

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

Performance report

**A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck
Antibody Test Kit**

**MANUFACTURED BY:
BIOGAL, GALED LABS.
KIBBUTZ GALED, 1924000, ISRAEL**

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

Summary:

A field and experimental trial to assess the performance of the ImmunoComb Canine VacciCheck Antibody Test Kit was done in comparison to the gold standard Virus Neutralization (VN) and Hemagglutination Inhibition (HI) assays. A total of 219 dog sera samples were collected at the Wisconsin University during 2007 till 2009. Of them, 199 were tested for CDV, 166 were tested for CAV and 186 were tested for CPV. The overall sensitivity and specificity for CDV compared to VN were 100% and 92% respectively. The overall sensitivity and specificity for CAV compared to VN were 94% and 93% respectively. The overall sensitivity and specificity for CPV compared to HI were 88% and 100% respectively. The overall accuracy was excellent for the three antigens, 97%, 93% and 91% for CDV, CAV and CPV respectively. In conclusion, the VacciCheck Test Kit showed excellent performance compared to the gold standard assays.

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

1. Objective:

To assess the specificity, sensitivity and overall accuracy of the ImmunoComb Canine VacciCheck Antibody Test Kit, in comparison to the gold standards Virus Neutralization (VN) or Hemagglutination Inhibition (HI) assays.

2. Introduction:

Infectious Canine Parvovirus (CPV), Canine Distemper Virus (CDV) and Infectious Canine Hepatitis (ICH) caused by Canine Adenovirus (CAV) are recognized as important causes of illness and death in dogs. Puppies are most susceptible to ICH, CPV and CDV, especially after weaning when protective maternally derived antibody (MDA) levels decrease. Sometimes MDA may actually interfere with vaccinations that are given for immunization. In many countries, vaccination programs have significantly curtailed, but not eliminated the incidence of these diseases. Thus, ICH, CPV and CDV continue to be of great clinical concern among veterinarians worldwide and still present a diagnostic challenge.

Use of serology has been established for use as an aid in the diagnosis of ICH, CPV and CDV in dogs. The gold standards methods are Hemagglutination Inhibition (HI) for CPV and Virus Neutralization (VN) for CAV and CDV. However, these assays are cumbersome and require a specialized laboratory.

The ImmunoComb Canine VacciCheck Antibody Test Kit (VacciCheck Test Kit) is designed to determine dog blood or serum IgG antibody titers to CDV, CAV and CPV. The VacciCheck Test Kit is a self-contained portable kit, sufficient for 12 individual tests, that enables the user to test dog blood or serum and have a result within 21 minutes. The main purpose of this kit is to provide a useful tool for assessing immunity status of dogs concerning these three pathogens. As such, it can either determine the IgG titer before and following vaccination or the state of immunity.

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

3. Glossary:

3.1. Abbreviations:

A Comb: An ImmunoComb Canine card.

VacciCheck Test Kit: ImmunoComb Canine VacciCheck Antibody Test Kit (US registered label: ImmunoComb Canine Distemper-Adenovirus type 2: Parvovirus Antibody Test Kit).

Wisconsin laboratory: Professor Schultz's lab at the Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin- Madison, USA. Principal contact investigator: Laurie J. Larson DVM.

3.2. Acronyms:

CAV: Canine Adenovirus

CDV: Canine Distemper

CPV: Canine Parvovirus

HI: Hemagglutination Inhibition assay

VN: Virus Neutralization assay

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

4. Materials and Methods:

4.1. The ImmunoComb Canine VacciCheck Antibody Test Kit:

Each kit contains a Comb, with attached antigens and control spots, and a developing plate, sufficient to perform triple test for 12 samples. The test is performed by adding five μ l of serum sample or 10 μ l of whole blood to the sample well (Row A). The Comb (12 teeth for 12 samples) or a single tooth are dipped in the series of wells for specific length of time: 5 min in the sample well (Row A), 2 min in the wash well (Row B), 5 min in the conjugate well (Row C), 2min in each sequential wash wells (Rows D-E), 5 min in the chromogen well (Row F) and then re-dipped in the last wash well (Row E) for 2 min fixation. The Combs are then allowed to dry before being read by comparison with a color scale supplied with each kit (CombScale). The CombScale enables to match the color tone of the test spot to a scale of 1-6. The positive reference spot on the Comb should be aligned with a reading scale of 3. The test spots are then read according to the aligned scale. Any reading of the test spot with score 3 or higher should be considered positive. Any reading with result lower than 3 should be considered negative.

4.2. Virus Neutralization (VN):

Done at Wisconsin laboratory, determine the antibody titer as the reciprocal of the highest dilution of the serum that neutralizes the infectivity of the virus. Dilutions of the serum are performed from 1:2 to 1:4096 for CDV and for CAV VN tests.

For CDV: any result equal or higher than 1:32 is considered positive and any result lower than 1:32 is considered negative.

For CAV: any result equal or higher than 1:16 is considered positive and any result lower than 1:16 is considered negative.

4.3. Hemagglutination Inhibition (HI) assay:

Done at Wisconsin laboratory, determine the antibody titer as the reciprocal of the highest dilution of the serum that prevents the agglutination of red blood cells by the virus. Dilutions are performed from 1:20 to 1:20,480 for CPV.

For CPV: Any result equal or higher than 1:80 is considered positive and any result lower than 1:80 is considered negative.

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

5. Specimens:

A total of 219 dog sera samples were collected at Wisconsin University from 2007 till 2009. All sera were kept at -80°C until testing was done.

2007 collection:

- 85 "field" sera from dogs with unknown vaccination status (Various vaccination protocols, bled after 14-21 days post vaccination) were tested for the 3 pathogens.
- 11 sera with negative status ("experimental") were tested for the 3 pathogens.
- 53 sera of challenged* dogs ("experimental"), 33 challenged to CDV and 20 challenged to CPV were tested only for the respective pathogen.

*Challenged post vaccinated.

