Sensitivity-specificity and accuracy of the ImmunoComb® Feline VacciCheck Antibody Test Kit for Feline Calici, Herpes and Panleukopenia Viruses (2011)

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Title:

Specificity, sensitivity and accuracy results of the ImmunoComb® Feline VacciCheck Antibody Test Kit in comparison to the Virus Neutralization (VN) or Hemagglutination Inhibition (HI) assays.

Summary:

Overall, 356 cat sera samples were used: the sera were derived from naturally infected or previously vaccinated cats[1]. All 356 sera samples were tested, , by the ImmunoComb® Feline VacciCheck Antibody Test Kit and by either VN for Feline Calici (FCV) and Feline Rhinotracheitis (FHV) or HI for Panleukopenia Virus (FPLV).

Sensitivity and specificity of the ImmunoComb® kit, in comparison to the VN/HI results, regarding each virus were calculated. Sensitivity calculated for FCV was 90%. Specificity calculated for FCV was 91%. Accuracy calculated for FCV was 88%. Sensitivity calculated for FHV was 93%. Specificity calculated for FHV was 96%. Accuracy calculated for FHV was 92%. Sensitivity calculated for FPLV was 89%. Specificity calculated for FPLV was 98%. Accuracy calculated for FPLV was 93%.

Introduction: The ImmunoComb® Feline VacciCheck Antibody Test Kit - is designed to determine cat blood or serum IgG antibody titer to Feline Calici (FCV), Rhinotracheitis (FHV) and Panleukopenia Virus (FPLV).

Background: Use of serology has been established for use as an aid in the diagnosis of Feline Calici, Rhinotracheitis and Panleukopenia Virus in cats[2].
The ImmunoComb® Feline VacciCheck 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.

Objectives - The study compared and analyzed the results accepted by the Biogal ImmunoComb® test kit in the Florida university lab to results accepted by Virus Neutralization (VN)/ Hemagglutination Inhibition (HI) assays performed in Cornell lab. The performance of the kit was established by its sensitivity and specificity towards the VN/HI results and the results accepted by the ImmunoComb®.

Materials:

Serum samples: collected by the Maddie's Shelter, in the College of Veterinary Medicine, University of Florida, from naturally infected or previously vaccinated cats. Sera were collected from May to August 2010, aliquated and kept in -20℃ since.

The ImmunoComb® Feline VacciCheck - the kits had been used according to manufacturer's instructions: kits, stored at 4℃, had been brought to room temperature before use. Five microliters of serum sample were added to each sample well. The combs were stripped of their protective cover and dipped in a series of wells for specific length of time: 5' at the serum diluent well, 2' at the wash well, 5' at the conjugate well, 2' and another 2' at another wash wells, 5' at the chromogen well, and than re-dipped in the last wash well for fixation. Overall, 30 kits were used. The combs were allowed to dry before being read by both CombScan – a software program, supplied by the manufacturer upon request, and visual comparison with a color scale supplied with each kit (CombScale) that enables obtaining results at a scale of 1-6. The positive reference spot should be aligned with a reading scale of 3, so that any reading of 3 or over 3, at the test spot, should be considered positive and any reading with result lower than 3 should be considered negative.

Virus Neutralization (VN) - assay performed in New York State Veterinary diagnostic Laboratory that tests antibody titer as the reciprocal of the highest dilution of the serum which neutralizes the infectivity of the virus. Dilutions are performed from 1:4 to 1:256 for FHV and from 4 to 6,144 for FCV. Since tests are performed in duplicates, median results may be accepted. For the comparison with the ImmunoComb® kit any result equal or higher than 1:16 is considered positive and any result lower than 1:16 is considered negative for FHV; any result equal or higher than 1:32 is considered positive and any result lower than 1:32 is considered negative for FCV.

Hemagglutination Inhibition (HI) - assay performed in New York State Veterinary diagnostic Laboratory that 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 FPLV. 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 for FPLV.

Methods:

Experimental methods - 356 sera were tested by both the kit in the Florida lab and by VN for FHV and FCV and HI for FPLV in Cornell diagnostic lab. The results of those tests were accepted in January 2011. Overall, results of 356 sera tested by both the ImmunoComb® and either VN or HI assays will be presented. The Florida laboratory testing was performed according to kit manual instructions.
Statistical analysis - Sensitivity for each assay was calculated as the number of cats with IC positive results (true-positives) divided by the total number of cats with positive results on the gold standard assay (true-positives) plus the total number of cats with positive results on the gold standard assay but negative results on the test assay (false-negatives). Specificity was calculated as the number of cats with negative results on the gold standard assay (true-negatives) divided by the total number of cats with negative results on the gold standard assay (true-negatives) plus the total number of cats with negative results on the gold standard assay but positive results on the test assay (false-positives). Accuracy was calculated as the number of true positive results plus the number of True negative results divided by the total number of results[1].

