Sensitivity-specificity-accuracy and difference between positive and negative mean results of the ImmunoComb® Canine VacciCheck Antibody Test Kit for Canine Distemper, Parvo and AdenoVirus. (2009) Mazar S.[1], Larson L.[2] and Lavi Y.[3]

[1] Biogal, Galed Labs. kit manufacturer.
[2] Professor Schultz's lab at the Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin - Madison.
[3] Genetics and statistics - Sha'ar Efraim 42835, Israel.

Summary: Overall, 219 dog sera samples were used: 149 sera had been tested during 2007, 40 sera during 2008 and 30 sera during 2009. Sera were derived from 3 groups: a negative group that included 41 sera derived from dogs that had been raised in barrier facilities, the positive group included 33 or 20 sera derived from dogs that had been experimentally infected with CDV or CPV, respectively, in various times post vaccination; the third "Un-known" group included 40 sera derived of 4 pups that had been vaccinated and bled at 10 intervals and 85 sera kept in the Wisconsin laboratory stock from various vaccination protocols after bled 14-21 days post vaccination. All sera samples were tested in Wisconsin by the ImmunoComb® Canine VacciCheck Antibody Test Kit, and by either VN for Canine Distemper (CDV) and Adenovirus Type1 (CAV-1) or HI for Parvovirus (CPV).
Sensitivity and specificity of the ImmunoComb® kit, in comparison to the VN/HI results, regarding each virus were calculated for each year, for each group and for all sera samples. Sensitivity calculated for CAV was 95% in 2007, 100% in 2008, and 96% using all sera samples. Specificity calculated for CAV was 100% in 2007, 54% in 2008, 100% in 2009, 100% using negative sera group and 82% using all sera samples. Sensitivity calculated for CPV was 85% in 2007, 100% in 2008, 100% using positive group and 88% using all sera samples. Specificity calculated for CPV was 100% in 2007, 100% in 2008, 100% in 2009, 100% using positive sera group and 100% using all sera samples. Sensitivity calculated for CDV was 100% in 2007, 100% in 2008, 100% using positive group and 100% using all sera samples. Specificity calculated for CDV was 64% in 2007, 86% in 2008, 100% in 2009, 100% using negative sera group and 83% using all sera samples.
The accuracy of the ImmunoComb® Kit, measured by ROC curve analysis was excellent regarding CDV and CPV (ACU>0.9) but only good - regarding CAV (0.99< ACU<0.79). A significant difference (P < 0.0001) was found between the ImmunoComb® means of positive and negative groups.

Summery of Results:

Tables 1: The summary of the agreement between the VN/HI and the ImmunoComb® results: using only 2007 results, using only 2008 results, using only 2009 results and using all trial results*.

CPV HI result	**ImmunoComb® result**	**2007 (n = 116)**	**2008 (n = 40)**	**2009 (n = 30)**	**All data (n = 186)**	**Conclusion**
Positive	Positive	89	27	-	116	True Positive
	Negative	16	-	-	16	False Negative
Negative	Positive	-	-	-	-	False Positive
	Negative	11	13	30	54	True Negative
CDV VN result	**ImmunoComb® result**	**2007 (n = 129)**	**2008 (n = 40)**	**2009 (n = 30)**	**All data (n = 199)**	**Conclusion**
Positive	Positive	101	26	-	127	True Positive
	Negative	-	-	-	-	False Negative
Negative	Positive	10	2	-	12	False Positive
	Negative	18	12	30	60	True Negative
CAV VN result	**ImmunoComb® result**	**2007 (n = 96)**	**2008 (n = 40)**	**2009 (n = 30)**	**All data (n = 199)**	**Conclusion**
Positive	Positive	80	14	-	94	True Positive
	Negative	4	-	-	4	False Negative
Negative	Positive	-	12	-	12	False Positive
	Negative	12	14	30	56	True Negative

Table 2: Sensitivity and specificity values, calculated using: only 2007 results (a); only 2008 results (b); only 2009 results (c); all data (d); only Negative sera groups (e); and only Positive sera group (f)*.

