THE VALIDATION PROTOCOL USED FOR BIO-RAD TESEE TEST: A PRACTICAL APPROACH

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Abstract. The present studies have the purpose to in house validation of the BioRad TeSeE ELISA test used for rapid TSE diagnosis. The samples used was: bovine brain homogenate, positive control, negative control and blank sample. The result of performance parameters evaluated from bovine brain homogenate was: repeatability 0.0108; intermediate precision 0.0247; accuracy 100%, Chi square almost absolute 0.00; sensitivity 100%; specificity 100%; linearity present, detection limit arbitrary estimated at dilution 1/8 and quantification limit at dilution 1/4. Considering this results, the validation protocol fulfill the quality management requirements.

INTRODUCTION

To assure a valid result from an measuremnet test is one of mainly purpose. Because in the course of the test, a lot of uncertainty factors can be involved, and many of them can not be appreciate, a validation protocol can be mandatary.

MATERIAL AND METHOD

The 15 different samples (Table 1) had been tested through Bio-Rad TeSeE test, for qualitative determination of PrP^{res} protein in the ruminant brainstem according the manufacturer specification. In the absence of certified positive control sample, we considered arbitrary the positive control from Bio-Rad TeSeE kit, and from it was made a serial dilution. The equipment, computer systems and software used are also according with manufacturer instructions. All equipment had been calibrated previously.

A 10 from sample no.1, 4 from sample no. 3, and 2 from samples no. 2, 4-14 has been tested by the first operator, and 10 from sample no.1, 4 from samples no. 3 and 15, and 2 from samples no. 2 has been tested by the second operator. The results were divided in: true positive N11; false positive N21; false negative N12; true negative 22. The performance of the method has been appreciated through: repeatability, intermediate precision, accuracy, sensitivity, specificity, Chi square, linearity, detection limit and quantification limit. For mathematical evaluation we used MS Office Excel software.

Table 1

The sample categories

Sample	Sample description

number	
1.	Bovine brain homogenate prior negative tested-20
2.	Control positive from Bio-Rad TeSeE kit-4
3.	Control negative from Bio-Rad TeSeE kit-8
4.	2/1 serial dilution of positive control from Bio-Rad TeSeE kit-2
5.	1/1 serial dilution of positive control from Bio-Rad TeSeE kit- 2
6.	1/2 serial dilution of positive control from Bio-Rad TeSeE kit- 2
7.	1/4 serial dilution of positive control from Bio-Rad TeSeE kit- 2
8.	1/8 serial dilution of positive control from Bio-Rad TeSeE kit- 2
9.	1/16 serial dilution of positive control from Bio-Rad TeSeE kit- 2
10.	1/32 serial dilution of positive control from Bio-Rad TeSeE kit- 2
11.	1/64 serial dilution of positive control from Bio-Rad TeSeE kit- 2
12.	1/128 serial dilution of positive control from Bio-Rad TeSeE kit- 2
13.	1/256 serial dilution of positive control from Bio-Rad TeSeE kit- 2
14.	1/512 serial dilution of positive control from Bio-Rad TeSeE kit- 2
15.	Blank –distillate water- 4

RESULTS AND DISCUSSIONS

Repeatability (r) expresses the precision under the same operation condition over a short interval of time, and represent the most close extreme in an independent measurement with 95% confidence level.[1].

$$r = t_{5\%}^{n-1} * S_D \qquad [1]$$

$t_{5\%}$ = Student coefficient
S_D = standard deviation

$$S_D = \sqrt{\frac{\sum (Xi - X)^2}{n-1}} \qquad [2]$$

$$\overline{X} = \frac{\sum Xi}{n} \qquad [3]$$

The trust limits calculated expressed like a value of mean ± r, for each one of the samples no. 1, 2, 3, and 15.

The results obtained by each operator are presented in the fallow tables:

