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Harmonisation of European tests for serological diagnosis of *Brucella* infection in bovines

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Summary

The principal methods for the serological diagnosis of bovine brucellosis are the complement fixation test (CFT), serum agglutination test (SAT), Rose-Bengal test (RBT), indirect enzyme-linked immunosorbent assay (iELISA) and more recently the competitive ELISA (cELISA) and the fluorescent polarisation assay (FPA). Guidelines set by the World Organisation for Animal Health (OIE) describe methods and diagnostic thresholds for each of these tests. Many countries have adopted these methods for the purposes of eradication of brucellosis and have legislated for the use of these tests (the CFT and SAT in particular) for the prevention of the spread of the disease through international trade. Within the European Union (EU) each member state has a National Reference Laboratory which regulates the quality of brucellosis diagnosis and works to the recommendations set by the OIE. This article describes the results from the first three EU ring trials assessing the harmonisation of diagnostic tests between each member state. The general level of harmony for SAT, CFT, and iELISA was found to be good, but issues of standardisation of the RBT, cELISA and FPA remain. The cELISA and FPA in particular need further work to create European harmony. The ring trials also proved successful at providing specific evidence of poor performance in some areas. The decision on whether or not to take action on the basis of these results rested with the individual laboratories concerned. The increase in the number of participants in these trials over time reflected the enlargement of the EU and increased the need for quality assurance.

Keywords

Brucella – Complement fixation test – Enzyme-linked immunosorbent assay – Harmonisation – Ring trial – Rose-Bengal test – Serum agglutination test.

Introduction

Serological techniques are the mainstay of diagnosis and mass testing programmes for brucellosis. The most successful of the serological diagnostic tests are based on the detection of antibodies to the lipopolysaccharide antigen of smooth *Brucella* strains. Specifically, this is the immunodominant part of the O-chain and is a homopolymer of 1,2-linked N-acylated 4-amino-4, 6-dideoxy-α-D-mannopyranosyl residues (1).

The complement fixation test (CFT), serum agglutination test (SAT), and Rose-Bengal test (RBT) are the conventionally used tests for diagnosis of bovine brucellosis. All three tests are described in the World Organisation for Animal Health (OIE) *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*) (9), which gives details of all diagnostic methods. It also describes the required strain of *Brucella* to be used for antigen preparation and standardisation for each test. To aid inter-laboratory harmonisation, the OIE International

Standard (anti-*Brucella*) Serum (OIEISS) is used (formerly ISaBS [International Standard anti-*Brucella* Serum]). The strength of this serum is set at 1,000 international units (IUs) for CFT, SAT and RBT. By establishing a titre for the OIEISS in the CFT and SAT, the titre for all other sera can be calculated in terms of IU. The RBT should be standardised such that the antigen is used at a dilution that will find a 1/45 dilution of the OIEISS in phenol saline positive and a 1/55 dilution negative. If all *Brucella* testing laboratories adopt the recommended methods standardised to the same serum and use appropriate internal quality controls for each test performed, then theoretically quantitative (SAT and CFT) and qualitative serology results should be the same in any laboratory.

Although the indirect enzyme-linked immunosorbent assay (iELISA) is a prescribed test that is described in the OIE *Terrestrial Manual* and commonly used within countries for eradication of disease and for maintenance of disease-free status, it is less frequently used for international trade. The development of the weak positive OIE ELISA standard serum ($OIEELISA_{WP}SS$) and the strong positive OIE ELISA standard serum ($OIEELISA_{SP}SS$) for brucellosis has provided a tool with which to harmonise the diagnostic interpretations for these methods. There is also a negative OIE ELISA standard serum ($OIEELISA_{N}SS$).

A collaborative project between the National Reference Laboratories (NRLs) for brucellosis from several European Union (EU) member states defined the use of these available standards to set minimum diagnostic requirements for a wide range of commercially available and locally produced ELISAs. These criteria have since been incorporated into the European Community (EC) Council Directive 64/432/EEC (Commission Regulation [EC] No. 535/2002). For individual serum samples, these are:

a) a 1/150 predilution of the OIEISS or a 1/2 predilution of the $OIEELISA_{WP}SS$ or a 1/16 predilution of the $OIEELISA_{SP}SS$ made up in negative serum (or in negative pool of sera) should give a positive reaction

b) a 1/600 predilution of the OIEISS or a 1/8 predilution of the $OIEELISA_{WP}SS$ or a 1/64 predilution of the $OIEELISA_{SP}SS$ made up in negative serum (or in negative pool of sera) should give a negative reaction

c) the $OIEELISA_{N}SS$ should always give a negative reaction.