Results:

Overall, for assessing each virus testing assay, 356 experimental unknown units were used: Although 356 sera were tested, twelve results were found inconclusive. Therefor the calculations of specificity and sensitivity represent only 344 sera. Accuracy on the other hand has been calculated for all 356 sera.

Results for FCV
The comparison between results of the VN and the ImmunoComb® of the sera (n=344) for FCV are summarized in Table 1.

Table 1: The summary of the agreement between the VN and the ImmunoComb® results for FCV.

VN result	ImmunoComb® result	Data (n = 344)	Conclusion
Positive	Positive	110	True Positive
	Negative	12	False Negative
Negative	Positive	19	False Positive
	Negative	203	True Negative

These results show Thirty-one cat sera with a mismatch between the VN and the ImmunoComb® results to FCV (nineteen false positive and twelve false negative).

The sensitivity and specificity values concerning data from 344 cat sera and accuracy calculated for all 356 sera are summarized in Table 2.

Table 2: Sensitivity, specificity and Accuracy values for FCV,

Sensitivity (n=344)	Specificity (n=344)	Accuracy (n=356)
90%	91%	88%

Results for FHV

The comparison between results of the VN and the ImmunoComb® of the sera (n=344) for FHV are summarized in Table 3.

Table 3: The summary of the agreement between the VN and the ImmunoComb® results for FHV:

VN result	ImmunoComb® result	Data (n = 344)	Conclusion
Positive	Positive	28	True Positive
	Negative	2	False Negative
Negative	Positive	13	False Positive
	Negative	301	True Negative

These results show twelve cat sera with mismatches between the VN and the ImmunoComb® results to FHV (thirteen false positive and two false negative).

The sensitivity and specificity values concerning data from 344 cat sera and accuracy calculated for all 356 sera are summarized in Table 4.

Table 4: Sensitivity and specificity values for FHV,

Sensitivity (n=344)	Specificity (n=344)	Accuracy (n=356)
93%	96%	92%

Results for FPLV

The comparison between results of the VN and the ImmunoComb® of the sera (n=344) for FCV are summarized in Table 5.

Table 5: The summary of the agreement between the HI and the ImmunoComb® results for FPLV.

HI result	ImmunoComb® result	Data (n = 344)	Conclusion
Positive	Positive	64	True Positive
	Negative	8	False Negative
Negative	Positive	6	False Positive
	Negative	266	True Negative

These results show fourteen cat sera with a mismatch between the HI and the ImmunoComb® results to FPLV (six false positive and eight false negative).

The sensitivity and specificity values concerning data from 344 cat sera and accuracy calculated for all 356 sera are summarized in Table 6.

Table 6: Sensitivity, specificity and accuracy values for FPLV.

Sensitivity (n=344)	Specificity (n=344)	Accuracy (n=356)
89%	98%	93%

Discussion:

356 serum samples had been tested, since 12 samples gave invalid data, that data could not be included in the sensitivity and specificity calculation.

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.

Specificity of the ImmunoComb® test for FCV was high (91%).
Specificity of the ImmunoComb® test for FHV was high (96%).

Specificity of the ImmunoComb® test for FPLV was high (98%).Sensitivity - The sensitivity determines how good the test is at picking out sera WITH a disease. The sensitivity of the ImmunoComb® test was calculated for each virus.

Sensitivity - The sensitivity determines how good the test is at picking out sera WITH a disease.

Sensitivity of the ImmunoComb® test for FCV was 90%.
Sensitivity of the ImmunoComb® test for FHV was 93%.
Sensitivity of the ImmunoComb® test for FPLV was 89%. According to the previous cut-off declared by the kit insert provided for the performance of the tests, the sensitivity was only 49%[1]. The current cut-off value brings the sensitivity to a value which is very close to 90%.

Accuracy - Accuracy indicates the proximity of accepted results to the values indicated by the reference assays.

Accuracy of the ImmunoComb® test for FCV was 88%.
Accuracy of the ImmunoComb® test for FHV was 92%.
Accuracy of the ImmunoComb® test for FCV was 93%.

Those results indicate that results accepted by the ImmunoComb® are accurate enough even when all data, are taken into account.

Bibliography

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