Table 1   Table 2

The results obtained for sample no.1

10 (op1)*	POSITIVE	NEGATIVE (2*10)
1.P	0	0
1.N	0	10
10 (op2)**		
1.P	10	0
1.N	0	10

*op 1= first operator
** op 2= second operator

The results obtained for sample no.2

2 (Op.1)	POSITIVE (2*2)	NEGATIVE
2.P	2	0
2.N	0	0
2 (Op.2)		
2.P	2	0
2.N	0	0

Table 3   Table 4

The results obtained for sample no.3

3 (Op1)	POSITIVE	NEGATIVE (2*4)
3.P	0	0
3.N	0	4

The results obtained for sample no.4

15 (Op1)	POSITIVE	NEGATIVE (1*4)
15.P	0	0
15.N	0	0

3 (Op2)	POSITIVE	NEGATIVE
3.P	0	0
3.N	0	4

15 (Op2)	POSITIVE	NEGATIVE
15.P	0	0
15.N	0	4

The first operator obtained 14 negative results and 2 positive results, and the second operator obtained 18 negative results and 2 positive results. The logical value had transformed in numeric value: each logical false value became value 0 and each true value become value 1. S_D (**standard deviation) =0, 00**, implicit **r (repeatability) =0, 00**, for each set, cu *repeatability limit = 1* (true value) ±0.

Table 5

The value of standard deviation, repeatability (r) and intermediate precision (R)

S_D =0.00

$t_{5\% \text{ for 16 measurement}}$= **2.12**

r= $t_{5\%}$ ***0.00**= 2.12*0.00=0.00

r=0.00

S_D =0.00.

r=0.00

S_{Dr} =0.00

$t_{5\% \text{ for 36 measurement}}$ = **2.03**

R= $t_{5\%}$ ***0.00**= 2.03*0.00=0.00

R=0.00

S_{Dr} =0.00.

R=0.00

The calculation of repeatability using the values of optical densities (DOP), showed the fallowing results for sample no. 1 S_D (**standard deviation) =0.0048**, implicit **r(repeatability)=0.0108.**

Table 6

Repeatability calculation for sample no. 1 bovine brain homogenate

Nr. crt	OP1	OP2
1	0.027	0.006
2	0.026	0.009
3	0.026	0.008
4	0.021	0.009
5	0.014	0.006
6	0.019	0.004
7	0.020	0.007
8	0.014	0.008
9	0.020	0.011
10	0.016	0.009
Mean	0.020	0.008
Standard deviation	0.0048	0.0020
Trust limit	0.02±0.005	0.01±0.002
tn-1 for. n measurement	2.23	2.23
repeatability	0.0108	0.0045
Uncertainty by repeatability:	0.020±0.011	0.008±0.004
RSD1	0.2380	0.2601
RSD= relative standard deviation		

Intermediate precision (R) had been calculated by multiplying the repeatability with 1.6 an accepted coefficient. The results are qualitative and are expressed like positive and negative. There was obtained 32 negative respective 4 positive results. The logical value had transformed in numeric value: each logical false value became value 0 and each true value become value 1. S_D (**standard deviation) =0, 00**, implicit **R (intermediary precision) =0. 00**, for each set, cu *intermediary precision limit = 1* (true value) ±0.

The calculation of repeatability using the values of optical densities (DOP), showed the fallowing results for bovine brain homogenate S_D (**standard deviation**) =**0.074**, implicit **R(intermediary precision)=0.0247**

Intermediate precision calculation for samples

Nr. crt	Sample no. 1 bovine brain homogenate	Sample no.2 Positive control	Sample no. 3 negative control	Sample no. 15 blank distillate water
1	0.027	1.513	0.015	0.011
2	0.026	1.377	0.025	0.012
3	0.026	1.323	0.024	0.010
4	0.021	1.498	0.021	0.010
5	0.014		0.009	
6	0.019		0.009	
7	0.020		0.011	
8	0.014		0.009	
9	0.020			
10	0.016			
11	0.006			
12	0.009			
13	0.008			
14	0.009			
15	0.006			
16	0.004			
17	0.007			
18	0.008			
19	0.011			
20	0.009			
Mean	0.014	1.428	0.015	0.011
Standard deviation	0.0074	0.0926	0.0070	0.0010
Trust limit	0.01±0.01	1.43±0.09	0.02±0.01	0.01±0.00
tn-1 for. n measurement	2.09	2.78	2.37	2.78
Intermediate precision(*1.6)	0.0247	0.4121	0.0264	0.0043
Uncertainty by intermediate precision	0.014±0.025	1.428±0.412	0.015±0.026	0.011±0.004
RSD	0.5285	0.0649	0.4532	0.0891

RSD= relative standard deviation

The accuracy (AC) is sometimes termed trueness. and result from the comparison of de values to the true value for the sample and had been 100%[4].

