

# IN-CLINIC TITER TESTS comparative study Results from 5 Clinics



## ABSTRACT

**OBJECTIVES:** To analyze the performance of three in-clinic test kits for the detection of protective serum antibody titers against:



**Canine  
Adenovirus  
(CAV)**



**Parvovirus  
(CPV)**



**Distemper  
Virus (CDV)**

**METHOD:** Serum samples from **46 vaccinated dogs**, were tested for the presence of protective antibodies using three commercial in-clinic test kits:

**VACCICheck<sup>®</sup>**  
by **Biogal** Galed Labs.

(Biogal, Israel)

**RapidStatus Titer Test**

(Biotech Laboratories USA)

**FASTest CDV-CPV Ab**

(Megacor, Austria)

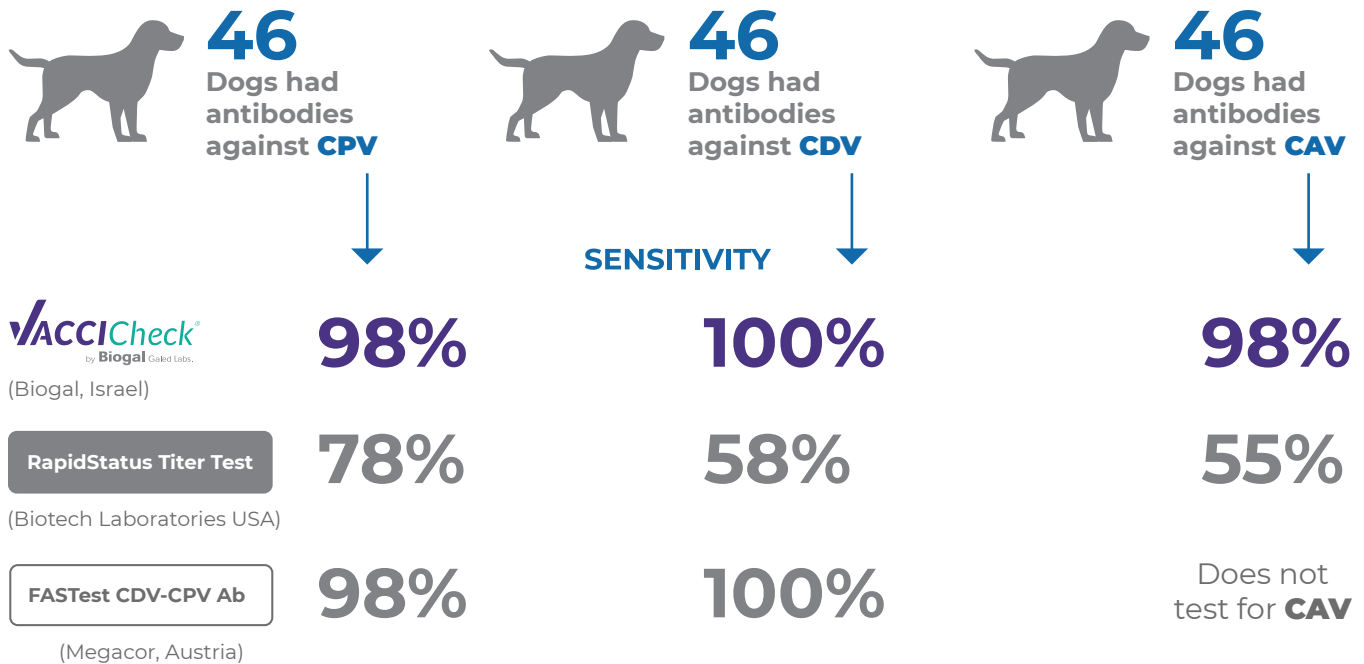
**46  
Vaccinated  
Dogs**



The accuracy of the **three in-clinic test kits** was evaluated against the Gold Standards

Serum Neutralization (CAV and CDV) and Hemagglutination Inhibition (CPV) performed at the Animal Health Diagnostic Center in Cornell University, New York.

**RESULTS:** The Gold Standard tests determined that:



## INTRODUCTION

According to the World Small Animal Veterinary Association (WSAVA) and the American Animal Hospital Association ((AAHA) Vaccination Guidelines<sup>1,2</sup>, 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 (CPV2). Both guidelines recommend that adult dogs be revaccinated with these core vaccines no more than every 3 years. Although this interval is recommended, it is evident by many studies that the modified attenuated virus vaccines confer protection for a period considerably longer than 3 years. The presence of serum antibody against CDV, CAV and CPV and the associated conferred protection against infection have been confirmed by numerous studies<sup>3,4,5,6</sup>. These studies concluded that the

vaccination protocol led to a long-lived persistence of vaccine-induced antibodies and, consequently, immune protection for up to 15 years after the last administration. The correlation between seropositivity for CDV, CAV and CPV and immune protection is substantial and this fact is reflected in both guidelines that supports the use of serological testing in place of conditioned revaccination<sup>1,2</sup>. The WSAVA guidelines specify that the presence of antibody (regardless of the titer) indicates protective immunity and immunological memory. According to Day et al, “giving more frequent vaccines to animals in an attempt to increase antibody titers is a pointless exercise”. When antibody is absent, (irrespective of the serological test used) the dog should be revaccinated unless there is a medical contraindications for not doing so.