2008 collection:

40 sera derived from 4 experimental pups that were vaccinated and bled at 10 intervals were tested for the 3 pathogens.

2009 collection:

30 SPF negative sera (experimental dogs that had been raised in a barrier facility) were tested for the 3 pathogens.

Of the 219 dog sera, 199 were tested for CDV, 166 were tested for CAV and 186 were tested for CPV. See appendix for samples lists by antigen and by year.

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

Data Analysis:

The sensitivity, specificity and accuracy for each virus (CDV, CAV and CPV) were analyzed separately. Sensitivity, specificity and accuracy were determined by standard calculation and quoted to the nearest integer according to the formulas in table 1:

Table 1: Sensitivity and specificity calculation formulas

		VN/Hi result		Total
		Positive	Negative	
VacciCheck Test Kit result	Positive	a	b	a+b
	Negative	c	d	c+d
Total		a+c	b+d	a+b+c+d
		% Sensitivity = $100 \times a / (a+c)$	% Specificity = $100 \times d / (b+d)$	% Accuracy = $100 \times (a+d) / (a+b+c+d)$

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

6. Results:

Note: Due to the limited number of the unknown "field" collected specimens with negative status; the analysis of the field specimens includes only sensitivity. The specificity for each antigen was analyzed on the entire specimens (both field collected and experimental animals) with the respective antigen designation. See separate and combined data tables below.

7.1. VacciCheck Test Kit sensitivity, specificity and accuracy to CDV:

The results of the VacciCheck Test Kit for CDV in comparison to the gold standard CDV VN are summarized in Table 2-4.

Table 2: The results of the VacciCheck Test Kit for CDV in comparison to the gold standard CDV VN: **Field samples, n=74.**

CDV		VN result
		Positive
VacciCheck Test Kit result	Positive	74
	Negative	0
	Total	74
		Sensitivity = 100 %

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

Table 3: The results of the VacciCheck Test Kit for CDV in comparison to the gold standard CDV VN: **Experimental samples, n=114.**

CDV		VN result		Total
		Positive	Negative	
VacciCheck Test Kit result	Positive	60	1	61
	Negative	0	53	53
	Total	60	54	114
		Sensitivity = 100%	Specificity = 98%	Accuracy = 99%

Table 4: The results of the VacciCheck Test Kit for CDV in comparison to the gold standard CDV VN: **Complete data, n=199.**

CDV		VN result		Total
		Positive	Negative	
VacciCheck Test Kit result	Positive	134	5	139
	Negative	0	60	60
	Total	134	65	199
		Sensitivity = 100%	Specificity = 92%	Accuracy = 97%

* 11 samples collected from unknown field with negative VN result were included in the combined analysis but not in the separate field analysis.

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

7.2. VacciCheck Test Kit sensitivity, specificity and accuracy to CAV:

The results of the VacciCheck Test Kit for CAV in comparison to the gold standard CAV VN are summarized in Tables 5-7.

Table 5: The results of the VacciCheck Test Kit for CAV in comparison to the gold standard CAV VN: **Field samples, n=85.**

CAV		VN result
		Positive
VacciCheck Test Kit result	Positive	80
	Negative	5
	Total	85
		Sensitivity = 94 %

Table 6: The results of the VacciCheck Test Kit for CAV in comparison to the gold standard CAV VN: **Experimental samples, n=81.**

CAV		VN result		Total
		Positive	Negative	
VacciCheck Test Kit result	Positive	22	4	26
	Negative	2	53	55
	Total	24	57	81
		Sensitivity = 92%	Specificity = 93%	Accuracy = 93%

Table 7: The results of the VacciCheck Test Kit for CAV in comparison to the gold standard CAV VN: **Complete data, n=166.**

CAV		VN result		Total
		Positive	Negative	
VacciCheck Test Kit result	Positive	102	4	106
	Negative	7	53	60
	Total	109	57	166
		Sensitivity = 94%	Specificity = 93%	Accuracy = 93%

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

7.3. VacciCheck Test Kit sensitivity, specificity and accuracy to CPV:

The results of the VacciCheck Test Kit for CPV in comparison to the gold standard CPV HI are summarized in Tables 8-10.

Table 8: The results of the VacciCheck Test Kit for CPV in comparison to the gold standard CPV HI: **Field samples, n=85.**

CPV		HI result
		Positive
VacciCheck Test Kit result	Positive	69
	Negative	16
	Total	85
		Sensitivity = 81 %

Table 9: The results of the VacciCheck Test Kit for CPV in comparison to the gold standard CPV HI: **Experimental samples, n=101.**

CPV		HI result		Total
		Positive	Negative	
VacciCheck Test Kit result	Positive	47	0	47
	Negative	0	54	54
	Total	47	54	101
		Sensitivity = 100%	Specificity = 100%	Accuracy = 100%

Table 10: The results of the VacciCheck Test Kit for CPV in comparison to the gold standard CPV HI: **Complete data, n=186.**

CPV		HI result		Total
		Positive	Negative	
VacciCheck Test Kit result	Positive	116	0	116
	Negative	16	54	70
	Total	132	54	186
		Sensitivity = 88%	Specificity = 100%	Accuracy = 91%

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

7. Discussion and conclusion:

This report compares the results obtained by the VacciCheck Test Kit to results obtained by VN/HI assays in a trial on dogs sera collected from the field and from experimental animals. The sensitivity of a test determines how good the test is at picking out sera with a disease or is at or above the cut-off value that provide protective antibody titer. The specificity is the ability of the test to identify sera that are negative to the disease or are at below the cut-off value that provide protective antibody titer. In this trial, the overall sensitivity and specificity for CDV compared to VN were 100% and 92% respectively. The overall sensitivity and specificity for CAV compared to VN were 94% and 93% respectively and the overall sensitivity and specificity for CPV compared to HI were 88% and 100% respectively. It should be noted that 8 of the 16 false negative for CPV in the VacciCheck Test Kit showed score 2, slightly fainter than the cut-off score 3. Six of these eight samples gave cut-off HI titer of 80. Due to the high specificity of the VacciCheck Test Kit for CPV and the fact that none of the negative samples (n=54) show score 2 on the VacciCheck Test Kit it is plausible to consider score 2 as inconclusive with high chance of positive HI.