The performance indicator after general classification of the samples

The samples status test	The obtained results		Total
	POSITIVE	Negative	
POSITIVE	$N_{11}=4$	$N_{12}=0$	$N_{1.}$
Negative	$N_{21}=0$	$N_{22}=32$	$N_{2.}$

Total	$N_{\cdot 1}$	$N_{\cdot 2}$	$N = N_{1\cdot} + N_{1\cdot}$ or $N_{\cdot 1} + N_{\cdot 2}$

$N_{11=}$ true positive; N_{12} = false negative; N_{21} = false positive; N_{22} = true negative

$$AC = \frac{N_{11} + N_{22}}{N_{11} + N_{12} + N_{21} + N_{22}} \qquad [4]$$

$$AC = \frac{4 + 32}{4 + 0 + 0 + 32} * 100 = 100\%$$

AC=100%

The Chi-square (^{2}) [5] reveal whether hypothesized results are verified by an experiment. and in our case it are almost absolute 0.00. (must be <3.84).

$$\text{Chi - square } (\,^2) = \frac{\left(\left|N_{12} - N_{21}\right| - 1\right)^2}{\left(N_{12} + N_{21}\right)} \qquad [5]$$

The sensitivity (p+/SE) [P(T$^+$|D$^+$)]calculated had been 1. or can be expressed like 100% and reveal the probability that a true positive sample will be tested positive. p+=4/4=1. SE=100% [6]

The specificity (p-/SP) [P(T$^-$|D$^-$)]calculated had been 1 or can be expressed like 100% and reveal the probability that a true negative sample will be tested negative. p-=32/32=1. SP=100% [7]

$$p+ = \frac{N_{11}}{N_{1\bullet}} \qquad [6]$$

$$p- = \frac{N_{22}}{N_{2\bullet}} \qquad [7]$$

Positive predictive value (D$^+$|T$^+$)=N_{11}/(N_{11}+N_{21}) is the proportion of positive test sample it is true positive. and it is 4/4=1.

Negative predictive value (D$^-$|T$^-$)=N_{22}/(N_{22}+N_{12}) is the proportion of negative test sample it is true negative. and it is 32/32=1.

False positive rate pf+ is the proportion of negative instances that were erroneously reported as being positive .It is equal to **1** minus the specificity of the test: pf+=0.[9]

False negative rate pf- is the proportion of positive instances that were erroneously reported as being negative .It is equal to **1** minus the sensibility of the test: pf-=0.[8]

$$pf- = \frac{N_{12}}{N_{\bullet 1}} \qquad [8]$$

$$\mathrm{pf+} = \frac{N_{21}}{N_{\bullet 2}} \qquad\qquad [9]$$

The detection limit had been estabileshed arbitrary at 1/8 serial dilution of positive control. because don't had been obatained any doubtful result. and at concentration higher than ¼ the results was positive.

The quantification limit had been estabileshed at 1/4 of serial dilution of positive control. because at concentration higher than ¼ the results was positive.

The DOP obtained from the serial dilution of the positive control. revealed a cvasilinear rising proportional related with the analit concentration. the fact can let to consider that bthe assay have linearity. The extreme value of DOP didn't respected this condition. and the value lower than 0.067 and greater than 2.014nm must be interprete carrefully according with this parameter. This particularity of ELISA assay can not affect the performance of the test. who is calibrated for detection even the PrP[res] is present at very low limit.

