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A proficiency testing method for detecting antibodies against *Brucella abortus* in quantitative and qualitative serological tests

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Summary

A proficiency testing panel for detecting antibodies against *Brucella abortus* was developed and evaluated by both primary binding and conventional serological tests, using the guidelines of the World Organisation for Animal Health and the International Organization for Standardization Guide 43-1.

All serological tests were judged satisfactory. Among the primary binding tests, the competitive enzyme-linked immunosorbent assay (ELISA 2) and the indirect enzyme-linked immunosorbent assay (ELISA 1), with standard deviation indices (z-scores) of –0.06 and 0.10, respectively, performed best. Similarly, E_n numbers (i.e. a way of comparing different measurements of performance) of 0 for both the competitive ELISA 2 and the indirect ELISA 1 indicated that these tests performed best in the initial round of proficiency testing. The conventional serological tests all passed the panel.

Comparing data from both the quantitative and qualitative tests demonstrated that this proficiency testing scheme was fit for the purpose for which it was designed.

Keywords

Brucella abortus – Brucellosis – E_n numbers – Fit for purpose – Proficiency testing – Z-scores.

Introduction

One of the stated purposes of proficiency testing is the overall improvement of laboratory diagnostic performance, thus providing the analyst, the laboratory and the client with the confidence that the results produced by the laboratory are indeed valid.

The challenge was to develop a proficiency panel suitable for use with the wide variety of serological tests available for the detection of antibody to *Brucella abortus*. These tests have a broad range of performance characteristics (i.e. sensitivity and specificity), as shown by Gall and Nielsen (14).

A proficiency panel fit for the purpose would require well-defined sera as described by the World Organisation for Animal Health (OIE) (33). The detection limits and detection of the four major bovine isotypes vary between these different serological tests (22, 24). As a result, it was

essential to ensure that positive sera included in this panel would react unequivocally.

Additionally, other requirements outlined by the OIE (33) were considered, including:

– that the sera were derived from single animals or a pool of animal sera

– that these sera were undiluted or diluted in negative sera

– that the sera were not lipaemic, haemolysed or contaminated

– that these sera were free of infectious agents.

Some of these factors could interfere with the subjective reading and interpretation of conventional serological tests, such as the:

– buffered antigen plate agglutination test (BPAT)

– card test (CT)

– complement fixation test (CFT)

– tube agglutination test (TAT).

Another consideration was the evaluation of results, as outlined by the International Organization for Standardization (ISO) Guide 43-1 (16), for both quantitative and qualitative tests. The choice of statistics for quantitative tests, including the competitive enzyme-linked immunosorbent assay (c-ELISA), the fluorescence polarisation assay (FPA) and the indirect ELISA (iELISA), was described in the ISO Guide 43-1 (16) and ISO 13528 (17). As a result of the descriptive nature of qualitative data (i.e. positive, negative or suspicious), the choice of statistics was limited.

With the above criteria for guidance, a proficiency panel, which included replicate samples, was developed to analyse repeatability within the laboratory and reproducibility between laboratories. This panel was also intended to provide performance indicators (i.e. z-scores, E_n numbers), to aid laboratories and analysts to assess their ability to perform tests competently (18), and to determine the degree of equivalence (19) between tests, analysts and laboratories.

Materials and methods

Participating laboratories

Three laboratories, all experienced and knowledgeable in laboratory diagnosis of *Brucella*, participated in the design, preparation and preliminary data evaluation of the proficiency panel.

These laboratories were:

– the Canadian Food Inspection Agency, Ottawa Laboratory Fallowfield, Ottawa, Ontario, Canada

– Servicio Nacional de Sanidad y Calidad Agroalimentaria (Senasa), Departamento Brucelosis, Dirección de Laboratorios y Control Técnico, Buenos Aires, Argentina

– Universidad Autónoma de Baja California, Mexicali, Mexico.

For the purposes of confidentiality, the results of c-ELISA, FPA and iELISA testing were not identified with any particular laboratory.

