

BioPro Rabies ELISA Ab kit

Blocking ELISA kit for detection of Rabies virus
antibodies in serum or plasma

SPECIFICITY AND SENSITIVITY RELATED
PARTS OF MASTER REGISTRATION FILE
(VALIDATION STUDY)

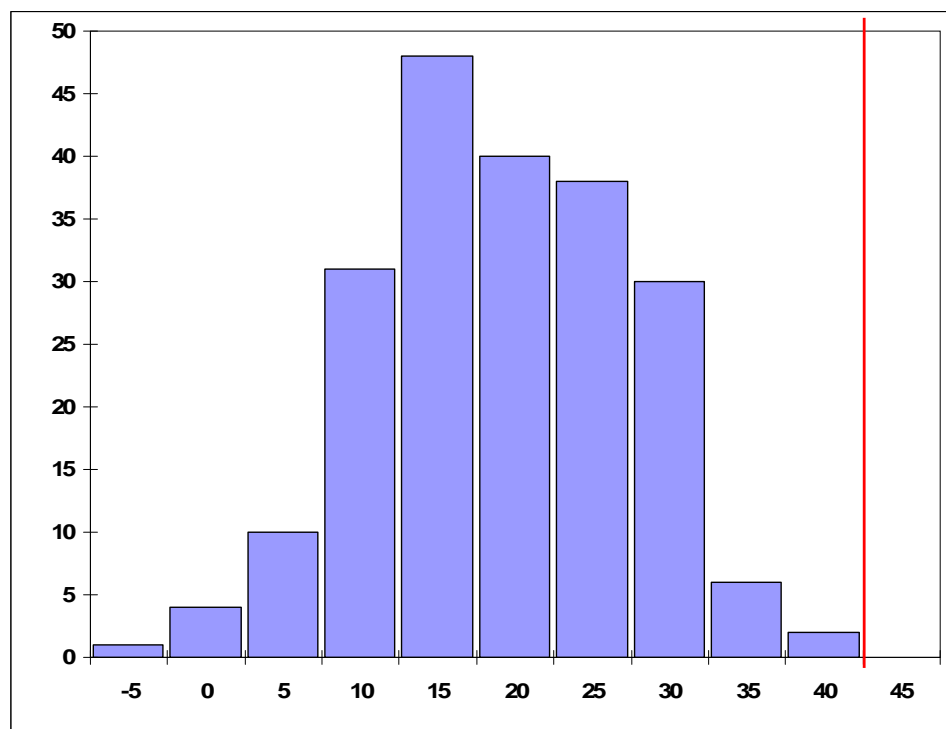
Product testing

18.1. Specificity

For the determination of BioPro Rabies ELISA Ab kit specificity following serum samples were used:

- 210 negative fox serum samples obtained from farming fur foxes (n=160) and from red foxes (n=50) kept in laboratory animal facility, both with no rabies vaccination and proven as rabies antibody free.
- 54 negative dog serum samples obtained from dogs reared in an experimental kennel, without rabies vaccination and proven as rabies antibody free.
- 125 negative wild fox serum samples obtained from nonvaccinated Rabies free area