Table 9

Results obtained from serial dilution of positive control

|	EST	Dilution	DOP	DOP	DOP-mean	Result	Dilution	DOP-mean
2	0	2	**2.078**	**2.022**	**2.050**	POZITIV	2	**2.05**
1	0	1	**1.973**	**2.055**	**2.014**	POZITIV	1	**2.014**
1	1	0.5	**1.572**	**1.565**	**1.569**	POZITIV	0.5	**1.5685**
1	2	0.33	**0.892**	**0.950**	**0.921**	POZITIV	0.33	**0.921**
1	4	0.2	**0.418**	**0.416**	**0.417**	POZITIV	0.2	**0.417**
1	8	0.111	**0.164**	**0.170**	**0.167**	NEGATIV	0.111	**0.167**
1	16	0.058	**0.062**	**0.071**	**0.067**	NEGATIV	0.058	**0.0665**
1	32	0.03	**0.028**	**0.026**	**0.027**	NEGATIV	0.03	**0.027**
1	64	0.015	**0.016**	**0.019**	**0.018**	NEGATIV	0.015	**0.0175**
1	128	0.0077	**0.009**	**0.015**	**0.012**	NEGATIV	0.0077	**0.012**
1	256	0.0038	**0.016**	**0.011**	**0.014**	NEGATIV	0.0038	**0.0135**
1	512	0.0019	**0.016**	**0.002**	**0.009**	NEGATIV	0.0019	**0.009**
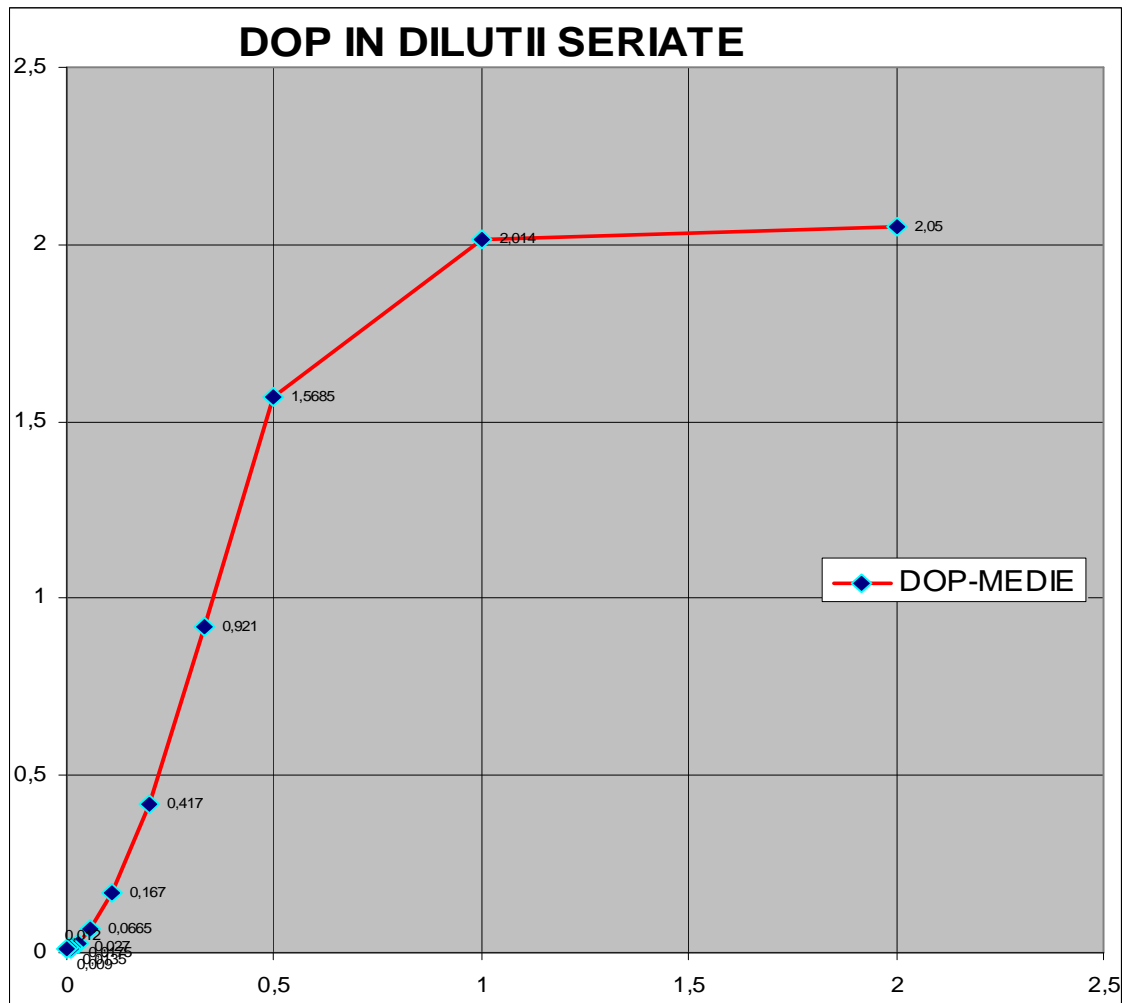
Fig. 1. The distribution of DOP proportional with serial dillution

CONCLUSION

The protocol revealed that the method is valid, and can be used proper in the laboratory for it purpose.

The values of the standard deviation. repeatability and intermediate precision are low. according with our expectation.

The high sensitivity and specificity is indicator of the high performances of the test.

BIBLIOGRAPHY

1. SR EN ISO 17025: 2005 General requirements for the competence of testing and calibration laboratories
2. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals:2006- CHAPTER 1.1.3.PRINCIPLES OF VALIDATION OF DIAGNOSTIC ASSAYS FOR INFECTIOUS DISEASES