Sera

Three defined groups of sera (n = 107) were initially assembled and referred to as:

– negative sera

– sera from animals from which *B. abortus* was isolated

– serologically positive sera.

The negative sera were from individual Canadian cows that tested negative for the presence of antibodies against *B. abortus* in all serological tests. Canada has been officially free of bovine brucellosis since 1984. The sera from culture-positive animals were from a pre-1984 serum bank of samples from individual cattle which had been naturally infected with *B. abortus*. The serologically positive sera were two CFT reactors from Argentina, containing greater than 288 and 1,323 CFT international units per millilitre of serum, respectively, and from which *B. abortus* had been isolated.

Serological tests

All sera were pre-screened at the Canadian Food Inspection Agency Ottawa Laboratory Fallowfield, to determine the suitability of each serum for proficiency panel inclusion, using the:

– BPAT

– TAT

– CFT

– iELISA

– c-ELISA

– FPA.

The above six tests were performed according to their respective standard operating procedures (SOP): BR-LD011 (7), BR-LD022 (8), BR-PR005 (6), BR-LD014 (5), BR-PR041 (10) and BR-PR040 (9). The diagnoses were

made according to the acceptance/rejection criteria approved in their respective SOP.

As described in the SOP, the BPAT and the TAT were performed according to the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (34).

The CFT SOP was adapted from procedures originally described by Samagh and Boulanger (28) and Alton *et al.* (2, 3).

Similarly, the c-ELISA, iELISA and FPA were performed as previously described by Nielsen *et al.* (23, 25, 26).

Panel design and preparation

After the initial pre-screening, the number of sera judged suitable for inclusion in the panel was reduced from 107 to 19. Further testing and evaluation resulted in the selection of 15 sera. These 15 sera consisted of nine positive sera and six negative sera. Each serum was aliquoted (0.6 ml) in triplicate (n = 45) into a 96-well plate and lyophilised, after being coded and randomised (1) for placement. These 45 coded sera, identified as an exploratory panel (ID 060116), were shipped to collaborating laboratories in Argentina and Mexico for evaluation by CT, the 2-mercaptoethanol agglutination test (2-ME), the rivanol agglutination test (RIV), c-ELISA and FPA.

In Argentina, the BPA and 2-ME were performed according to standard procedures (29), and the antigens used were prepared by Senasa, while the c-ELISA and FPA were performed as described by Nielsen *et al.* (23, 24).

The CT antigen in Mexico, prepared by Productora Nacional de Biológicos Veterinarios (PRONABIVE), was Rose-Bengal stained, buffered at a pH of 3.65 ± 0.05 and adjusted to 3% cell concentration.

For the RIV test, the method described by Morilla and Bautista (20) was employed. The antigen and the rivanol reagent were provided by PRONABIVE and the serum dilutions used were 1:25, 1:50, 1:100 and 1:200.

After the results from Panel ID 060117 were collated and evaluated, the number of sera for inclusion in the final version of the proficiency panel was further reduced from 15 to 12. The 12 sera consisted of six positive sera and six negative sera. Two of the positive sera were from Argentina while the remainder were sera from the pre-1984 serum bank. All the negative sera were derived from individual Canadian cows.

A second evaluation panel (ID 060403) was prepared and evaluated by the participating laboratories.

Data expression and evaluation

Only the data from the final version of the panel design (Panel ID 060403) were considered for data analysis.

The determination of an assigned value and its uncertainty were the consensus values from the test results as defined by Thompson and Wood (32), ISO Guide 43-1 (16), the Standards Council of Canada (30), and ISO 13528 (17).

For the iELISA, optical density (OD) results for each individual test sample were expressed as a percentage of the mean of the positive controls included on each 96-well microplate after a development time of ten minutes. The percentage of positivity (% P) was calculated as follows:

% P = (OD of the test sample ÷ mean OD of the positive control) × 100

Similarly, for the c-ELISA, individual test sample OD results were expressed as a percentage of the mean of the buffer controls included on each 96-well microplate after a development time of ten minutes. Percentage of inhibition (% I) was calculated as follows:

% I = 100 – (OD of the test sample ÷ mean OD of the buffer control) × 100

For summary statistics (see 'Results' below), the data for each test were analysed for outliers using the individual test mean plus three standard deviations (31). However, for performance indicators (i.e. z-scores, E_n numbers), outliers must be evaluated within the proficiency scheme and should not be removed (16, 30).