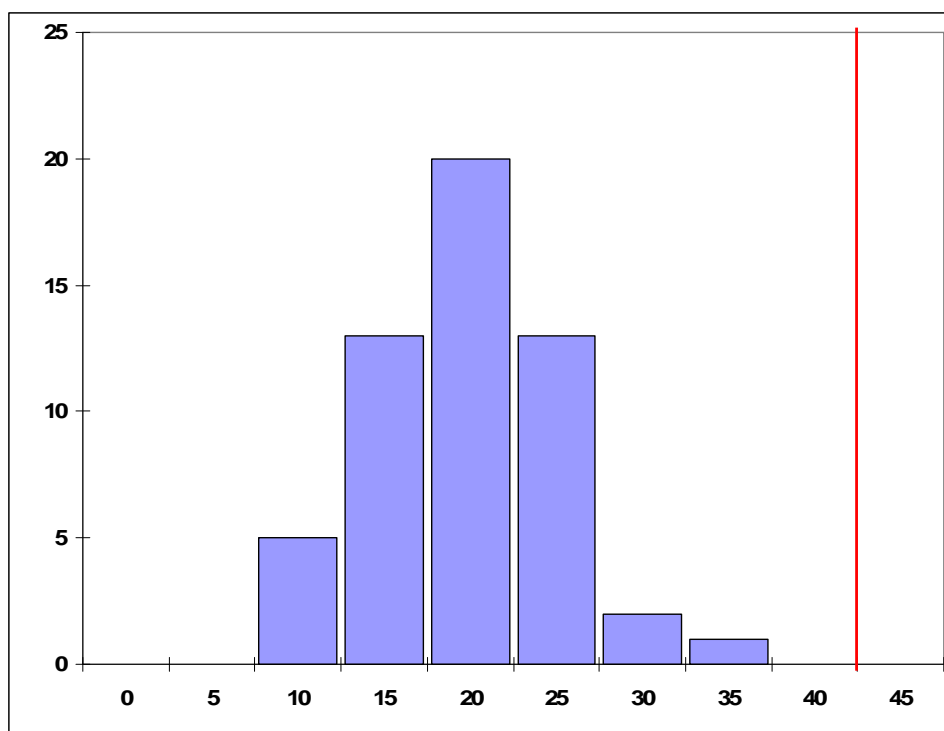
Fig.4 Frequency distribution within fox rabies negative sera



$$\begin{aligned} R-SP &= \frac{TN}{(TN+FP)} \\ R-SP &= \frac{210}{(210+0)} \end{aligned}$$

$$R-SP = 100\%$$

Fig.5 Frequency distribution within dog rabies negative sera

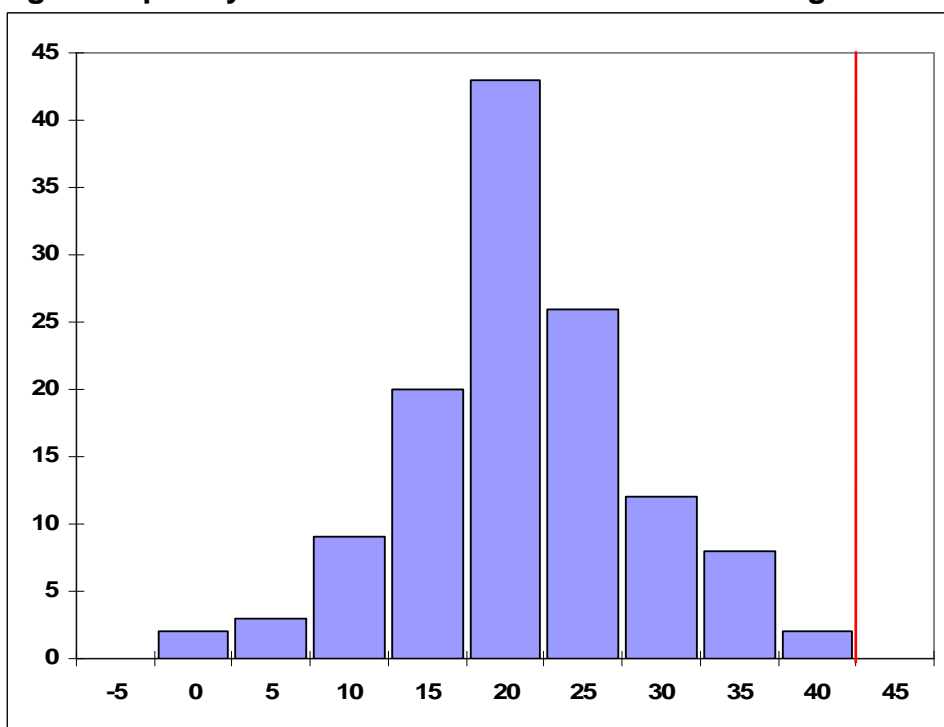


$$R-SP = \frac{TN}{(TN+FP)}$$

$$R-SP = \frac{54}{(54+0)}$$

R-SP = 100%

Fig.6 Frequency distribution within wild fox rabies negative sera



$$R-SP = \frac{TN}{(TN+FP)}$$

$$R-SP = \frac{125}{(125+0)}$$

R-SP = 100%

Conclusion

Based on the test results of fox, dog and wild fox serums the specificity of the BioPro Rabies ELISA Ab kit was assessed to 100%.