The accuracy of a test determines how accurate the test is in providing a correct result, either negative or positive. The overall accuracy of the VacciCheck Test Kit was excellent for the three antigens, 97%, 93% and 91% for CDV, CAV and CPV respectively.

This evaluation has few minor limitations. First, the number of specimens with negative status collected from the field was limited and could not provide sufficient data for specificity analysis. The reason for that is that dogs from the "field" are actually normal dogs from households, they are routinely vaccinated and the rate of failed vaccination is anticipated to be low. Since this cohort cannot provide negative un-vaccinated dogs, the only source for negative un-vaccinated puppies can be find under experimental conditions. Therefore, the analysis of specificity was done to the entire specimens, both from the field and from the experimental animals. Second, the sera in the study were not randomly tested, yet the technicians that performed the assays were blinded to the results of the other assays. And last, discordant results between VN/HI and the VacciCheck Test Kit, were not verified by a repeated tests. Due to the nature of the VN/HI assays, it is not implausible to obtain a different titer result on repeated test.

VN and HI are considered as the gold-standard to determine the protective antibody titer and are used in reference laboratories. However, these assays are very complicated, take several hours to perform and require a specialized laboratory with high level of biosafety. On the other hand, the VacciCheck Test Kit is very simple to operate, does not require any laboratory tools and take less than half an hour to perform and to obtain results.

This evaluation shows that the VacciCheck Test Kit provides an excellent performance compared to the gold standard assays with a much simpler and accessible method.

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

8. Appendix:

9.1. CDV data

9.1.1 CDV 2007 results

Serum ID	Dog ID	Group	VN titer	VN result	IC score	IC result
1	1	Un-known	128	Positive	6	Positive
2	2	Un-known	8	Negative	2	Negative
3	3	Un-known	256	Positive	5	Positive
4	7	Un-known	64	Positive	4	Positive
5	8	Un-known	64	Positive	4	Positive
6	10	Un-known	64	Positive	5	Positive
7	11	Un-known	8	Negative	1	Negative
8	12	Un-known	128	Positive	4	Positive
9	13	Un-known	256	Positive	5	Positive
10	15	Un-known	128	Positive	4	Positive
11	16	Un-known	128	Positive	4	Positive
12	17	Un-known	64	Positive	5	Positive
13	18	Un-known	8	Negative	2	Negative
14	19	Un-known	128	Positive	4	Positive
15	20	Un-known	1024	Positive	5	Positive
16	22	Un-known	128	Positive	3	Positive
17	23	Un-known	64	Positive	4	Positive
18	24	Un-known	256	Positive	5	Positive
19	25	Un-known	64	Positive	3	Positive
20	26	Un-known	32	Positive	3	Positive
21	27	Un-known	256	Positive	4	Positive
22	28	Un-known	512	Positive	5	Positive
23	29	Un-known	16	Negative	1	Negative
24	30	Un-known	16	Negative	4	Positive
25	32	Un-known	64	Positive	4	Positive
26	33	Un-known	64	Positive	6	Positive
27	35	Un-known	1024	Positive	6	Positive
28	36	Un-known	128	Positive	5	Positive
29	37	Un-known	256	Positive	5	Positive
30	40	Un-known	256	Positive	5	Positive
31	41	Un-known	128	Positive	5	Positive
32	44	Un-known	128	Positive	5	Positive
33	45	Un-known	32	Positive	3	Positive
34	47	Un-known	128	Positive	3	Positive
35	48	Un-known	512	Positive	6	Positive
36	49	Un-known	1024	Positive	6	Positive
37	50	Un-known	32	Positive	4	Positive
38	51	Un-known	256	Positive	6	Positive
39	52	Un-known	64	Positive	4	Positive
40	57	Un-known	128	Positive	4	Positive
41	58	Un-known	256	Positive	4	Positive
42	60	Un-known	64	Positive	5	Positive
43	61	Un-known	256	Positive	5	Positive
44	63	Un-known	8	Negative	3	Positive

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

45	64	Un-known	8	Negative	3	Positive
46	65	Un-known	128	Positive	5	Positive
47	66	Un-known	256	Positive	5	Positive
48	67	Un-known	128	Positive	5	Positive
49	68	Un-known	512	Positive	5	Positive
50	69	Un-known	64	Positive	4	Positive
51	70	Un-known	128	Positive	4	Positive
52	71	Un-known	32	Positive	4	Positive
53	72	Un-known	128	Positive	6	Positive
54	73	Un-known	128	Positive	5	Positive
55	74	Un-known	16	Negative	4	Positive
56	75	Un-known	256	Positive	5	Positive
57	78	Un-known	128	Positive	4	Positive
58	79	Un-known	16	Negative	1	Negative
59	81	Un-known	512	Positive	6	Positive
60	82	Un-known	128	Positive	6	Positive
61	84	Un-known	512	Positive	5	Positive
62	89	Un-known	64	Positive	5	Positive
63	90	Un-known	1024	Positive	5	Positive
64	91	Un-known	128	Positive	5	Positive
65	92	Un-known	256	Positive	5	Positive
66	93	Un-known	256	Positive	5	Positive
67	95	Un-known	128	Positive	5	Positive
68	98	Un-known	128	Positive	6	Positive
69	99	Un-known	32	Positive	4	Positive
70	100	Un-known	128	Positive	4	Positive
71	101	Un-known	512	Positive	6	Positive
72	102	Un-known	1024	Positive	6	Positive
73	103	Un-known	32	Positive	5	Positive
74	104	Un-known	128	Positive	5	Positive
75	105	Un-known	8	Negative	1	Negative
76	106	Un-known	512	Positive	5	Positive
77	107	Un-known	256	Positive	6	Positive
78	108	Un-known	512	Positive	6	Positive
79	109	Un-known	128	Positive	5	Positive
80	110	Un-known	64	Positive	4	Positive
81	111	Un-known	128	Positive	4	Positive
82	112	Un-known	8	Negative	2	Negative
83	113	Un-known	128	Positive	4	Positive
84	114	Un-known	64	Positive	4	Positive
85	115	Un-known	64	Positive	4	Positive
86	H0 K038	Negative	4	Negative	0	Negative
87	H0 L028	Negative	0	Negative	0	Negative
88	H0 L164	Negative	4	Negative	0	Negative
89	H0 M100	Negative	2	Negative	0	Negative
90	H0 K023	Negative	2	Negative	0	Negative
91	H0 K109	Negative	0	Negative	0	Negative
92	H0 L015	Negative	0	Negative	0	Negative
93	H0 K013	Negative	4	Negative	1	Negative
94	H0 L127	Negative	16	Negative	0	Negative
95	H0 L027	Negative	0	Negative	1	Negative

