

**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)

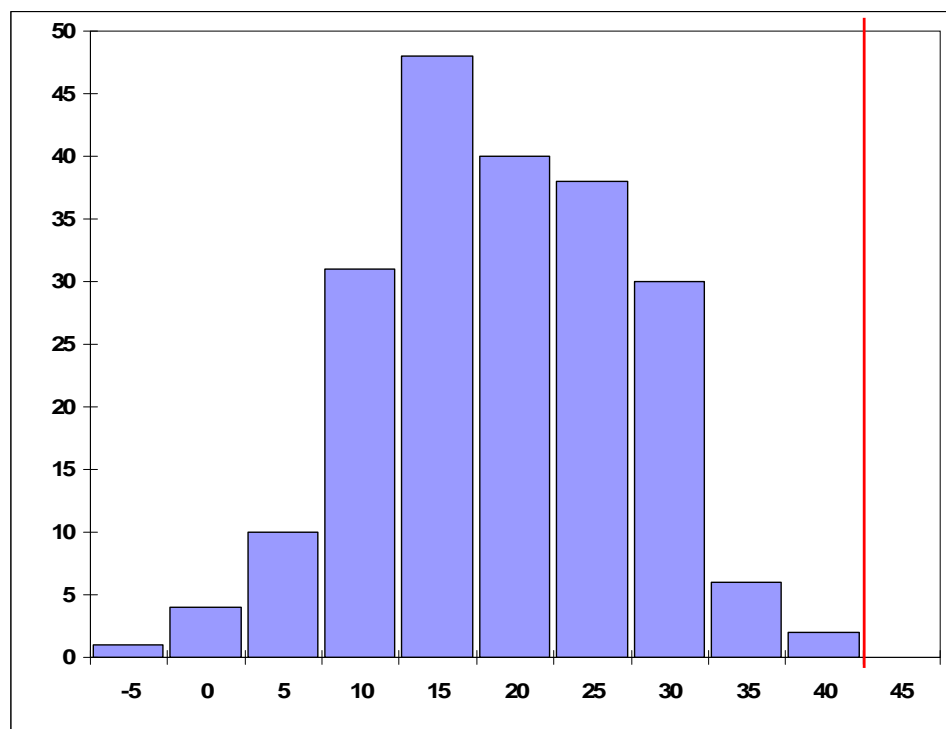
## Product testing

### 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

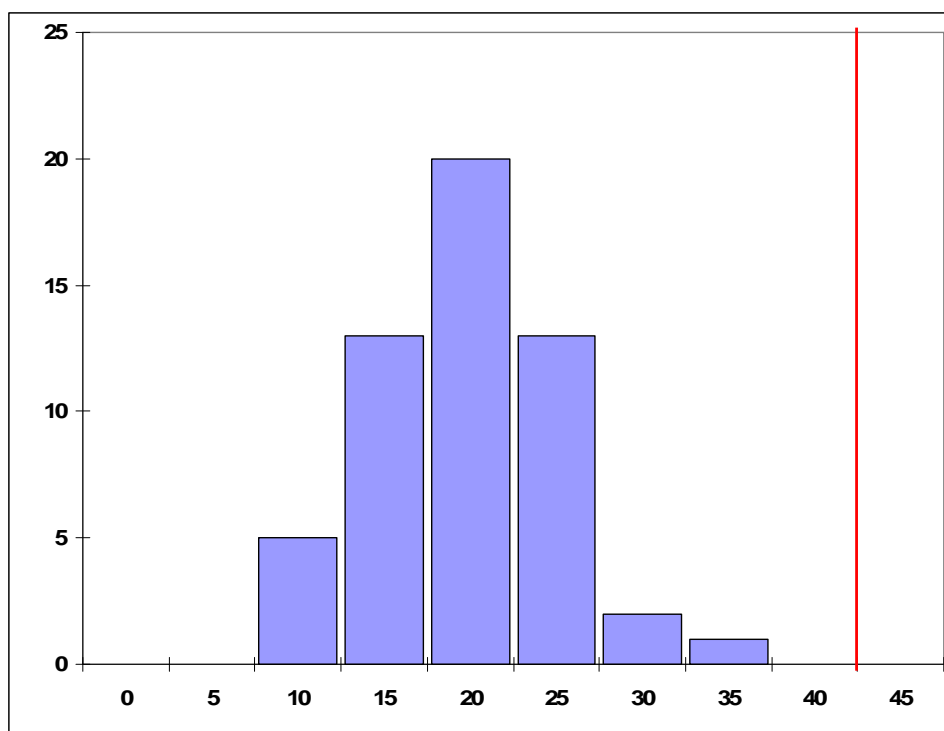
**Fig.4 Frequency distribution within fox rabies negative sera**