The requirements stated in the current OIE *Terrestrial Manual* are not so tightly defined. Despite this, the use of the iELISA as a test for international trade is not common due to the individual legislation in place in each country.

The use of the competitive ELISA (cELISA) within the EU is also governed by the above directive. The test can also be successfully used on samples of poor quality that may be unsuitable for other serological tests (8). The use of the fluorescent polarisation assay (FPA) is not described in the European Directive but is in the *Terrestrial Manual* (9). It has the advantage that there is no separation step and it has been reported to work on whole blood (7).

Harmonisation is important for trade purposes. Within the EU, where animals may be moved between countries of brucellosis-free and non-free status, diagnostic serological thresholds are set whereby animals can be classified as either positive or negative. As such, they may also be regarded as infected or uninfected, subject to the usual uncertainties of sensitivity and specificity of diagnostic tests (3), and the clinical and epidemiological picture. Such thresholds have been set on the basis of validation studies (not all of which have been published) and others are historically based. More thorough validation of the conventional tests has in some cases been retrospectively conducted (2).

Three pan-European ring trials for bovine brucellosis serology have been conducted to date: in 2000, 2002 and 2003. They were conducted with the aim of establishing the degree of diagnostic accuracy in each laboratory and the level of diagnostic harmonisation between the NRLs for brucellosis of all EU member states. The data may also show if some tests are better standardised and harmonised than others. The aim was not to compare the sensitivity or specificity of the tests – this was not a validation exercise.

A complete report for each ring trial, giving the full set of results, a written summary and a conclusion, can be found on the website of the OIE Reference Laboratory for Brucellosis in the United Kingdom (Veterinary Laboratories Agency – www.vla.gov.uk/aboutus/publicat.htm).

Materials and methods

Ring trial panel composition

The three ring trials were conducted using differing panels of serum. This was due to the development of the OIE ELISA standards during the trials and the desire to rationalise the number of samples used. It also reflected feedback from each ring trial where improvements were incorporated into selection and preparation of the panel for the subsequent trial(s).

For the first ring trial 31 bovine serum samples were sent to each of the participating NRLs. These included 16 samples from animals in Britain (formally recognised by the EU as officially brucellosis-free since 1991), eight dilutions in negative sera of the OIEISS (duplicates of 1/10, 1/20, 1/50, and 1/60 dilutions), sera from four animals experimentally infected with *B. abortus* strain 544 and serologically positive to CFT, SAT, RBT, and iELISA (each

further diluted in negative sera), two dilutions of the $OIEELISA_{WP}SS$ (neat and 1/4) and the $OIEELISA_{SP}SS$ (neat). The samples were re-labelled to ensure that tests were performed blind and dispatched, without preservation, to each of the 16 recipient laboratories.

The 31 samples in the first ring trial were divided into three categories. There were nine samples for which it was reasonable to expect a positive result from all tests. There were 16 samples from non-infected cattle for which the expected result from all tests was negative. The remaining six samples had titres for which the expected qualitative results from the tests were likely to vary according to the test in use (the titre in IUs would remain constant).

In the second ring trial, 2002, a different panel of 31 samples was dispatched. There were 11 samples from non-infected animals and nine samples from infected animals (three duplicates and three single samples) which displayed a range of positive titres. Seven samples were prepared from diluting the OIEISS (1/45, 1/55, 1/150, and to give 42.43, 28.28, 21.28, and 14.41 IUs) and four from diluting the $OIEELISA_{SP}SS$ (1/4, 1/16, 1/64, 1/128) in negative serum. The sera were all freeze-dried with the intention of minimising the risks of sample degradation during transit. Expected results for each sample were calculated according to the infection status of the donor animal, the titre (in IUs) or dilution of the $OIEELISA_{SP}SS$, and the test in use. Sixteen laboratories participated in this trial.

For the third ring trial a panel of 24 samples was sent to each of the 20 participating laboratories. This consisted of eight samples from *Brucella*-free cattle in Britain, seven samples from naturally infected cattle and one sample from a cow experimentally infected. Eight samples prepared from OIE standard sera were also included. These were six dilutions of the OIEISS and two dilutions of the $OIEELISA_{SP}SS$. Each was diluted in negative serum. Each sample was randomly assigned a number so that the trial was conducted blind. The identity of each sample for this ring trial is shown in Table I.