CONFIDENTIAL

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

96	H0 K015	Negative	4	Negative	1	Negative
97	R042	Positive	4096	Positive	6	Positive
98	R041	Positive	512	Positive	6	Positive
99	R014	Positive	2048	Positive	5	Positive
100	R016	Positive	2048	Positive	6	Positive
101	R029	Positive	4096	Positive	5	Positive
102	R032	Positive	1024	Positive	5	Positive
103	R007	Positive	1024	Positive	5	Positive
104	R008	Positive	512	Positive	5	Positive
105	R009	Positive	1024	Positive	4	Positive
106	R013	Positive	128	Positive	4	Positive
107	R010	Positive	256	Positive	4	Positive
108	R025	Positive	2048	Positive	5	Positive
109	R023	Positive	1024	Positive	4	Positive
110	R028	Positive	2048	Positive	6	Positive
111	R027	Positive	1024	Positive	6	Positive
112	R006	Positive	4096	Positive	6	Positive
113	R047	Positive	2048	Positive	5	Positive
114	R052	Positive	4096	Positive	5	Positive
115	R055	Positive	4096	Positive	6	Positive
116	R015	Positive	512	Positive	5	Positive
117	R046	Positive	2048	Positive	6	Positive
118	R034	Positive	4096	Positive	5	Positive
119	R022	Positive	4096	Positive	6	Positive
120	R024	Positive	4096	Positive	6	Positive
121	R012	Positive	2048	Positive	5	Positive
122	R060	Positive	4096	Positive	6	Positive
123	R056	Positive	1024	Positive	6	Positive
124	R037	Positive	2048	Positive	6	Positive
125	R039	Positive	1024	Positive	6	Positive
126	R038	Positive	2048	Positive	5	Positive
127	R036	Positive	64	Positive	3	Positive
128	R043	Positive	2048	Positive	6	Positive
129	R005	Positive	2048	Positive	6	Positive

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

9.1.2. CDV 2008 results

Serum ID	Dog ID	Group	VN titer	VN result	IC score	IC result
150	pup542 13/07/2004	Un-known	8	Negative	0	Negative
151	pup542 15/07/2004	Un-known	0	Negative	0	Negative
152	pup542 17/07/2004	Un-known	0	Negative	0	Negative
153	pup542 22/07/2004	Un-known	64	Positive	3	Positive
154	pup542 26/07/2004	Un-known	64	Positive	5	Positive
155	pup542 29/07/2004	Un-known	64	Positive	6	Positive
156	pup542 02/08/2004	Un-known	128	Positive	6	Positive
157	pup542 05/08/2004	Un-known	512	Positive	6	Positive
158	pup542 12/08/2004	Un-known	512	Positive	6	Positive
159	pup542 19/08/2004	Un-known	256	Positive	6	Positive
160	pup555 13/07/2004	Un-known	4	Negative	1	Negative
161	pup555 15/07/2004	Un-known	0	Negative	0	Negative
162	pup555 17/07/2004	Un-known	0	Negative	1	Negative
163	pup555 22/07/2004	Un-known	64	Positive	4	Positive
164	pup555 26/07/2004	Un-known	128	Positive	6	Positive
165	pup555 29/07/2004	Un-known	128	Positive	6	Positive
166	pup555 02/08/2004	Un-known	512	Positive	6	Positive
167	pup555 05/08/2004	Un-known	256	Positive	6	Positive
168	pup555 12/08/2004	Un-known	256	Positive	6	Positive
169	pup555 19/08/2004	Un-known	256	Positive	6	Positive
170	pup573 13/07/2004	Un-known	4	Negative	1	Negative
171	pup573 15/07/2004	Un-known	4	Negative	1	Negative
172	pup573 17/07/2004	Un-known	0	Negative	1	Negative
173	pup573 22/07/2004	Un-known	16	Negative	5	Positive
174	pup573 26/07/2004	Un-known	256	Positive	5	Positive
175	pup573 29/07/2004	Un-known	128	Positive	6	Positive
176	pup573 02/08/2004	Un-known	128	Positive	6	Positive
177	pup573 05/08/2004	Un-known	256	Positive	6	Positive
178	pup573 12/08/2004	Un-known	512	Positive	6	Positive
179	pup573 19/08/2004	Un-known	512	Positive	6	Positive
180	pup551 13/07/2004	Un-known	8	Negative	0	Negative
181	pup551 15/07/2004	Un-known	4	Negative	1	Negative
182	pup551 17/07/2004	Un-known	0	Negative	1	Negative
183	pup551 22/07/2004	Un-known	32	Positive	4	Positive
184	pup551 26/07/2004	Un-known	512	Positive	6	Positive
185	pup551 29/07/2004	Un-known	512	Positive	6	Positive
186	pup551 02/08/2004	Un-known	512	Positive	6	Positive
187	pup551 05/08/2004	Un-known	512	Positive	6	Positive
188	pup551 12/08/2004	Un-known	512	Positive	6	Positive
189	pup551 19/08/2004	Un-known	1024	Positive	6	Positive