Virus	Year / Group	Sensitivity	Specificity
CDV	a) 2007 (n = 129)	100%	64%
	b) 2008 (n = 40)	100%	86%
	c) 2009 (n = 30)	--	100%
	d) All data (n = 100)	100%	83%
	e) Negative sera groups (n = 41)	--	100%
	f) Positive sera group (n = 33)	100%	--
CPV	a) 2007 (n = 116)	85%	100%
	b) 2008 (n = 40)	100%	100%
	c) 2009 (n = 30)	--	100%
	d) All data (n = 186)	88%	100%
	e) Negative sera groups (n = 41)	--	100%
	f) Positive sera group (n = 20)	100%	--
CAV	a) 2007 (n = 96)	95%	100%
	b) 2008 (n = 40)	100%	54%
	c) 2009 (n = 30)	--	100%
	d) All data (n = 166)	96%	82%
	e) Negative sera groups (n = 41)	--	100%
	f) Positive sera group (n = 0)	--	--

Discussion:

Specificity - Specificity is the ability of the test to pick out sera that do NOT have the disease. The specificity of the ImmunoComb® test was calculated for each virus for each year of testing (2007, 2008 and 2009) and for each of 2 sera groups nominated according to the certainty of results (i.e. positive and negative).
At 2007, specificity of the ImmunoComb® test for CDV was only 64% but at 2008 it was 86% and at 2009 the specificity was high (100%). Merging all three years, the specificity was 89%. The negative group, that included 41 SPF sera, gave specificity of 100%.
At 2007, 2008 and 2009 specificity of the ImmunoComb® test for CPV was high (100%) so using all data, the specificity accepted remained 100%, so did the negative group that gave 100% specificity.
At 2007 and 2009, specificity of the ImmunoComb® test for CAV was high (100%) but at 2008 was only 54%. But using all data, the specificity was 82%; the negative group gave 100% specificity.

VN and HI are used in all reference laboratories, and this is why those 2 tests have been picked to be the "gold-standard" for this study, yet since no test in "immune" from false results, and since 41 SPF sera are pooled to a substantial statistical group, it seems that although not all sera groups maintained study criteria for CAV, the 100% specificity accepted for the 41 negative sera assures that true false positive results would not be accepted with the ImmunoComb®.

Sensitivity - The sensitivity determines how good the test is at picking out sera with a disease. The specificity of the ImmunoComb® test was calculated for each virus for each year of testing (2007 and 2008) and for each of 2 sera groups nominated according to the certainty of results (i.e. positive and negative).
At 2007 and 2008, specificity of the ImmunoComb® test for CDV was high (100%) and using all data, the sensitivity remained 100%; so did the positive group that gave 100% sensitivity.
At 2007, sensitivity of the ImmunoComb® test for CPV was only 85%: at 2008 the sensitivity accepted was 100%. Overall sensitivity, using all data, was 88%. Sensitivity of the positive group was 100%.
At 2007 and 2008 sensitivity of the ImmunoComb® test for CAV was high (95% and 100%). Although the data lack a "Positive group", using all data, the sensitivity remained high (100%).

Accuracy - The accuracy of the ImmunoComb® test depends on how well the test separates the dog sera into positive or negative groups. The results of this trial showed that the accuracy was excellent (AUC > 0.9) for CDV and CPV and for all three viruses tested by the ImmunoComb® except for the test for CAV done in 2008*.

Difference between Positive and Negative ImmunoComb® means – A significant difference ($P < 0.0001$) was found between the ImmunoComb® means of positive and negative groups. The difference of 3.46 S, 4.07 S and 4.16 S for CAV, CPV and CDV respectively, was higher than the satisfactory conclusion criteria (1 S)*.

* Crude data, figures and statistical analysis may be supplied upon request.