Table I
A description of the samples used in the 2003 ring trial and their expected classification per test

Sample ID	Sample description	Expected test classification					
		CFT	SAT	RBT	iELISA	cELISA	FPA
1	Field infected	Pos	Pos	Pos	Pos	Pos	Pos
2	Non-infected	Neg	Neg	Neg	Neg	Neg	Neg
3	Field infected	Pos	Pos	Pos	Pos	Pos	Pos
4	42.42 IUs (OIEISS)	Pos	Pos	Pos	Pos	Unknown	Unknown
5	Field infected	Pos	Pos	Pos	Pos	Pos	Pos
6	6.66 IUs (OIEISS at 1/150 dilution)	Neg	Neg	Neg	Pos *	Unknown [b]	Unknown
7	22.22 IUs (OIEISSat 1/45 dilution)	Borderline [a]	Neg	Pos	Pos	Unknown	Unknown
8	28.28 IUs (OIEISS)	Pos	Borderline	Pos	Pos	Unknown	Unknown
9	$OIEELISA_{SP}SS$ at 1/16 dilution	Unknown	Unknown	Unknown	Pos *	Unknown	Unknown
10	$OIEELISA_{SP}SS$ at 1/64 dilution	Unknown	Unknown	Unknown	Neg	Unknown	Unknown
11	Field infected	Pos	Pos	Pos	Pos	Pos	Pos
12	Non-infected	Neg	Neg	Neg	Neg	Neg	Neg
13	Non-infected	Neg	Neg	Neg	Neg	Neg	Neg
14	Field infected	Pos	Pos	Pos	Pos	Pos	Pos
15	Non-infected	Neg	Neg	Neg	Neg	Neg	Neg
16	Experimentally infected	Pos	Pos	Pos	Pos	Pos	Pos
17	Non-infected	Neg	Neg	Neg	Neg	Neg	Neg
18	Field infected	Pos	Pos	Pos	Pos	Pos	Pos
19	Non-infected	Neg	Neg	Neg	Neg	Neg	Neg
20	Non-infected	Neg	Neg	Neg	Neg	Neg	Neg
21	Field infected	Pos	Pos	Pos	Pos	Pos	Pos
22	14.14 IUs (OIEISS)	Neg	Neg	Neg	Pos	Unknown	Unknown
23	Non-infected	Neg	Neg	Neg	Neg	Neg	Neg
24	18.18 IUs (OIEISS at 1/55 dilution)	Borderline	Neg	Neg	Pos	Unknown	Unknown

CFT: complement fixation test
cELISA: competitive enzyme-linked immunosorbent assay
FPA: fluorescent polarisation assay
iELISA: indirect enzyme-linked immunosorbent assay
IU: international unit
OIEISS: OIE International Standard (anti-*Brucella*) Serum
$OIEELISA_{SP}SS$: strong positive OIE enzyme-linked immunosorbent assay standard serum
RBT: Rose-Bengal test
SAT: serum agglutination test
a) Samples are defined as 'Borderline' when the expected titre is close to the test positive/negative threshold
b) Samples are defined as 'Unknown' where there is insufficient justification to have a test expectation
* The minimum diagnostic requirements set out in EC Directive 64/432/EEC state that only one of these two results needs to be positive

Also included in Table I is the expected test classification for each sample for each test. Owing to varying diagnostic thresholds for each test the dilutions of the standard sera have been assigned an expectation on a test-by-test basis.

Each sample for the 2003 ring trial was prepared, tested by all methods included in the trial, dispensed into 1 ml aliquots and then frozen. A randomly selected frozen aliquot from each sample was thawed and tested to ensure the stability of the sample. The results demonstrated that the titre had remained stable for each sample. Samples were dispatched to the participating laboratories packed in dry ice within a polystyrene box to prolong the stability of the serum. Each laboratory was notified in advance of dispatch to ensure that they were prepared to store or test the samples at the earliest opportunity.

Test methods

Participating laboratories were asked to perform each of their routine serological tests for bovine brucellosis on the ring trial samples plus any additional methods available within their expertise. Each laboratory was asked to perform each test according to its normal protocol and to note when returning their data any deviations from the method in the *Terrestrial Manual* or other significant factors such as the choice of antigen or kit used. Many laboratories performed variations on the tests such as, for example, the SAT with addition of ethylene diamine tetra-acetic acid (5) or used antigens from different commercial suppliers. The choice of iELISA method varied between laboratories. Many laboratories used commercially available iELISA kits and some used their own laboratory prepared iELISAs.