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

9.1.3. CDV 2009 results

Serum ID	Dog ID	Group	VN titer	VN result	IC score	IC result
190	1	Negative	0	Negative	0	Negative
191	2	Negative	0	Negative	0	Negative
192	3	Negative	0	Negative	1	Negative
193	4	Negative	0	Negative	1	Negative
194	5	Negative	0	Negative	1	Negative
195	6	Negative	0	Negative	1	Negative
196	7	Negative	0	Negative	0	Negative
197	8	Negative	0	Negative	0	Negative
198	9	Negative	0	Negative	0	Negative
199	10	Negative	0	Negative	0	Negative
200	11	Negative	0	Negative	0	Negative
201	12	Negative	0	Negative	0	Negative
202	13	Negative	0	Negative	0	Negative
203	14	Negative	0	Negative	0	Negative
204	15	Negative	0	Negative	0	Negative
205	16	Negative	0	Negative	0	Negative
206	17	Negative	0	Negative	0	Negative
207	18	Negative	0	Negative	0	Negative
208	19	Negative	0	Negative	0	Negative
209	20	Negative	0	Negative	0	Negative
210	21	Negative	0	Negative	0	Negative
211	22	Negative	0	Negative	0	Negative
212	23	Negative	0	Negative	0	Negative
213	24	Negative	0	Negative	0	Negative
214	25	Negative	0	Negative	0	Negative
215	26	Negative	0	Negative	0	Negative
216	27	Negative	0	Negative	0	Negative
217	28	Negative	0	Negative	0	Negative
218	29	Negative	0	Negative	0	Negative
219	30	Negative	0	Negative	0	Negative

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacchiCheck Antibody Test Kit

9.2. CAV data

9.2.1. CAV 2007 results

Serum ID	Dog ID	Group	VN titer	VN result	IC score	IC result
1	1	Un-known	1024	Positive	5	Positive
2	2	Un-known	512	Positive	4	Positive
3	3	Un-known	4096	Positive	5	Positive
4	7	Un-known	4096	Positive	6	Positive
5	8	Un-known	2048	Positive	4	Positive
6	10	Un-known	2048	Positive	4	Positive
7	11	Un-known	4096	Positive	5	Positive
8	12	Un-known	2048	Positive	4	Positive
9	13	Un-known	2048	Positive	5	Positive
10	15	Un-known	16	Positive	2	Negative
11	16	Un-known	2048	Positive	4	Positive
12	17	Un-known	4096	Positive	5	Positive
13	18	Un-known	512	Positive	1	Negative
14	19	Un-known	1024	Positive	4	Positive
15	20	Un-known	1024	Positive	3	Positive
16	22	Un-known	1024	Positive	3	Positive
17	23	Un-known	1024	Positive	3	Positive
18	24	Un-known	2048	Positive	3	Positive
19	25	Un-known	256	Positive	5	Positive
20	26	Un-known	128	Positive	1	Negative
21	27	Un-known	2048	Positive	3	Positive
22	28	Un-known	2048	Positive	3	Positive
23	29	Un-known	1024	Positive	5	Positive
24	30	Un-known	256	Positive	3	Positive
25	32	Un-known	64	Positive	3	Positive
26	33	Un-known	2048	Positive	4	Positive
27	35	Un-known	1024	Positive	4	Positive
28	36	Un-known	512	Positive	4	Positive
29	37	Un-known	512	Positive	4	Positive
30	40	Un-known	128	Positive	1	Negative
31	41	Un-known	1024	Positive	1	Negative
32	44	Un-known	256	Positive	4	Positive
33	45	Un-known	4096	Positive	4	Positive
34	47	Un-known	512	Positive	4	Positive
35	48	Un-known	256	Positive	3	Positive
36	49	Un-known	2048	Positive	4	Positive
37	50	Un-known	4096	Positive	4	Positive
38	51	Un-known	1024	Positive	4	Positive
39	52	Un-known	4096	Positive	6	Positive
40	57	Un-known	1024	Positive	3	Positive
41	58	Un-known	1024	Positive	5	Positive
42	60	Un-known	1024	Positive	5	Positive
43	61	Un-known	1024	Positive	6	Positive
44	63	Un-known	256	Positive	3	Positive
45	64	Un-known	4096	Positive	5	Positive
46	65	Un-known	256	Positive	5	Positive

