary of each evaluation.

DISCUSSION

Using challenge experiments, Jensen *et al.* (17) and Abedelmagid *et al.* (18) demonstrated that the presence of serum antibodies to CAV, CDV and CPV-2 as a positive predictor of protection in dogs that have been vaccinated for, or previously infected with one of these diseases. With this in mind, it is reasonable to assume that measurement of these antibodies can be used to assess the individual immune status of dogs for CAV, CDV and CPV-2 (18, 19).

Sensitivity values were calculated for all three in-clinic POC kits using the reference gold standard laboratory tests regularly performed at the Cornell Animal Health Diagnostic Center. Despite their different technologies, VacciCheck and FASTest demonstrated equal and high sensitivities for the CPV (97.8%) and CDV (100%) tests compared to Serum Neutralization and Haemagglutination Inhibition tests. Three false positives were registered with VacciCheck (2 for CAV and one for CDV). According to RapidStatus, one of the CAV samples (D10) was also a "false positive" while

the second (D6) was negative (same as the gold standard). Further examination of the entire set of results for this sample revealed that, while RapidStatus did identify the CAV titer in D6, it missed the correct response for both CPV and CDV, which the other two kits identified correctly. Sample D1 registered a positive titer for CDV with all POC kits but was negative with Serum Neutralization. The three discordant results between SN and the POC kits were not verified with repeated testing and therefore it is impossible to determine which result is correct. A single CPV-2 sample (C1) registered negative for all three tests kits but was positive by HI at a low titer of 1:40 (1:10-1:80 fall into the low titer range). This was the lowest and only CPV-2 titer generated from all of the samples and may indicate the limits-of-detection of all three of POC test kits.

VacciCheck correctly identified 43 (96.6%) out of the 45 gold standard positive SN CAV samples while RapidStatus identified only 26 (56.5%). Adenovirus antibody test is not available with the FASTest kit. Infectious hepatitis caused by canine adenovirus type 1 (CAV-1) and infectious respiratory disease complex of which type 2 adenovirus (CAV-2) is associated with, have largely disappeared after the introduction of CAV-1 vaccines. Despite the fact that many CAV-2 infections are subclinical and usually do not require treatment, lack of vaccination and co-infections are the main risk factors for infection and progression to clinical disease. The causative agents are still prevalent in developing countries where only a small percentage of dogs are vaccinated and in feral carnivore populations worldwide (19, 20, 21). According to the WSAVA guidelines, vaccination to protect against Adenovirus infections must be continued in order to prevent outbreaks of this disease (1). Although population density present in many shelters and kennel environments are thought to increase the risk of transmission, Schultz *et al.* (22) suggested that, privately owned dogs should be considered to be potential reservoirs of susceptibility and subclinical carriers of Adenoviurs.

When examining the combined results for the presence of serum antibodies to CPV2, CDV, and CAV, it was evident that the performance of the RapidStatus test was relatively low compared to VacciCheck and FASTest. Of the 46 tested samples, only 27 samples were reactive for CDV, 36 for CPV and 25 for CAV with the RapidStaus test. When these results were compared to the gold standards, the sensitivities of the RapidStatus test were calculate to be 58%, 78.3% and 54.6% for CDV, CPV and CAV respectively.

The administration of core vaccines aims to generate significant levels of serum antibodies for all three diseases (CPV2, CAV 1 and 2, CDV) resulting in long-term protection against infectious diseases of global importance. The RapidStatus test yielded positive results for only a subset of the samples tested (Table 3). Only 12 samples showed positive reaction for all 3 antigens, and 22 samples were positive for both CDV and CPV alone. As a result, 25 cases would be interpreted as necessitating revaccination due to a negative CDV, CPV, or both. In the case of false negative CAV tests, the clinician would have to decide whether the result would warrant revaccination considering that in some circles immunity to CAV is less critical.

In reference to Duration of Immunity, 20 out of the 46 animals tested had not received the recommended 3 year booster (lag times ranging between >3-9 years) but still tested positive for protective antibody titers with both FASTest and VacciCheck. Seventeen of these dogs which had not received the recommended 3-year booster were negative for CDV or CPV only on the RapidStatus.

Antibody testing is used for verifying response after initial core vaccination, evaluating the need for revaccination, and assessing risk levels during outbreak management. The major limitations of titer testing include performance and interpretation challenges. The VacciCheck and FASTest in-clinic test kits provided accurate indication of presence of protective titers when compared with the gold standard tests. Test accuracy is imperative to enable the clinician to make proper decisions relating to canine core revaccination. In comparison, the RapidStatus kit was found to be less reliable in identifying true positive titers which could result in unnecessary revaccination.

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