The results for all three ring trials were analysed on a qualitative level (positive or negative) for all tests. For the CFT and SAT the results were also evaluated on a quantitative basis using the system of International CFT and SAT Units. The SAT and CFT results were also analysed by converting the IUs into a representative dilution within the serial doubling dilution method stated in the *Terrestrial Manual* for the CFT and commonly used for the SAT. This was achieved by taking the log (to the base of 2) of the IUs of each sample. A difference of 1 between results from this method represents a difference of one dilution in a doubling dilution series. This allowed for errors for low titre samples and for higher titre samples to be expressed equivalently.

Results

2000 ring trial

Table II shows that the samples with the expected classification of negative and positive for all tests are correctly identified in the majority of cases, the percentage of outliers being 0.32% and 1.13% respectively (the overall rate was 0.61%). No single laboratory stood out as being consistently poor for all the tests although some were poor in certain areas (laboratory 'E' in the CFT, and laboratory 'J' for the SAT). Table II also shows that for the RBT, two laboratories failed to correctly classify one sample expected to be positive by all tests, but results were otherwise good. The iELISA was the only test to correctly classify all the samples expected to be positive by all tests. However, one laboratory classified two samples from uninfected animals as positive. Of the four tests, the iELISA classified the most samples as positive. Neither the cELISA or the FPA was used by any of the laboratories in this ring trial.

Table III shows that of the samples where the expected results were qualitatively known (the dilutions of the OIEISS), two laboratories for the CFT had an average error of one or more dilutions (one laboratory was a dilution higher on average, and one was lower) and one laboratory for the SAT had an average over one dilution below the expected result. The overall averaged results from all laboratories for these samples were very close to the expected titre (data not shown). The arithmetic average tended to be just above the expected values whereas the geometric mean was usually just below.

2002 ring trial

For the CFT, all the 14 laboratories that returned data were, on average, within one dilution from the overall trial average (consensus) result for each sample. One laboratory reported two samples from non-infected animals as positive and another laboratory reported two samples from infected animals as negative. There were no other outliers from these sample categories. Although the general level of harmonisation was good, the average result from the samples prepared using the OIEISS was lower than that expected by nearly one dilution.

From the 12 laboratories performing SAT, two laboratories reported results that were on average greater than one dilution from the overall trial average for each sample (–1.39 and 1.70). However, the reported SAT results for the dilutions of the OIEISS were closer to the expected results than were the CFT results. None of the samples from infected or non-infected animals were incorrectly classified.

For the RBT, only three laboratories from the 15 that performed this test classified the 1/45 and 1/55 dilutions of the OIEISS as positive and negative respectively. Although no laboratories incorrectly classified any of the samples from the non-infected animals, some of the lower titre samples from the infected animals were misclassified as negative, with one laboratory doing this twice.

Table II

Table of outliers (incorrect classifications where a classification was possible) from the first ring trial in 2000

(Outliers are in bold type)

Lab ID	Number of samples that gave incorrect results											
	CFT			SAT			RBT			iELISA		
	SP [a]	WP [b]	Neg [c]	SP	WP	Neg	SP	WP	Neg	SP	WP	Neg
A	0	1	0	0	2	0	**1**	4	0	0	1	0
B	0	2	0	0	6	0	0	6	0	0	0	0
C	0	6	0	0	6	0	0	6	0			
D	0	6	0	0	6	0	**1**	6	0	0	1	0
E	**2**	6	0	0	4	0	0	2	0	0	0	0
F	0	6	0	0	6	0	0	6	0	0	0	0
G	0	6	0	0	5	0	0	5.5*	0	0	1	0
H	0	2	0	0	5	0	0	2	0	0	0.5*	0
I	0	0	0				0	2	0	0	0	0
J				**2**	6	0	0	6	0	0	1	0
K	0	3	0	0	2	0	0	3	0	0	0	0
L	0		0	0	6	0	0	6	0	0	1	0
M	0	4	0	0	6	0	0	2	0			
N	0	1	0				0	2	**1**	0	1	0
O	0	2	0	0	3	0	0	3	0	0	0	0
P	0	4	0	0	6	0	0	3	0	0	0	**2**

a) SP: strong positive (n = 9), table lists number of samples found negative
b) WP: weak positive (n = 6), table lists number of tests found negative
c) Neg: negative (n = 16), table lists number found positive
* in duplicate testing one test result was positive and the other negative
CFT: complement fixation test
iELISA: indirect enzyme-linked immunosorbent assay
RBT: Rose-Bengal test
SAT: serum agglutination test
Number of outliers from Neg samples = 3, total number of tests = 944, percentage of outliers = 0.32
Number of outliers from SP samples = 6, total number of tests = 531, percentage of outliers = 1.13