The Gold Standard serological tests used to determine antibody titers generated from core vaccine antigens are the Virus Neutralization (VN) test for CDV and CAV and the Haemagglutination Inhibition (HI) test for CPV. 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. These test kits can rapidly, determine the presence of serum antibody against canine core vaccine antigens providing valuable information within 20-25 minutes.

The aim of the present study was to evaluate 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 3 different POC titer test kits using samples collected from vaccinated clients received in their clinics.

## STUDY DESIGN AND METHOD

The study took place at 5 veterinary clinics, 4 in the Netherlands and one in Belgium (Table 1). Each clinic collected fresh serum from healthy adult dogs with known vaccination histories. In total 46 samples were collected. Each clinic tested 7-10 fresh 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 manufacturer's instructions. An aliquot of the same (0.5 ml) serum samples was labeled with the clinic code (A-E) and sample number 1-10. The samples were kept frozen prior to processing. An aliquot of each serum was sent to the AHDC at Cornell University for Gold Standard titer tests. Sensitivity values were calculated by comparing the positive/negative results against the Gold Standard tests performed at AHDC. This study is limited to sensitivity evaluation only. The specificity of the different kits could not be evaluated due to the absence of non-vaccinated "Naïve" dogs in this study.

**Table 1: Veterinary clinics**

Clinic code	Investigator	Clinic name
A	Dr. Karin Salomon, DVM	Dierenkliniek Lunetten, Netherlands
B	Dr. Thamar de Lange, DVM	A7 Noord Drachten, Netherlands
C	Dr. Annemieke Calis, DVM	Annemieke Calis, Netherlands
D	Dr. Odette Radier, DVM	Dap Den Bommel, Netherlands
E	Dr. Jolanda Krosse, DVM	Jolanda Krosse, Belgium

## RESULTS



The 46 serum samples collected from dogs in 5 clinics (Table 1) were tested using the 3 commercial POC rapid test kits. Protective antibodies against CPV2 were detected in all of the samples. Only forty-five (45) had protective antibodies against CDV and 44 against CAV. The 2 dogs that were seronegative for CAV and the one dog seronegative for CDV had been last vaccinated between 3 to 9 years previously.

In comparison to the Gold Standard CPV2 HI test performed at the AHDC, the sensitivities of VacciCheck, FASTest and RapidSTATUS were 98, 98 and 78 percent respectively. The calculated sensitivities for the same three POC tests compared to the Gold Standard CDV SN test were 100%, 100% and 60% respectively. The FASTest does not test for CAV. Results obtained from the VacciCheck and RapidSTATUS tests were compared to the Gold Standard CAV SN test generating sensitivities of 98 and 57 percent respectively. Results are summarized in Tables 2a, 2b and 2c.

### Table 2a, b and c: Sensitivity evaluation of three Point of Care Titer testing kits compared to the laboratory Gold Standard tests, Serum Neutralization and Haemagglutination Inhibition.

(\*Individual sample results can be acquired upon request from Biogal.)

Table 2a

		 <b>CANINE DISTEMPER VIRUS</b>		
	 (Biogal, Israel)	FASTest CDV-CPV Ab (Megacor, Austria)	RapidStatus Titer Test (Biotech Laboratories USA)	
True Positive	<b>45</b>	<b>45</b>	<b>27</b>	
True Negative	<b>0</b>	<b>0</b>	<b>1</b>	
False Positive	<b>0</b>	<b>0</b>	<b>0</b>	
False Negative	<b>1</b>	<b>1</b>	<b>18</b>	
<b>Sensitivity</b>	<b>100%</b>	<b>100%</b>	<b>60%</b>	

**Table 2b**

**CANINE  
PARVOVIRUS**
  
 (Biogal, Israel)

**FASTest CDV-CPV Ab**  
 (Megacor, Austria)

**RapidStatus Titer Test**  
 (Biotech Laboratories USA)

True Positive	<b>45</b>	<b>45</b>	<b>36</b>
True Negative	<b>0</b>	<b>0</b>	<b>0</b>
False Positive	<b>0</b>	<b>0</b>	<b>0</b>
False Negative	<b>1</b>	<b>1</b>	<b>10</b>
<b>Sensitivity</b>	<b>97.8%</b>	<b>97.8%</b>	<b>78.3%</b>

**Table 2c**

**CANINE  
PARVOVIRUS**
  
 (Biogal, Israel)

**FASTest CDV-CPV Ab**  
 (Megacor, Austria)

**RapidStatus Titer Test**  
 (Biotech Laboratories USA)

True Positive	<b>43</b>	Does not test for <b>CAV</b>	<b>25</b>
True Negative	<b>0</b>	Does not test for <b>CAV</b>	<b>1</b>
False Positive	<b>2</b>	Does not test for <b>CAV</b>	<b>1</b>
False Negative	<b>1</b>	Does not test for <b>CAV</b>	<b>19</b>
<b>Sensitivity</b>	<b>97.7%</b>	Does not test for <b>CAV</b>	<b>56.8%</b>