The c-ELISA, FPA and iELISA data were tested for normal distribution using the D'Agostino and Pearson test (Medcalc 2006, version 8.2.10. for Windows, Mariakerke, Belgium). Before using parametric statistics, such as z-scores or E_n numbers, normality was verified (12, 16, 30).

The calculation of the standard deviation index (SDI) or z-scores (16, 30, 32) reduces the data to the same standardised values, making it possible to compare results of different quantitative tests without referring to the means, standard deviations or different units of measurement (32). As a measure of relative accuracy, the SDI was calculated as follows:

SDI (z-scores) = (individual test mean – mean of all the data values)/standard deviation of all the data values

Similarly, a measure of relative precision, the co-efficient of variation (% CV) index (CVI) was calculated as follows:

CVI = % CV of the individual test ÷ % CV of all the data values

In a similar way, E_n numbers (21, 30, 31) also transform data into standardised values, taking into account the measures of uncertainty for both the test result and the assigned value, thus allowing for the comparison of various tests with different units of measurement, such as the SDI. Thus, E_n numbers were calculated as follows:

$E_n = (x - X)/\text{square root of } (U^2_{Lab} + U^2_{Ref})$

Where:

x = the mean of the results from a test

X = the mean of all the values (i.e. the assigned value by consensus)

U^2_{Lab} = the uncertainty of the results of a test or the results from a laboratory

U^2_{Ref} = the uncertainty of all the values (i.e. the assigned value by consensus)

A one-way analysis (ANOVA) was adapted for the comparison of qualitative and quantitative tests.

Prior to performing an ANOVA (Medcalc 2006, version 8.2.10.), the test results for each test were transformed (4) into one dichotomous variable, containing the code 1 for positive values and the code 0 for negative values when compared with the negative cut-off of each test. The ANOVA was performed on the transformed data of 36 samples of each test (n = 22 tests) to accept or reject the null hypotheses that the individual test means were equal.

The ANOVA F statistic or ratio was calculated by dividing the mean of the squares between groups by the mean of the squares within groups. The tabulated F statistic was calculated using the FINV function of Microsoft Excel (2002, version 10.5313.4219 SP2 for Windows, Washington, United States of America).

Results

Two of the three c-ELISA results had 100% agreement, whereas the third c-ELISA gave 97.2% agreement (i.e. 35/36 results). All c-ELISA tests used the same cut-off value of 30% inhibition. The one discrepant value was 34% for a negative serum, which was 4% above the cut-off value.

Similarly, two of the three iELISA results, with cut-off values of 53% and 46% positivity, respectively, had 100% agreement. A third iELISA, using recombinant protein A/G, gave 97.2% agreement with a negative cut-off of 20% positivity (27). The aberrant result was 24% positivity for a negative serum.

There was 100% agreement between the BPAT performed in Canada and that performed in Argentina.

The remaining conventional serological tests (i.e. the CFT, Rose-Bengal test, TAT and RIV) correctly identified all the sera.

No outliers were detected prior to the calculation of the summary statistics (Table I), based on the mean plus three standard deviations (31).

A total of nine FPA were performed (Table I), using different instrumentation and analysts. The FPA 1, 2, 6, 7 and 9, as shown in Table I, were performed on the same type of instrument (Instrument 1) by five different analysts. There was 100% agreement between FPA 1, 2, 6 and 7. For FPA 9, one triplicate value for one positive serum and three negative sera disagreed (Table I), resulting in an 88.9% agreement.

The data for FPA 3 and 8 gave 100% agreement. Both of these FPA were performed using a different instrument from that in the earlier FPA tests (i.e. Instrument 2) by two different analysts.

