Performance Report

ImmunoComb Feline Panleukopenia Virus Antibody Test Kit

MANUFACTURED BY:

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Purpose of Report:

The purpose of the performance report was to submit data to the USDA in order to gain USDA approval in the United States for the ImmunoComb Feline Panleukopenia Virus Antibody Test Kit.

Personnel:

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Proffesor Mike Lappin of the College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Department of Clinical Sciences, Fort Collins, CO 80523 – the cooperator of the ImmunoComb tests performed in Colorado and VN and HI tests performed in Cornell.

Dr. Julie Levy and Dr. Brain DiGangi from The Department of Small Animal Clinical Sciences lab and the Maddie's Shelter Medicine Program, in the College of Veterinary Medicine, University of Florida - the cooperators of the ImmunoComb tests performed in Florida and HI tests performed in Cornell.

Dr. Edward J. Dubovi in Cornell University Animal Health Diagnostic Center - PO Box 5786 - Ithaca, NY 14852 - the supervisor of HI tests done.

US Cooperator – Dr. Vergil Davis President Lasher Associates, inc. 28300 DuPont Hwy, Millsboro, DE 19966- USA.

Summary:

A field trial to assess the performance of the ImmunoComb Feline Panleukopenia Virus Antibody Test Kit was done in comparison to the gold standard Hemagglutination Inhibition (HI) assays. Overall, 456 cat sera samples were used: the sera were derived from naturally infected or previously vaccinated cats. Of them, 50 sera had been tested during 2006, 50 sera during 2009 and 356 sera were tested in 2010. Sera were derived from 3 groups: a negative group that included 40 sera derived from cats that had been raised in a barrier facility, the positive group included 10 sera derived from cats that had been experimentally infected in various times post vaccination; the third “un-known" group included 406 sera derived from naturally infected or previously vaccinated cats. The 456 sera samples were tested in two different US locations by the ImmunoComb Feline Panleukopenia Virus Antibody Test Kit, Dot Blot and in one location by HI for Panleukopenia Virus (FPLV). The overall sensitivity, specificity and accuracy for FPLV compared to HI were 90.4%, 97.8% and 97.3% respectively.

1. Objective:

To assess the specificity, sensitivity and overall accuracy of the ImmunoComb Feline Panleukopenia Virus Antibody Test Kit, in comparison to the gold standards Hemagglutination Inhibition (HI) assays.

2. Introduction:

The ImmunoComb Feline Panleukopenia Virus Antibody Test Kit is designed to determine cat blood or serum IgG antibody titer to Feline Panleukopenia Virus (FPLV). Use of serology has been established for use as an aid in the diagnosis of Feline Panleukopenia Virus in cats. The ImmunoComb Feline Panleukopenia Virus Antibody Test Kit is a self-contained portable kit, sufficient for 12 individual tests, that enables the user to test cat blood or serum by themselves and have a result within 21 minutes.

3. Materials and Methods:

The ImmunoComb Feline Panleukopenia Virus 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 µl of serum sample or 10 µl 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 were allowed to dry before being read by the CombScan (a software program, supplied by Biogal) and visual comparison with a color scale supplied with each kit (CombScale) that enables obtaining results at a scale of 1-6. 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 spot is 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 where considered negative.

Hemagglutination Inhibition (HI) assay:
Done at the Cornell University, Animal Health Diagnostic Center (New York State Veterinary diagnostic Laboratory). HI tests 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 1:10 to 1:10,240. For the comparison with the ImmunoComb kit any result equal or higher than 1:80 is considered positive and any result lower than 1:80 is considered negative.

4. Specimens:

Specimens had been collected in two USA locations Colorado and Florida.
Colorado: The Medicine and Biomedical Sciences lab in the College of Veterinary, Colorado State University.
Florida: The Department of Small Animal Clinical Sciences lab in the College of Veterinary Medicine, University of Florida.
Specimens collected from naturally infected or previously vaccinated cats. In total, 456 sera tested by both the ImmunoComb and by HI assays.

2006 collection:
The Colorado laboratory tested 50 sera, collected at that laboratory for various studies and tittered by HI for FPLV in the Cornell diagnostic lab. Of the 50 sera, 10 sera derived from cats that had been experimentally infected in various times post vaccination

2009 collection:
The Colorado laboratory tested additional 50 sera (25 negative and 25 positive to FPLV) by both the kit and by HI for FPLV in Cornell diagnostic lab. The results of those tests were accepted in January 2009.

2010 collection:
The Florida lab tested 356 sera from naturally infected or previously vaccinated cats. The sera were sent to be tested by HI for FPLV in Cornell diagnostic lab.

5. Data analysis

The sensitivity, specificity and accuracy for the IC FPLV 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

		HI result		**Total**
		Positive	Negative	
IC FPLV Test result	Positive	a	b	a+b
	Negative	c	d	c+d
	Total	a+c	b+d	a+b+c+d
		% Sensitivity = 100 x a/(a+c)	% Specificity = 100 x d/(b+d)	% Accuracy= 100x(a+d)/(a+b+c+d)

6. Results:

Overall 456 experimental units were used. Although 456 sera were tested, twelve results gave invalid result. Therefore the calculations of specificity and sensitivity represent only 444 sera. The sensitivity, specificity and accuracy are summarized in Table 2.

Table 2: The results of the IC FPLV Test Kit fin comparison to the gold standard HI:

FPLV		**HI result**		**Total**
		Positive	Negative	
IC FPLV Test result	Positive	113	7	120
	Negative	12	312	324
	Total	125	319	444
		Sensitivity = 90.4%	Specificity = 97.8%	Accuracy = 97.3%

7. Discussion:

This report compares the results obtained by the ImmunoComb Feline Panleukopenia Virus Antibody Test Kit to results obtained by HI assay in a trial on Cats sera collected from the field and from experimental animals. Overall, 456 serum samples had been tested, yet 12 samples gave invalid results, and were omitted from the sensitivity and specificity calculation.

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 FPLV compared to HI were 90.4% and 97.8% respectively.

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 ImmunoComb Feline Panleukopenia Virus Test Kit was excellent 97.3%.

HI is considered as the gold-standard to determine the protective antibody titer and are used in reference laboratories. However, this assay is very complicated, take several hours to perform and require a specialized laboratory with high level of biosafety. On the other hand, the ImmunoComb Feline Panleukopenia Virus Antibody 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 ImmunoComb Feline Panleukopenia Virus Antibody Test Kit provides an excellent performance compared to the gold standard assay with a much simpler and accessible method.