CONFIDENTIAL

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

47	66	Un-known	1024	Positive	5	Positive
48	67	Un-known	2048	Positive	5	Positive
49	68	Un-known	1024	Positive	5	Positive
50	69	Un-known	512	Positive	5	Positive
51	70	Un-known	512	Positive	5	Positive
52	71	Un-known	2048	Positive	6	Positive
53	72	Un-known	2048	Positive	5	Positive
54	73	Un-known	4096	Positive	6	Positive
55	74	Un-known	4096	Positive	4	Positive
56	75	Un-known	4096	Positive	5	Positive
57	78	Un-known	512	Positive	4	Positive
58	79	Un-known	4096	Positive	5	Positive
59	81	Un-known	512	Positive	4	Positive
60	82	Un-known	256	Positive	3	Positive
61	84	Un-known	512	Positive	3	Positive
62	89	Un-known	2048	Positive	6	Positive
63	90	Un-known	1024	Positive	5	Positive
64	91	Un-known	512	Positive	5	Positive
65	92	Un-known	512	Positive	6	Positive
66	93	Un-known	128	Positive	4	Positive
67	95	Un-known	1024	Positive	5	Positive
68	98	Un-known	256	Positive	4	Positive
69	99	Un-known	4096	Positive	6	Positive
70	100	Un-known	512	Positive	5	Positive
71	101	Un-known	256	Positive	4	Positive
72	102	Un-known	2048	Positive	6	Positive
73	103	Un-known	4096	Positive	6	Positive
74	104	Un-known	512	Positive	5	Positive
75	105	Un-known	256	Positive	5	Positive
76	106	Un-known	512	Positive	4	Positive
77	107	Un-known	1024	Positive	6	Positive
78	108	Un-known	512	Positive	5	Positive
79	109	Un-known	512	Positive	3	Positive
80	110	Un-known	1024	Positive	5	Positive
81	111	Un-known	1024	Positive	5	Positive
82	112	Un-known	1024	Positive	3	Positive
83	113	Un-known	1024	Positive	3	Positive
84	114	Un-known	2048	Positive	6	Positive
85	115	Un-known	2048	Positive	5	Positive
86	H0 K038	Negative	0	Negative	0	Negative
87	H0 L028	Negative	0	Negative	0	Negative
88	H0 L164	Negative	0	Negative	0	Negative
89	H0 M100	Negative	0	Negative	0	Negative
90	H0 K023	Negative	0	Negative	0	Negative
91	H0 K109	Negative	0	Negative	0	Negative
92	H0 L015	Negative	0	Negative	0	Negative
93	H0 K013	Negative	0	Negative	0	Negative
94	H0 L127	Negative	0	Negative	0	Negative
95	H0 L027	Negative	0	Negative	0	Negative
96	H0 K015	Negative	0	Negative	0	Negative

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacchiCheck Antibody Test Kit

9.2.2. CAV 2008 results

Serum ID	Dog ID	Group	VN titer	VN result	IC score	IC result
150	pup542 13/07/2004	Un-known	0	Negative	0	Negative
151	pup542 15/07/2004	Un-known	4	Negative	0	Negative
152	pup542 17/07/2004	Un-known	4	Negative	0	Negative
153	pup542 22/07/2004	Un-known	4	Negative	1	Negative
154	pup542 26/07/2004	Un-known	8	Negative	6	Positive
155	pup542 29/07/2004	Un-known	16	Positive	6	Positive
156	pup542 02/08/2004	Un-known	4	Negative	5	Positive
157	pup542 05/08/2004	Un-known	16	Positive	5	Positive
158	pup542 12/08/2004	Un-known	256	Positive	5	Positive
159	pup542 19/08/2004	Un-known	512	Positive	5	Positive
160	pup555 13/07/2004	Un-known	8	Negative	1	Negative
161	pup555 15/07/2004	Un-known	8	Negative	1	Negative
162	pup555 17/07/2004	Un-known	4	Negative	0	Negative
163	pup555 22/07/2004	Un-known	4	Negative	3	Positive
164	pup555 26/07/2004	Un-known	64	Positive	6	Positive
165	pup555 29/07/2004	Un-known	32	Positive	6	Positive
166	pup555 02/08/2004	Un-known	16	Positive	6	Positive
167	pup555 05/08/2004	Un-known	32	Positive	6	Positive
168	pup555 12/08/2004	Un-known	128	Positive	6	Positive
169	pup555 19/08/2004	Un-known	256	Positive	6	Positive
170	pup573 13/07/2004	Un-known	4	Negative	0	Negative
171	pup573 15/07/2004	Un-known	32	Positive	0	Negative
172	pup573 17/07/2004	Un-known	32	Positive	0	Negative
173	pup573 22/07/2004	Un-known	16	Positive	3	Positive
174	pup573 26/07/2004	Un-known	64	Positive	6	Positive
175	pup573 29/07/2004	Un-known	64	Positive	6	Positive
176	pup573 02/08/2004	Un-known	64	Positive	6	Positive
177	pup573 05/08/2004	Un-known	64	Positive	6	Positive
178	pup573 12/08/2004	Un-known	256	Positive	6	Positive
179	pup573 19/08/2004	Un-known	512	Positive	6	Positive
180	pup551 13/07/2004	Un-known	8	Negative	0	Negative
181	pup551 15/07/2004	Un-known	4	Negative	0	Negative
182	pup551 17/07/2004	Un-known	4	Negative	1	Negative
183	pup551 22/07/2004	Un-known	8	Negative	1	Negative
184	pup551 26/07/2004	Un-known	8	Negative	6	Positive
185	pup551 29/07/2004	Un-known	64	Positive	6	Positive
186	pup551 02/08/2004	Un-known	16	Positive	6	Positive
187	pup551 05/08/2004	Un-known	32	Positive	6	Positive
188	pup551 12/08/2004	Un-known	64	Positive	5	Positive
189	pup551 19/08/2004	Un-known	64	Positive	5	Positive

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacchiCheck Antibody Test Kit

9.2.3. CAV 2009 results

Serum ID	Dog ID	Group	VN titer	VN result	IC score	IC result
190	1	Negative	0	Negative	0	Negative
191	2	Negative	2	Negative	0	Negative
192	3	Negative	0	Negative	0	Negative
193	4	Negative	0	Negative	0	Negative
194	5	Negative	4	Negative	0	Negative
195	6	Negative	8	Negative	0	Negative
196	7	Negative	0	Negative	0	Negative
197	8	Negative	0	Negative	0	Negative
198	9	Negative	2	Negative	0	Negative
199	10	Negative	4	Negative	0	Negative
200	11	Negative	0	Negative	0	Negative
201	12	Negative	0	Negative	0	Negative
202	13	Negative	0	Negative	0	Negative
203	14	Negative	0	Negative	0	Negative
204	15	Negative	2	Negative	0	Negative
205	16	Negative	0	Negative	0	Negative
206	17	Negative	0	Negative	0	Negative
207	18	Negative	0	Negative	0	Negative
208	19	Negative	0	Negative	0	Negative
209	20	Negative	0	Negative	0	Negative
210	21	Negative	0	Negative	0	Negative
211	22	Negative	0	Negative	0	Negative
212	23	Negative	0	Negative	0	Negative
213	24	Negative	0	Negative	0	Negative
214	25	Negative	0	Negative	0	Negative
215	26	Negative	0	Negative	0	Negative
216	27	Negative	0	Negative	0	Negative
217	28	Negative	0	Negative	0	Negative
218	29	Negative	0	Negative	0	Negative
219	30	Negative	0	Negative	0	Negative