Tests FPA 4 and FPA 5 were performed on two different instruments again (Instruments 3 and 4), using the microplate format, by the same analyst. Once more, there was 100% agreement, although the negative sera produced negative data values with FPA 4. However, these negative values did not affect the final outcome, although the summary statistics were affected, as shown in Table I.

Based on a calculated p-value greater than 0.05 (Table II), the c-ELISA, FPA and iELISA data were confirmed as having a normal distribution (Medcalc, 2006, version 8.2.10.) before determining their performance measures, such as z-scores and E_n numbers.

In Figure 1, the FPA test results are compared with those of the c-ELISA and iELISA, using the SDI or z-scores as a measure of accuracy. An SDI value of 2 indicates that the c-ELISA, FPA or iELISA individual test means are two standard deviations from their respective mean of all the test values for the c-ELISA, FPA or iELISA. On the other hand, SDI values (z-scores) greater than +2 or less than –2 indicate that the individual test means are not in good agreement with the mean of all the values and are questionable. However, SDI values between –2 and +2 are considered satisfactory (12, 16, 30).

Figure 2 compares the c-ELISA, FPA and iELISA values using the co-efficient of variation index (CVI) as a precision index (a relative measure of precision). Using this measure, CVI values of less than two are considered satisfactory. The majority of the tests gave CVI values of less than one.

Table I
The summary statistics of nine fluorescence polarisation assay tests (FPA), using four different instruments and five different analysts

Index	Status	ID	FPA1	FPA2	FPA3	FPA4	FPA5	FPA6	FPA7	FPA8	FPA9	Mean	SD	%CV
1	+	**P9**	**226**	**175**	**169**	**181**	**213**	**217**	**221**	**227**	**168**	**200**	**26**	**13**
2	+	**P9**	**221**	**187**	**179**	**192**	**203**	**214**	**221**	**234**	**178**	**203**	**20**	**10**
3	+	**P9**	**221**	**192**	**187**	**188**	**208**	**215**	**226**	**229**	**153**	**202**	**24**	**12**
4	+	P10	225	188	177	206	206	220	226	236	183	207	21	10
5	+	P10	227	192	184	204	235	221	220	229	169	209	23	11
6	+	P10	224	193	190	210	230	218	232	239	117	206	37	18
7	+	**P13**	**136**	**109**	**105**	**143**	**180**	**130**	**156**	**135**	**112**	**134**	**24**	**18**
8	+	**P13**	**134**	**108**	**112**	**138**	**173**	**132**	**145**	**134**	**89**	**129**	**24**	**19**
9	+	**P13**	**129**	**113**	**107**	**153**	**186**	**131**	**229**	**135**	**91**	**142**	**43**	**30**
10	+	P15	254	218	210	198	230	250	251	258	209	231	23	10
11	+	P15	252	221	215	201	226	250	225	262	198	228	23	10
12	+	P15	254	217	211	199	226	249	257	262	228	234	23	10
13	+	**P17**	**250**	**221**	**214**	**190**	**204**	**251**	**262**	**265**	**185**	**227**	**31**	**14**
14	+	**P17**	**253**	**212**	**206**	**188**	**229**	**252**	**258**	**274**	**197**	**230**	**31**	**13**
15	+	**P17**	**241**	**216**	**205**	**196**	**224**	**252**	**260**	**275**	**195**	**229**	**29**	**13**
16	+	P18	246	197	194	154	199	243	237	242	176	210	33	16
17	+	P18	244	160	162	141	194	237	243	247	176	200	43	21
18	+	P18	233	212	200	160	199	237	246	252	170	212	33	16
19	–	**N1**	**69**	**59**	**59**	**–9**	**40**	**61**	**89**	**62**	**34**	**52**	**28**	**54**
20	–	**N1**	**72**	**56**	**55**	**–6**	**25**	**69**	**78**	**62**	**27**	**49**	**28**	**57**
21	–	**N1**	**69**	**47**	**49**	**–29**	**40**	**71**	**81**		**56**	**47**	**37**	**78**
22	–	N2	53	58	60	29	50	66	83	68	48	57	15	26
23	–	N2	73	68	62	15	39	64	85	60	147	68	36	53
24	–	N2	69	57	54	–9	21	74	85	57	53	51	29	56
25	–	**N3**	**60**	**67**	**59**	**–15**	**15**	**53**	**73**	**52**	**15**	**42**	**30**	**71**
26	–	**N3**	**76**	**62**	**55**	**–40**	**32**	**66**	**82**	**41**	**105**	**53**	**41**	**77**
27	–	**N3**	**62**	**65**	**55**	**–36**	**28**	**70**	**77**	**54**	**74**	**50**	**35**	**71**
28	–	N4	64	63	57	–14	12	72	89	60	61	52	32	62
29	–	N4	61	54	50	–22	6	67	79	58	9	40	34	84
30	–	N4	69	65	59	5	60	63	82	63	77	60	22	37
31	–	**N5**	**72**	**64**	**57**	**7**	**31**	**64**	**78**	**67**	**31**	**52**	**24**	**45**
32	–	**N5**	**73**	**63**	**62**	**22**	**72**	**68**	**78**	**60**	**52**	**61**	**17**	**27**
33	–	**N5**	**67**	**58**	**58**	**–10**	**48**	**65**	**76**	**64**	**78**	**56**	**26**	**47**
34	–	N7	70	58	60	14	67	66	76	62	129	67	29	44
35	–	N7	57	67	61	16	48	69	76	66	48	56	18	32
36	–	N7	65	66	62	8	47	67	81	63	68	59	21	36
		Mean	144	123	118	88	124	142	155	147	114	128	28	34
		SD:	83	68	67	96	89	82	78	92	65	79	7	24
		%CV:	58	56	56	109	72	58	51	63	57	61	25	69