18.2. *Diagnostic sensitivity*

18.3. *Comparison of BioPro Rabies ELISA Ab kit with FAVN*

Blocking format of BioPro Rabies ELISA Ab kit enables its use for Rabies antibody detection of many animal species. Quality of fox serum samples may be decreased by bacterial contamination or autolysis, which disable usage of such sera in golden standard methods FAVN or RFFIT.

For determination of BioPro Rabies ELISA Ab kit diagnostic sensitivity panel of 83 dog sera obtained from dog after vaccination was used. Positive cut off for BioPro Rabies ELISA Ab kit was 40% of blocking, for indirect Rabies ELISA kit it was 0,5 EU/ml and for FAVN it was 0,5 IU/ml.

Fig.7 Comparison of BioPro Rabies ELISA Ab kit with FAVN on the panel of 83 sera obtained from dogs after vaccination

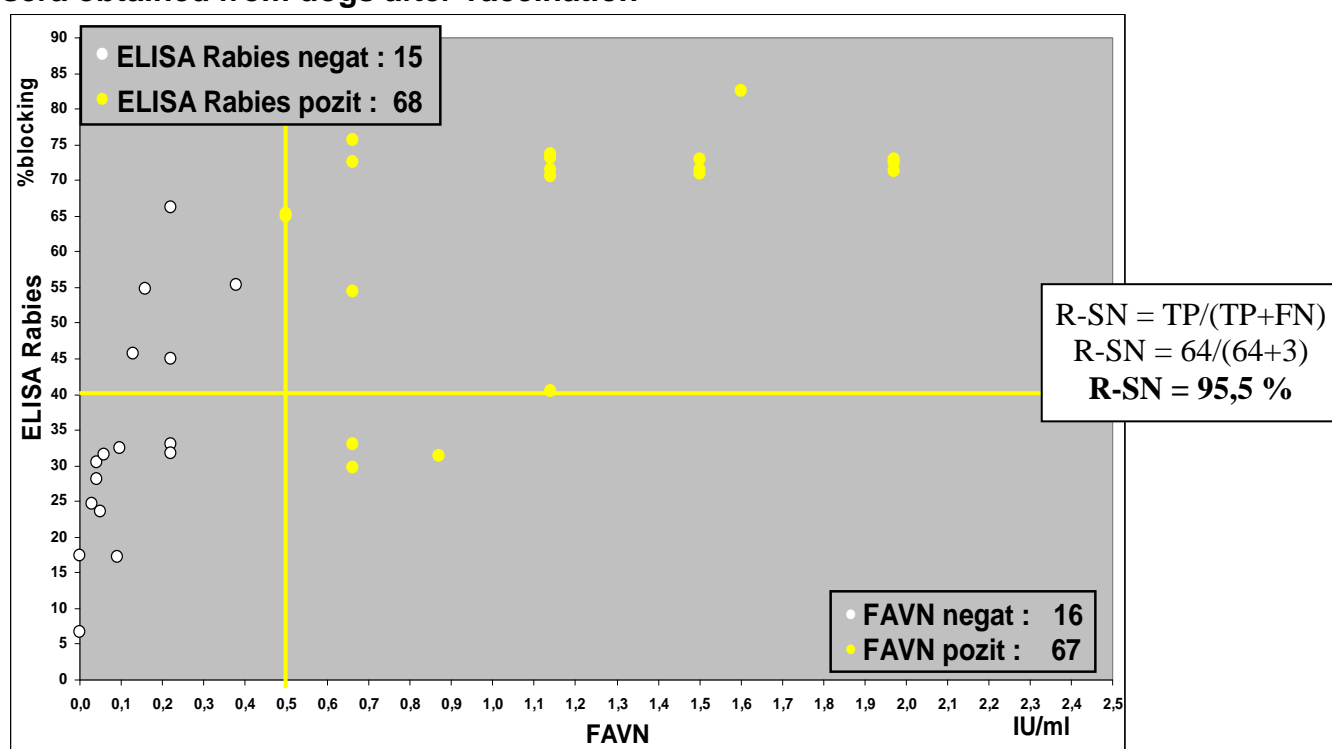


Table 4 Comparison of BioPro Rabies ELISA Ab kit with commercial indirect Rabies ELISA and FAVN on the panel of 83 sera obtained from dogs after vaccination

a) Summary tables

	negative	positive
FAVN	16	67
ELISA Rabies	14	69
Indirect ELISA	11	72

FAVN		ELISA Rabies	Indirect ELISA
negative	negative	11	9
16	positive	5	7
positive	negative	3	2
67	positive	64	65