## DISCUSSION

### CPV2 and Distemper

All three in-clinic POC kits were validated against the CPV2 and Distemper Virus Gold Standard titer tests regularly performed at the Cornell Animal Health Diagnostic Center. VacciCheck and FasTest showed excellent sensitivity for both CPV2 and CDV. One sample which was collected from a senior canine registered a negative result for CPV2 and Distemper titers with both VacciCheck and FastTest. The Gold standard recorded a low titer dilution of 1:40 which may indicate a limit of detection for these two in-clinic tests. In contrast the RapidStatus test performed less well with 60% sensitivity for CDV (18 false negatives) and 78% sensitivity for CPV2 (10 false positives).

### CAV

VacciCheck correctly identified 43 out of the 46 samples positive for CAV while RapidStatus reacted to only 25 out of the 46 samples. A target test is not available with the FasTest kit. When compared to the Gold Standard, the calculated sensitivity of VacciCheck was 97.7%. The titer test results for CAV using RapidStatus had a significantly lower sensitivity level of 56.8%.

**CLINICAL SIGNIFICANCE:** The VacciCheck and FASTest in-clinic test kits provided an accurate measurement of protective titers, allowing informed decision-making about canine core revaccination. In the present study, the RapidSTATUS test failed to provide

consistent accurate results which could lead to unnecessary revaccination of dogs. Although both VacciCheck and FASTest test results had excellent positive correlation with the Gold Standard test unfortunately Fastest does not covers Adenovirus giving VacciCheck an advantage for core vaccine titer testing.

Technically all the POC kits were easy to use. VacciCheck involved more hands-on management with a slightly longer time to results than the two lateral flow kits. Considering the fact that the test is semi-quantitative with high accuracy, the addition time spent is repaid in terms of the final results obtained.

VacciCheck and Fastest both produced visually clear results which could be easily interpreted. The dark purple spots produced by VacciCheck are permanent long-term while the lines which appeared next to the control and test areas with the Fastest faded overtime. The visual appearance of the test lines of RapidTest were in some cases very light resulting in difficulty in interpretation of the result as positive, negative or background noise.

This study reconfirmed the Duration of Immunity (DOI) in adult dogs which do not have annual revaccinations for many years following the initial core puppy vaccination. Thirteen (13) dogs who were last vaccinated between 3 - 6 years previously and 7 dogs with a lapse of 7 - 9 years post vaccination. These canines demonstrated positive titers for all three diseases when tested by Fastest and VacciCheck but were negative with the RapidStatus test.

## CONCLUSIONS

1. The **VacciCheck** and **FasTest kits** have excellent agreement between themselves and with the **Gold standard laboratory methods**. Compared to these two kits, the RapidStatus is less reliable in identifying true positive titers. The high rate of false negative reading may lead to unnecessary revaccination.



FASTest CDV-CPV Ab

(Megacor, Austria)

Vs

RapidStatus Titer Test

(Biotech Laboratories USA)

2. There are several advantages of the use of **VacciCheck in-clinic**: (i) the kit includes CAV titer testing which is part of the recommendation by the WSAVA Guidelines. CAV vaccination protects against Canine Infectious Hepatitis, a prevalent disease in many countries; (ii) VacciCheck test spots are stable, while the FasTest results have no diagnostic value after 20 min. This is especially important when there is a need for a second review of the results; (iii) VacciCheck results can be digitally recorded when using the handheld CombCam reader which provides accurate objective readings.



## REFERENCES

1. Day, M.J., Horzinek, M.C., Schultz, R.D., et al. (2016) Guidelines for the vaccination of dogs and cats. *Journal of Small Animal Practice* 57, E1 - E45.
2. AAHA Canine Vaccination Guidelines 2017.  
<https://www.aaaha.org/aaaha-guidelines/vaccination-canine-configuration/vaccination-canine/>
3. Bohm, M., Thompson, H., Weir, A., et al. (2004) Serum antibody titres to canine parvovirus, adenovirus and distemper virus in dogs in the UK which had not been vaccinated for at least three years. *Veterinary Record* 154, 457-463.
4. Ottiger, H.P., Neimeier-Forster, M., Stark, K. D. C., et al. (2006) Serological responses of adult dogs to revaccination against distemper, parvovirus and rabies. *Veterinary Record* 159, 7-12.
5. Schultz, R. D. (2006) Duration of immunity for canine and feline vaccines: a review. *Veterinary Microbiology* 117, 75 – 79.
6. Schultz, R.D., Thiel, B., Mukhtar, E., et al. (2010) Age and long-term protective immunity in dogs and cats. *Journal of Comparative Pathology* 142 (Suppl. 1), S102-S108.
7. AHDC Website  
<https://www.vet.cornell.edu/animal-health-diagnostic-center/testing/protocols-and-interpretations>
8. Waner, T., Mazar, S. & Keren-Kornblatt, E. (2006) 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. *Journal of Veterinary Diagnostic Investigation* 18, 267 – 270.