SD: standard deviation
%CV: co-efficient of variation

As with the SDI scores, E_n numbers can be used to compare tests that use different methods of data expression. Thus, Figure 3 compares the c-ELISA, FPA and iELISA tests. Values less than or equal to one are considered satisfactory (16), indicating that, in this exploratory round of proficiency testing, all the tests were acceptable.

There was no significant difference ($p = 1.0$) between qualitative and quantitative tests, as shown by ANOVA in

Table II
A comparison of the calculated p-values from the D'Agostino and Pearson test for the normal distribution of the proficiency test data from the competitive enzyme-linked immunosorbent assay (c-ELISA), fluorescence polarisation assay (FPA) and indirect enzyme-linked immunosorbent assay (iELISA)
P-values greater than 0.05 indicate normal distribution

Test	P-value
c-ELISA1	0.1284
c-ELISA2	0.1212
c-ELISA3	0.1468
FPA1	0.1190
FPA2	0.1164
FPA3	0.1172
FPA4	0.1488
FPA5	0.1431
FPA6	0.1179
FPA7	0.1280
FPA8	0.1319
FPA9	0.2601
iELISA1	0.1243
iELISA2	0.1247
iELISA3	0.1549

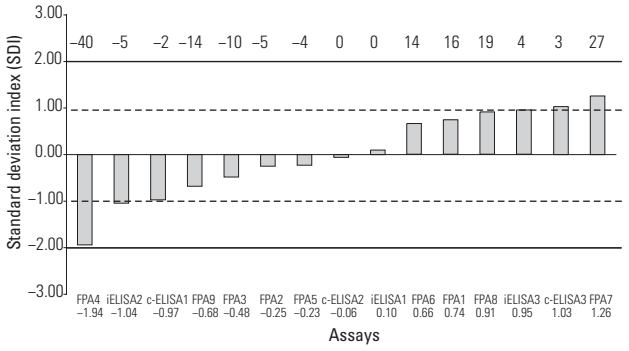

Fig. 1
A comparison of the competitive enzyme-linked immunosorbent assay (c-ELISA), fluorescence polarisation assay (FPA) and indirect enzyme-linked immunosorbent assay (iELISA) tests, using the standard deviation index (z-scores)
The standard deviation index values between –2 and +2 were considered satisfactory. The differences between the test means and their respective assigned value were aligned from the lowest to the highest value within each test method

Table III. Additionally, the calculated F statistic (0.072) was less than the tabulated F statistic (1.570), indicating that the individual population means were equal. Thus, the null hypothesis (H) (i.e. H_0: $\mu_1 = \mu_2 = \mu_3$ = etc.), that the population means were essentially the same, was not rejected.