Tables of discrepancies between

b) FAVN and ELISA Rabies

FAVN IU / ml	ELISA Rabies %blocking
0,13	45,7
0,16	54,8
0,22	44,9
0,22	66,3
0,38	55,3

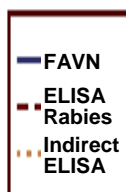
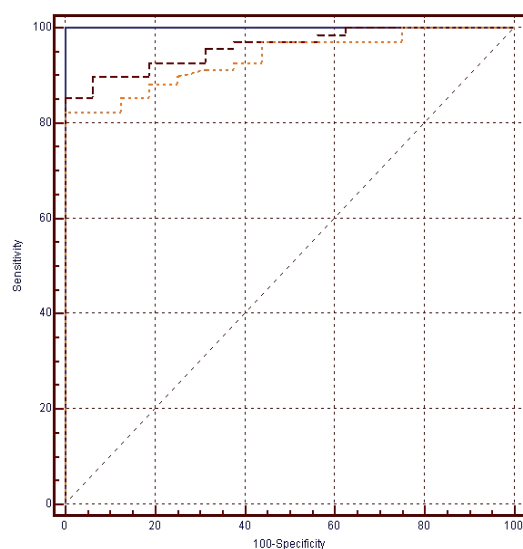
FAVN IU / ml	ELISA Rabies %blocking
0,66	29,8
0,66	33,0
0,87	31,5

c) Tables of discrepancies between FAVN and indirect ELISA

FAVN IU / ml	Indirect ELISA EU / ml
0,09	0,638
0,096	0,886
0,16	0,581
0,22	0,869
0,22	0,566
0,22	0,701
0,22	0,580

FAVN IU / ml	Indirect ELISA EU / ml
0,66	0,340
1,14	0,293

Fig.10 ROC analysis between FAVN, BioPro Rabies ELISA Ab kit and commercial indirect Rabies ELISA on the panel of 83 sera obtained from dogs after vaccination



ROC curve for FAVN

Area under the ROC curve = 1,000
Standard error = 0,000

ROC curve for ELISA Rabies

Area under the ROC curve = 0,960
Standard error = 0,020

ROC curve for Indirect ELISA

Area under the ROC curve = 0,936
Standard error = 0,026

Pair wise comparison of ROC curves

FAVN vs. ELISA Rabies

Difference between areas = 0,040
Standard error = 0,020
Significance level P = 0,041

FAVN vs. Indirect ELISA

Difference between areas = 0,064
Standard error = 0,026
Significance level P = 0,014

ELISA Rabies vs. Indirect ELISA

Difference between areas = 0,023
Standard error = 0,023
Significance level P = 0,318

Conclusion

Percentages of blocking results obtained in BioPro Rabies ELISA Ab kit were compared with values of EU/ml obtained with indirect Rabies ELISA (Fig.7) and IU/ml obtained with FAVN test (Fig.8). Numbers of sera which were found negative or gave positive results with BioPro Rabies ELISA Ab kit, indirect Rabies ELISA and FAVN are shown in Tabla.3a or respectively in Table.4a. Discrepancies are shown Tabla.3b or respectively in Table.4b and Table 4c. **Diagnostic sensitivity of BioPro Rabies ELISA Ab kit was on this panel of sera was assessed to 95,5%.**

Comparison of all three tests on this population was also done with receiver operating characteristic (ROC) curve analysis (Fig.10). FAVN was used as a reference test. When both ELISA tests were compared with FAVN significant difference between tests was found $p=0,014$ (BioPro Rabies ELISA Ab kit) and $p=0,041$ (Indirect ELISA)). Par wise comparison of both ELISA test founded no significant difference between ELISA tests ($p=0,318$).

Contact

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