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

$$R-SP = 100\%$$

**Fig.5 Frequency distribution within dog rabies negative sera**

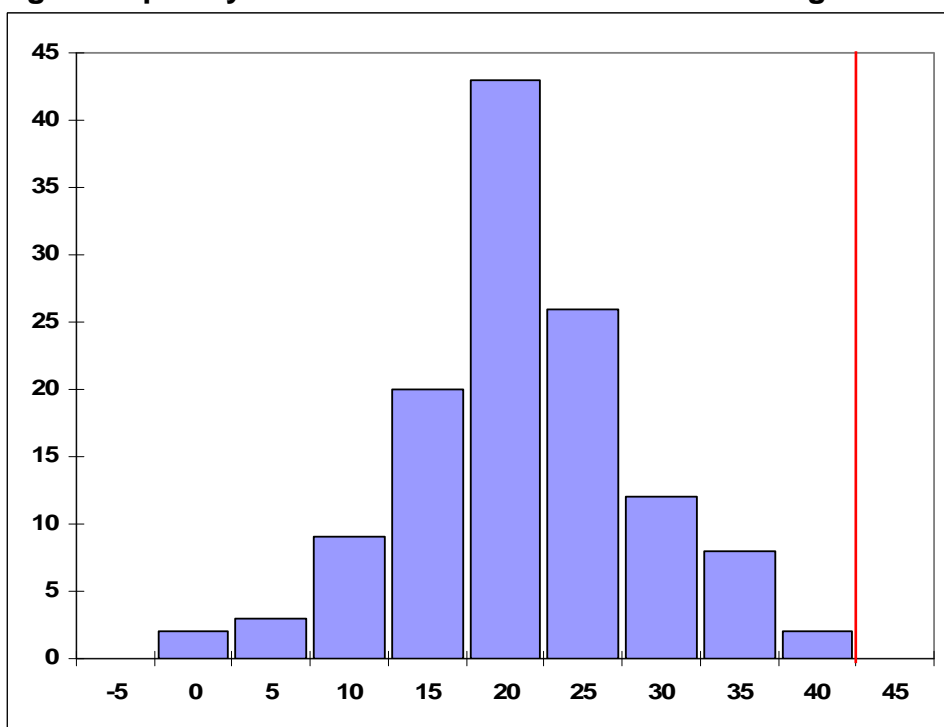


$$R-SP = \frac{TN}{(TN+FP)}$$

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

**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%.

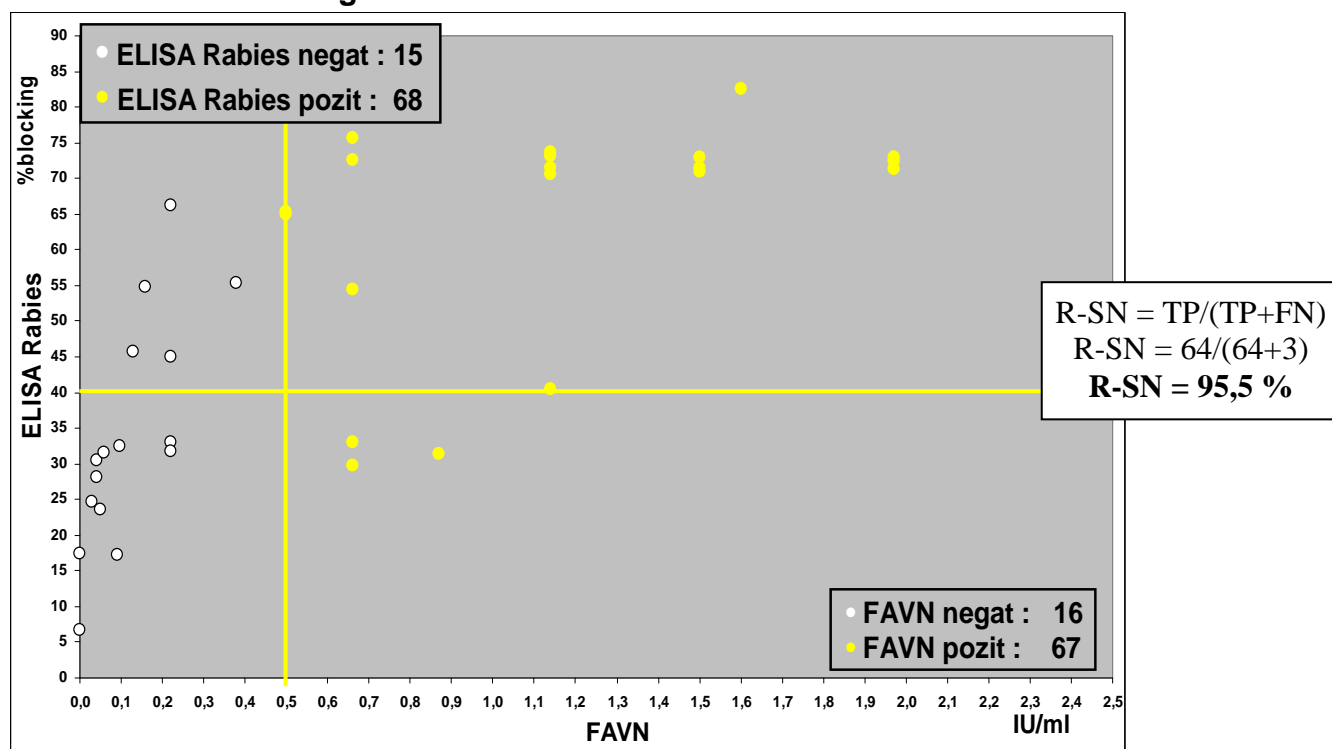
## 18.2. *Diagnostic sensitivity*

## 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

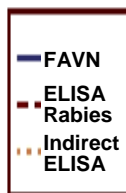