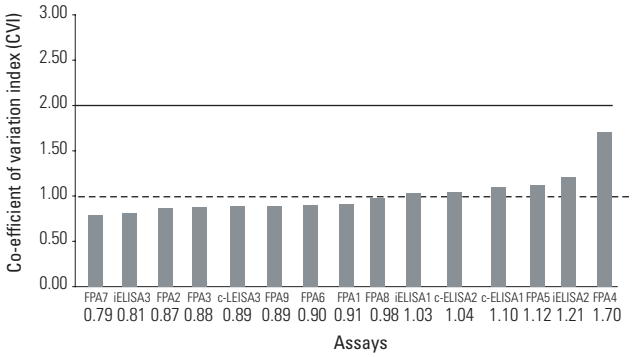

Fig. 2
A comparison of the competitive enzyme-linked immunosorbent assay (c-ELISA), fluorescence polarisation assay (FPA) and indirect enzyme-linked immunosorbent assay (iELISA), using the co-efficient of variation index (a measure of precision)

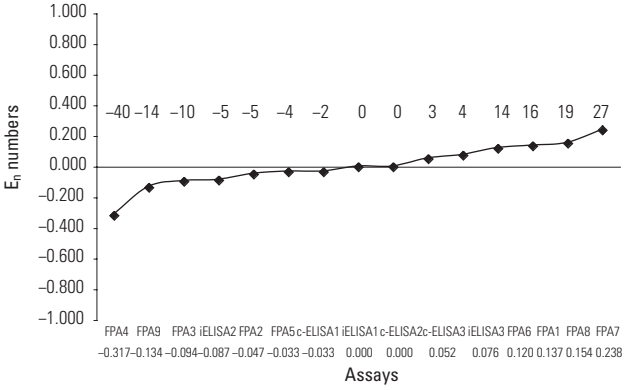

Fig. 3
An evaluation of the competitive enzyme-linked immunosorbent assay (c-ELISA), fluorescence polarisation assay (FPA) and indirect enzyme-linked immunosorbent assay (iELISA), through the use of E_n numbers
E_n values between –1 and +1 were judged to be satisfactory. Unlike the standard deviation index, the differences between the test means and their respective assigned values were ordered from the lowest value to the highest value, regardless of test method

Discussion

Although not a requirement of ISO Guide 43-1 (16), triplicate samples of each positive and negative serum selected for Panel ID 060304 were included in the proficiency panel. By including triplicate samples, a measure of variation within a test or laboratory could be ascertained and used to help resolve anomalous results. For instance, the discrepancy in the results obtained for both the c-ELISA and the iELISA could be due to insufficient mixing of the sample or improper pipette technique, such as an air bubble in the pipette tip, leading to improper volumes. Discrepant results obtained for FPA 9 suggest similar possibilities or that the instrument was not functioning correctly. The use of duplicate or triplicate samples assists the analyst or laboratory to 'troubleshoot'

Table III
A one-way analysis of variance, showing no significant difference between tests (p = 1.0)
The population means for each test are equal
($F_{0.05, 21, 769}$ = 0.027; n = 36)

Source of variation	Sum of squares	Degrees of freedom	Mean square
Between groups (influence factor)	0.1482	21	0.0071
Within groups (other fluctuations)	197.5762	769	0.2569
Total	**197.7244**	**790**	

