Internal control for the search of Brucellosis into cow milk by ELISA method

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Introduction

Brucellosis is a cattle disease transmissible to humans and dangerous for them. If contamination occurs, it could have a significant economic and health impact. Every year, approximately 9000 cow milk samples are analyzed in the GALILAIT laboratory with ELISA test to detect Brucellosis. Internal controls allow to confirm a positive sample in case of doubt, as well as watch micro-plates and control samples stability.

Material and methods

Internal control making was composed of five steps: reconstitution of pure positive serum, serum dilution of 1/10 in water, second serum dilution in negative milk to be determined, ELISA test to validate dilutions and to finish aliquoting. Note that a second dilution was performed into milk, it will allows to have an internal control which has the same matrix than the analyzed samples.
An ELISA test was carried out with micro-plates sensitized with a Brucellosis lipopolysaccharide. Each micro-plate contained 96 micro-wells. Milk samples were put into wells. If the milk was contaminated, the sample contained anti-brucellosis antibodies which made a complex with the antigens present into the wells. After micro-plate incubation and washing, a conjugate was added, it bound to the complex antigens-antibodies present. After a second incubation and washing, a substrate was added into wells. If there conjugate bound was present, the substrate became blue then yellow after adding a stop solution. The color intensity resultant was proportional to the antibodies quantity into the tested milk. This intensity was read with a spectrophotometer which gives optical density (DO) of each micro-well. Two controls provided in the ELISA kits, were placed into every plate, one positive and one negative. These controls allowed to calculate %E/P of each well and so to determine if they were positive or not using the formula:

$$\%E/P = \frac{(DO\ (sample)-DO(\ TN))}{(DO\ (TP)-DO(\ TN))*100}$$

TN: Negative control
TP: positive control

If this percentage was higher than 55%, the sample was positive. If it was lower than 45% the sample was negative and if it was between 45 and 55%, the sample was dubious.

Results and discussion

Dilutions of 1/10, 1/50, 1/100, 1/150 and 1/200 were performed into negative powder milk reconstituted to determine which dilution factor will be applied. Then each sample was analyzed by ELISA test. These tests were divided in three parts, therefore three negative controls and three positive controls were used. After reading the DO of each diluted milk and %E/P calculation (figures 1, 2 and 3), we can deduct that the dilution which will have permitted to have a %E/P greater than 55% but still close to this value, is a 1/150 dilution.

So pure serum was diluted at 1/10 into pure water then at 1/150 into negative milk, then it was aliquoted in 0.5 ml and used for each Brucellosis ELISA test.

1/10	%E/P	1/50	%E/P	1/100	%E/P
3.324	175.52	3.295	173.92	2.199	113.55
3.15	165.93	2.949	154.86	2.159	111.35

Figure 1: ELISA test, 1st dilution series 1/10, 1/50, 1/100
TN value: 0,138-TP value: 1,953

TN	TP	1/150	%E/P
0.083	1.27	1 .266	76.62
		1.28	77.53

Figure 2: ELISA test, 2nd dilution series 1/150

TN	TP	1/200	%E/P
0.116	1.969	0.825	38.27
		0.721	32.66

Figure 3: ELISA test, 3rd dilution series 1/200

Conclusion

The internal controls used in the search of cattle diseases like Brucellosis is mandatory and required by the standard COFRAC 109-05. ELISA method is used for cattle disease search because this method is simple, reliable and inexpensive.

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