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacchiCheck Antibody Test Kit

9.3. CPV data

9.3.1. CPV 2007 results

Serum ID	Dog ID	Group	HI titer	HI result	IC score	IC result
1	1	Un-known	160	Positive	6	Positive
2	2	Un-known	160	Positive	2	Negative
3	3	Un-known	2560	Positive	6	Positive
4	7	Un-known	160	Positive	3	Positive
5	8	Un-known	320	Positive	4	Positive
6	10	Un-known	320	Positive	4	Positive
7	11	Un-known	640	Positive	4	Positive
8	12	Un-known	160	Positive	3	Positive
9	13	Un-known	160	Positive	4	Positive
10	15	Un-known	80	Positive	1	Negative
11	16	Un-known	160	Positive	3	Positive
12	17	Un-known	320	Positive	4	Positive
13	18	Un-known	160	Positive	4	Positive
14	19	Un-known	320	Positive	3	Positive
15	20	Un-known	1280	Positive	3	Positive
16	22	Un-known	80	Positive	1	Negative
17	23	Un-known	1280	Positive	3	Positive
18	24	Un-known	2560	Positive	5	Positive
19	25	Un-known	80	Positive	1	Negative
20	26	Un-known	640	Positive	5	Positive
21	27	Un-known	80	Positive	3	Positive
22	28	Un-known	320	Positive	4	Positive
23	29	Un-known	80	Positive	0	Negative
24	30	Un-known	160	Positive	5	Positive
25	32	Un-known	160	Positive	3	Positive
26	33	Un-known	160	Positive	4	Positive
27	35	Un-known	320	Positive	3	Positive
28	36	Un-known	80	Positive	1	Negative
29	37	Un-known	160	Positive	3	Positive
30	40	Un-known	80	Positive	2	Negative
31	41	Un-known	160	Positive	1	Negative
32	44	Un-known	320	Positive	4	Positive
33	45	Un-known	80	Positive	1	Negative
34	47	Un-known	80	Positive	2	Negative
35	48	Un-known	80	Positive	2	Negative
36	49	Un-known	640	Positive	4	Positive
37	50	Un-known	80	Positive	2	Negative
38	51	Un-known	80	Positive	5	Positive
39	52	Un-known	640	Positive	3	Positive
40	57	Un-known	1280	Positive	3	Positive
41	58	Un-known	160	Positive	3	Positive
42	60	Un-known	160	Positive	4	Positive
43	61	Un-known	640	Positive	6	Positive
44	63	Un-known	80	Positive	4	Positive
45	64	Un-known	160	Positive	1	Negative
46	65	Un-known	80	Positive	2	Negative

CONFIDENTIAL

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

47	66	Un-known	640	Positive	5	Positive
48	67	Un-known	640	Positive	5	Positive
49	68	Un-known	640	Positive	6	Positive
50	69	Un-known	1280	Positive	4	Positive
51	70	Un-known	160	Positive	4	Positive
52	71	Un-known	320	Positive	5	Positive
53	72	Un-known	160	Positive	4	Positive
54	73	Un-known	80	Positive	4	Positive
55	74	Un-known	640	Positive	5	Positive
56	75	Un-known	640	Positive	5	Positive
57	78	Un-known	320	Positive	4	Positive
58	79	Un-known	1280	Positive	5	Positive
59	81	Un-known	320	Positive	4	Positive
60	82	Un-known	160	Positive	3	Positive
61	84	Un-known	10240	Positive	5	Positive
62	89	Un-known	160	Positive	5	Positive
63	90	Un-known	320	Positive	4	Positive
64	91	Un-known	80	Positive	3	Positive
65	92	Un-known	160	Positive	5	Positive
66	93	Un-known	80	Positive	3	Positive
67	95	Un-known	160	Positive	3	Positive
68	98	Un-known	320	Positive	5	Positive
69	99	Un-known	80	Positive	2	Negative
70	100	Un-known	80	Positive	4	Positive
71	101	Un-known	80	Positive	5	Positive
72	102	Un-known	640	Positive	5	Positive
73	103	Un-known	80	Positive	4	Positive
74	104	Un-known	160	Positive	3	Positive
75	105	Un-known	320	Positive	4	Positive
76	106	Un-known	160	Positive	2	Negative
77	107	Un-known	1280	Positive	5	Positive
78	108	Un-known	320	Positive	4	Positive
79	109	Un-known	1280	Positive	5	Positive
80	110	Un-known	640	Positive	3	Positive
81	111	Un-known	640	Positive	5	Positive
82	112	Un-known	640	Positive	4	Positive
83	113	Un-known	640	Positive	4	Positive
84	114	Un-known	320	Positive	4	Positive
85	115	Un-known	160	Positive	3	Positive
86	H0 K038	Negative	0	Negative	0	Negative
87	H0 L028	Negative	0	Negative	0	Negative
88	H0 L164	Negative	0	Negative	0	Negative
89	H0 M100	Negative	0	Negative	0	Negative
90	H0 K023	Negative	0	Negative	0	Negative
91	H0 K109	Negative	0	Negative	0	Negative
92	H0 L015	Negative	0	Negative	0	Negative
93	H0 K013	Negative	0	Negative	0	Negative
94	H0 L127	Negative	0	Negative	0	Negative
95	H0 L027	Negative	0	Negative	0	Negative
96	H0 K015	Negative	0	Negative	0	Negative
130	TQJ	Positive	1280	Positive	6	Positive