Factor	Mean
2ME	0.5000
BPA	0.5000
BPAT	0.5000
CARD	0.5000
c-ELISA1	0.5000
c-ELISA2	0.5000
c-ELISA3	0.5278
CFT	0.5000
FPA1	0.5000
FPA2	0.5000
FPA3	0.5000
FPA4	0.5000
FPA5	0.5000
FPA6	0.5000
FPA7	0.5000
FPA8	0.5143
FPA9	0.5556
iELISA1	0.5000
iELISA2	0.5278
iELISA3	0.5000
RIVANOL	0.5000
TAT	0.5000

The results for each test were classified as one dichotomous variable containing the code 1 for positive and the code 0 for negative when compared with predetermined status and the negative cut off of each test. The sample size was 791 samples

tests and identify various random and systemic sources of variation, as described by Gall and Nielsen (13) and Dimech *et al.* (11).

The calculation of SDI values or normal deviates or z-scores is a statistical method of standardising or normalising a test result relative to standard deviation. Since the SDI units are in standard deviations, this standardisation allows comparisons of the relative positions of tests with different measurement units (15, 32), such as quantitative serological assays (i.e. FPA, c-ELISA and iELISA), by reducing the data to the same value indicators. A positive SDI value indicates that the test mean was larger than the mean of all the values, whereas a negative SDI value indicates that the test mean was less than the mean of all the values. For instance, the SDI for FPA 4 was –1.94 standard deviations below the mean for all the FPA values. The SDI for the c-ELISA 2 was –0.06 standard deviations below the mean of all the c-ELISA values, a score substantially better than that of the FPA 4. From these standard scores, it is clear that the performance of the c-ELISA 2 was better, relative to the mean of the c-ELISA values, than the FPA 4, relative to the mean of all the FPA values (Fig. 1). Similarly, an SDI of 0.10 standard deviations for iELISA 1 indicated a better performance than that of FPA 4 but slightly lesser than the c-ELISA 2, relative to their respective group means (assigned values).

Relative performance of the quantitative tests, such as the c-ELISA, FPA and iELISA, may also be compared using E_n numbers (16, 30). In a similar way to the SDI, test results are transformed or reduced to the same value, thus making comparisons between the various tests easier. In this study, the assigned value (i.e. the mean of all the FPA tests combined) and its uncertainty (i.e. the standard deviation of all the FPA tests combined) are consensus values derived from the tests of the participating laboratories (16, 30). All the tests were considered acceptable, since all the E_n numbers were less than or equal to one.

It was interesting to observe the tests using E_n numbers as opposed to the tests using SDI. In both data evaluations, the c-ELISA 2 and iELISA 1 had the lowest values, while the FPA 4 and FPA 7 were at the extremes of the abscissa (or *x* co-ordinate), as shown in Figures 1 and 3. This suggests that both the SDI and E_n numbers were similar when identifying the closeness of agreement between the mean value of a test result and the assigned (consensus) value. Tests between lowest values and the extremes were exactly the same, except that their ranking was different. For instance, FPA 8 had a higher E_n number than c-ELISA 3, whereas the emphasis for SDI was the opposite for both tests (e.g. for c-ELISA 3, the SDI was higher than the FPA 8). In fact, the data range between the mean of the test and their respective group means (assigned values) aligned perfectly from the lowest value to the highest value with the E_n numbers, regardless of the test. However, for the SDI, the same values were similarly aligned but grouped by test type. This suggests that the E_n numbers were better suited for ranking tests or laboratories. For instance, the test with the greatest difference between the mean and the assigned value was FPA 4, with an E_n number of –0.317, while the test with the least difference was the c-ELISA 2 or iELISA 1, both of which centred on the abscissa.

Co-efficients of variation index values are a measure of relative precision, indicating the amount of variation or dispersion about the tested sample. Low CVI values indicate a smaller variation from the individual test mean, whereas high CVI values indicate more variation or dispersion of results about the individual test mean. For instance, although both the iELISA 1 and c-ELISA 2 had the best SDI values, their CVI values were 1.03 and 1.04,

respectively, suggesting more dispersion about the individual test means than the iELISA 3 and FPA 7, which both had higher SDI values. So, excellent SDI values (a measure of relative accuracy) do not necessarily translate into good CVI values (a measure of relative precision).

