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Certificate of the research project completion

This is to certify that the research project entitled "Determination of Bioguard FIV Ab test performance" according to the agreement between Faculty of Veterinary Science, Chulalongkorn University and Future Intrend co., ltd. and Bioguard Corporation (Chulalongkorn University Announcement no: RES_63_046_31_010) has been completed. The full report of the research project is appended with this certificate.

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(Navapon Techakriengkrai, DVM MSc PhD)

Project name Determination of Bioguard FIV Ab test performance

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Objective

The aim of this study is to determine performance of Bioguard FIV Ab test for the diagnosis of feline immunodeficiency virus infection.

Materials and methods

To determine the sensitivity and specificity of the Bioguard FIV Ab test. Serum or plasma samples used in this study were collected from cats previously visited CU small animal hospital or submitted for diagnosis of FIV/ FeLV infection. Genomic DNA was extracted from buffy coats and used for the confirmation of true FIV infection status by detecting FIV provirus using nested PCR with primer pairs specific to *gag* gene and to *env* gene. Only samples which were positive for both gene were considered true positive. In total, 101 samples (51 negative and 50 positive) were selected and tested for FIV antibodies using the Bioguard VetLab FIV Ab test and other FIV Ab test. Samples were tested according to manufacturer's protocol at the same time and read by 3 independent trained technicians. Samples which were read positive by 2 out of 3 readers were considered positive regardless of the intensity of the band.

Statistical analysis

Sensitivity, specificity, was calculated by the following formulas:

Sensitivity = number of true positive / number of true positive + false negative)

Specificity = number of true negative / number of true negative + false positive)

The required sample size for statistical comparison of sensitivity and specificity between two tests was calculated by the following formula:

$$n = \frac{\left[Z_{\frac{\alpha}{2}}\sqrt{2 \times \overline{P}(1-\overline{P})} + Z_{\beta}\sqrt{P_1(1-P_1)+P_2(1-P_2)}\right]^2}{(P_1-P_2)^2}$$

where $\overline{P}$ is the average of P_1 and P_2. P_1 and P_2 are sensitivity or specificity of the tests. Z_α and Z_β are the standard normal Z values corresponding to type I and type II errors, respectively. Using above formula the estimated sample size th can detect 10% difference in diagnostic performance between 2 tests (hypothetically one at 100% vs. 90%) at $p < 0.05$ and power of 80% is 73.29. Therefore, a sample size of 100 used in this study has adequate statistical power to perform the comparison. However, this study only determined and compared the diagnostic sensitivity and specificity between 2 tests. To determine the true performance, further study should be performed in a prospective manner with unknown samples.

Results

Of the 50 FIV proviral positive samples tested, all tested positive on Bioguard while one tested negative on other (Table 1). For the FIV proviral negative samples, 4 out of 51 tested positive on Bioguard while all were negative on other (Table 1). Altogether, Bioguard showed a 100% sensitivity as compared with other at 98% (1 false negative). However, with 4 false positive, Bioguard specificity was lower than other at 92.15% vs. 100% (Table 2). Nevertheless, these differences in sensitivity and specificity did not reach statistical significance as evidenced by their overlapped 95%CI (Table 2).

Table 1 4x4 table comparing Bioguard and other results with FIV provirus PCR **FIV provirus***

		positive	negative
Bioguard	positive	50	4
FIV Ab	negative	0	47

		FIV provirus*	
		positive	negative
Other	positive	49	0
FIV Ab	negative	1	51

Table 2 Sensitivity and specificity of Bioguard and other FIV Ab test

	Sensitivity	**95% CI**	**Specificity**	**95% CI**
Bioguard	100%	92.89-100	92.15%	81.12-97.82
Other	98%	89.35-99.95	100%	93.02-100

Conclusion

In this study, all FIV provirus positive samples were read positive, giving Bioguard sensitivity of 100% in comparison with other 98% (49/50). Altogether, this study shows that the performances of Bioguard and other are statistically comparable, although the lower specificity observed on Bioguard might be more apparent with larger sample size.