CONFIDENTIAL

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

131	TSI	Positive	2560	Positive	6	Positive
132	TTI	Positive	1280	Positive	6	Positive
133	TUI	Positive	2560	Positive	6	Positive
134	TWJ	Positive	5120	Positive	6	Positive
135	TXJ	Positive	2560	Positive	5	Positive
136	UBI	Positive	2560	Positive	6	Positive
137	UDI	Positive	1280	Positive	6	Positive
138	UTJ	Positive	2560	Positive	6	Positive
139	UUJ	Positive	1280	Positive	6	Positive
140	UWI	Positive	2560	Positive	6	Positive
141	UXI	Positive	5120	Positive	6	Positive
142	UZI	Positive	2560	Positive	6	Positive
143	VAI	Positive	20480	Positive	6	Positive
144	VDJ	Positive	2560	Positive	6	Positive
145	VEJ	Positive	5120	Positive	6	Positive
146	VGI	Positive	5120	Positive	6	Positive
147	VVJ	Positive	1280	Positive	6	Positive
148	VWJ	Positive	5120	Positive	6	Positive
149	VXI	Positive	5120	Positive	6	Positive

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacchiCheck Antibody Test Kit

9.3.2. CPV 2008 results

Serum ID	Dog ID	Group	HI titer	HI result	IC score	IC result
150	pup542 13/07/2004	Un-known	0	Negative	0	Negative
151	pup542 15/07/2004	Un-known	0	Negative	0	Negative
152	pup542 17/07/2004	Un-known	0	Negative	0	Negative
153	pup542 22/07/2004	Un-known	40	Negative	1	Negative
154	pup542 26/07/2004	Un-known	640	Positive	5	Positive
155	pup542 29/07/2004	Un-known	640	Positive	5	Positive
156	pup542 02/08/2004	Un-known	320	Positive	6	Positive
157	pup542 05/08/2004	Un-known	320	Positive	6	Positive
158	pup542 12/08/2004	Un-known	640	Positive	6	Positive
159	pup542 19/08/2004	Un-known	1280	Positive	6	Positive
160	pup555 13/07/2004	Un-known	0	Negative	0	Negative
161	pup555 15/07/2004	Un-known	0	Negative	0	Negative
162	pup555 17/07/2004	Un-known	0	Negative	1	Negative
163	pup555 22/07/2004	Un-known	320	Positive	4	Positive
164	pup555 26/07/2004	Un-known	640	Positive	5	Positive
165	pup555 29/07/2004	Un-known	320	Positive	5	Positive
166	pup555 02/08/2004	Un-known	320	Positive	5	Positive
167	pup555 05/08/2004	Un-known	640	Positive	6	Positive
168	pup555 12/08/2004	Un-known	640	Positive	6	Positive
169	pup555 19/08/2004	Un-known	1280	Positive	6	Positive
170	pup573 13/07/2004	Un-known	40	Negative	1	Negative
171	pup573 15/07/2004	Un-known	0	Negative	1	Negative
172	pup573 17/07/2004	Un-known	0	Negative	1	Negative
173	pup573 22/07/2004	Un-known	80	Positive	3	Positive
174	pup573 26/07/2004	Un-known	160	Positive	4	Positive
175	pup573 29/07/2004	Un-known	640	Positive	6	Positive
176	pup573 02/08/2004	Un-known	640	Positive	6	Positive
177	pup573 05/08/2004	Un-known	640	Positive	6	Positive
178	pup573 12/08/2004	Un-known	1280	Positive	6	Positive
179	pup573 19/08/2004	Un-known	640	Positive	6	Positive
180	pup551 13/07/2004	Un-known	0	Negative	0	Negative
181	pup551 15/07/2004	Un-known	0	Negative	0	Negative
182	pup551 17/07/2004	Un-known	0	Negative	1	Negative
183	pup551 22/07/2004	Un-known	640	Positive	3	Positive
184	pup551 26/07/2004	Un-known	160	Positive	5	Positive
185	pup551 29/07/2004	Un-known	640	Positive	6	Positive
186	pup551 02/08/2004	Un-known	160	Positive	6	Positive
187	pup551 05/08/2004	Un-known	160	Positive	6	Positive
188	pup551 12/08/2004	Un-known	2560	Positive	6	Positive
189	pup551 19/08/2004	Un-known	2560	Positive	6	Positive

A field and experimental trial to assess the performance of the
ImmunoComb Canine VacciCheck Antibody Test Kit

9.3.3. CPV 2009 results

Serum ID	Dog ID	Group	HI titer	HI result	IC score	IC result
190	1	Negative	0	Negative	0	Negative
191	2	Negative	0	Negative	0	Negative
192	3	Negative	0	Negative	1	Negative
193	4	Negative	0	Negative	1	Negative
194	5	Negative	0	Negative	1	Negative
195	6	Negative	0	Negative	1	Negative
196	7	Negative	0	Negative	1	Negative
197	8	Negative	0	Negative	1	Negative
198	9	Negative	0	Negative	1	Negative
199	10	Negative	0	Negative	1	Negative
200	11	Negative	0	Negative	0	Negative
201	12	Negative	0	Negative	0	Negative
202	13	Negative	0	Negative	0	Negative
203	14	Negative	0	Negative	0	Negative
204	15	Negative	0	Negative	0	Negative
205	16	Negative	0	Negative	0	Negative
206	17	Negative	0	Negative	0	Negative
207	18	Negative	0	Negative	0	Negative
208	19	Negative	0	Negative	0	Negative
209	20	Negative	0	Negative	0	Negative
210	21	Negative	0	Negative	0	Negative
211	22	Negative	0	Negative	0	Negative
212	23	Negative	0	Negative	0	Negative
213	24	Negative	0	Negative	0	Negative
214	25	Negative	0	Negative	0	Negative
215	26	Negative	0	Negative	0	Negative
216	27	Negative	0	Negative	0	Negative
217	28	Negative	0	Negative	0	Negative
218	29	Negative	0	Negative	0	Negative
219	30	Negative	0	Negative	0	Negative