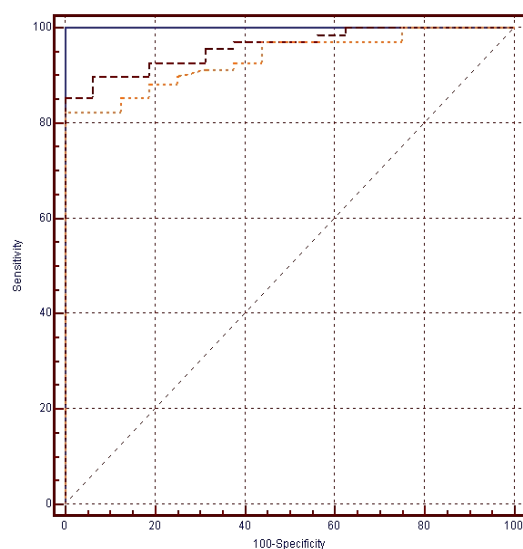
FAVN IU / ml	ELISA Rabies %blocking
0,66	29,8
0,66	33,0
0,87	31,5

**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*

#### **Manufacturer**



#### **O.K. SERVIS BioPro, s.r.o.**

Boretická 2668/1, 193 00 Praha 9 – Horní Počernice, Czech republic  
tel.: +420 281 091 460, fax: +420 281 866 264, [info@oks.cz](mailto:info@oks.cz)

**Infolinka:** +420 841 111 114  
[www.biopro.cz](http://www.biopro.cz)