While SDI values or E_n numbers are useful to compare quantitative tests, there is no similar methodology for qualitative tests (i.e. BPAT, CFT, RBT, RIV), or for comparing quantitative and qualitative tests with one another. By reducing the various test results to one dichotomous variable, as explained above in 'Data expression and evaluation', an ANOVA was used to show that there was no significant difference (p = 1.0) between qualitative and quantitative tests for Panel ID 060403. Although the F statistic shows no difference in the individual test means, this figure may be biased, due to the method of data reduction. However, as a method for comparing qualitative and quantitative tests, or laboratories using different test systems, it summarises the data adequately for comparison purposes and could aid the diagnostician in troubleshooting tests that were inconsistent.

Based on the definitions for repeatability and reproducibility as described by the OIE (33), and data evaluation according to the ISO Guide 43-1 (16) and ISO 13528 (17), it was concluded that the proficiency panel described in this article was fit for the purpose for which it was designed and prepared.

Un essai d'aptitude pour les épreuves sérologiques quantitatives et qualitatives de détection des anticorps dirigés contre *Brucella abortus*

D. Gall, K. Nielsen, A. Nicola & T. Renteria

Résumé

Un ensemble de tests pour la détection d'anticorps dirigés contre *Brucella abortus* a été mis au point, évalué et validé en utilisant des réactions de liaison primaire et de tests sérologiques classiques, conformément aux Lignes directrices de l'Organisation mondiale de la santé animale et au Guide 43-1 de l'Organisation internationale de normalisation.

Toutes les épreuves sérologiques ont été jugées satisfaisantes. Parmi les réactions de liaison primaire, les meilleures performances ont été celles de l'épreuve immuno-enzymatique (ELISA) de compétition (ELISA 2) et l'ELISA indirect (ELISA 1), avec des écarts-types (*Z-scores*) respectifs de –0,06 et 0,10. De même, les valeurs d'E_n (une mesure de performance) égales à 0,0 pour les deux épreuves ELISA 2 de compétition et ELISA 1 indirect, indiquent que ces tests ont donné de meilleurs résultats lors du premier cycle de l'essai d'aptitude. Les épreuves sérologiques classiques ont toutes réussi en utilisant le processus d'évaluation.

La comparaison des données obtenues par les épreuves quantitatives et qualitatives montre l'aptitude du procédé de validation à l'emploi qui lui est assigné.

Mots-clés

Aptitude à l'emploi – Brucella abortus – Brucellose – Essai d'aptitude – Valeur d'E_n – Z-score.

Método de análisis de la eficacia de detección de anticuerpos contra *Brucella abortus* de pruebas serológicas cuantitativas y cualitativas

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Resumen

Los autores describen la elaboración y evaluación de un cuadro de análisis de la eficacia en la detección de anticuerpos contra *Brucella abortus* por medio de pruebas serológicas, tanto de unión primaria como convencionales, utilizando las Directrices de la Organización Mundial de Sanidad Animal y la Guía 43-1 de la Organización Internacional de Normalización.

Todas las pruebas serológicas fueron juzgadas satisfactorias. De entre las técnicas de unión primaria, las más eficaces resultaron el ensayo inmunoenzimático de competición (ELISA 2) y el ensayo inmunoenzimático indirecto (ELISA 1), con índices de desviación típica (*z-scores*) de –0,06 y 0,10 respectivamente. Análogamente, el valor E_n (esto es, la medida de rendimiento) de 0,0 obtenido con el ELISA 2 (de competición) y el ELISA 1 (indirecto) denota una eficacia óptima de ambas pruebas en la ronda inicial de análisis. Todas las pruebas serológicas convencionales superaron el examen.

La comparación de los datos correspondientes a las pruebas cuantitativas y cualitativas demuestra que este sistema de análisis se ajusta a los fines con que fue concebido.

Palabras clave

Adecuación a una finalidad – Análisis de la eficacia – Brucella abortus – Brucelosis – Valor E_n – Z-score.

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