Table III

Average deviation from expected titres for the complement fixation test and serum agglutination test per laboratory in the 2000 ring trial

Complement fixation test				Serum agglutination test			
Lab ID	IUs	Lab ID	Log_2 IUs	Lab ID	IUs	Lab ID	Log^2 IUs
N	161	N	1.25	A	59	A	0.82
B	127	O	0.83	H	57	H	0.81
O	34	I	0.73	B2*	42	G	0.52
F	31	B	0.38	O	32	K	0.51
I	25	K	0.36	L1	22	L1	0.43
K	16	A	0.28	L2	13	O	0.38
A	– 1	F	0.08	G	12	D	0.26
G	– 24	G	0.01	K	1	L2	0.20
H	– 37	H	0.01	D	1	B1	– 0.13
D	– 39	P	– 0.22	B1	– 1	F1	– 0.19
L	– 48	L	– 0.39	F1	– 13	F2	– 0.26
M	– 57	M	– 0.42	F2	– 18	E	– 0.27
P	– 57	C	– 0.53	C	– 18	B2	– 0.37
C	– 60	D	– 1.00	M	– 41	M	– 0.46
E	– 74	E	– 1.41	P	– 41	P	– 0.48
				E	– 43	C	– 0.98
				J	– 44	J	– 1.11

IU: international unit
* Where a laboratory has been listed more than once (e.g. B1 and B2) this is due to multiple sets of results being returned from that laboratory, each representing a variation of test method

Most of the 15 participating laboratories successfully passed the criteria for the iELISA as laid out in the Council Directive 64/432/EEC, with the exception of laboratories C, H (insufficiently sensitive), I and K (over sensitive). Laboratory H in particular misclassified six samples expected to give positive results. For four of these samples, every other laboratory classified them as positive. Eleven of the 15 laboratories provided perfectly satisfactory results.

Overall, the cELISA results from the six laboratories that returned data for this test, classified fewer samples as positive than did the iELISA, including the dilutions of the OIEISS and OIEELISA$_{SP}$SS, which a test must classify correctly if it is to conform with the requirements of Council Directive 64/432/EEC. Laboratory J returned results that classified many of the samples from infected animals as negative. As with the iELISA, a variety of cELISA test kits were used by the participants. A similar pattern was seen with the results from the four laboratories that returned results for the FPA. Two laboratories returned results which incorrectly classified several of the samples from the infected samples and none of the results conformed with the standards for the ELISA.

2003 ring trial

Table IV shows the number of outliers, defined as incorrect classifications for those samples where an expected classification was possible, from a total of 1,898 results for

Table IV
Number of outliers (incorrect classifications) from a total of 1,898 results for which there was an expected classification (2003 ring trial)

Lab ID	Number of outliers per test						Total number of outliers
	CFT	SAT	RBT	iELISA	cELISA	FPA	
1 a*	1		2	0	0	0	3
1 b			2				2
1 c			2				2
2	0	1	4	0			5
3	0	2	1	0			3
4				0			0
5		0	1	1			2
6	1		0	1			2
7	0	0	0	0		1	1
8	0	1	0	0	0		1
9	1	0	0				1
10	0	1	2	2			5
11 a	0	0	1	0	0		1
11 b			1	0	0		1
11 c				0			0
11 d				0			0
12	0		2	0	0		2
13	0	0	0	0			0
14	1		2	0	0	0	3
15	1	1	1	0			3
16	0	0	1	1	0	0	2
17	0	0	3	0	0		3
18 a	0	0	2	0	0		2
18 b				0			0
19	1	0	1	1	0		3
20	0	0	1	0	0	0	1
Total							**48 (2.58%)**

* Where a laboratory has been listed more than once (e.g. 1a, 1b and 1c) this is due to multiple sets of results being returned from that laboratory, each representing a variation of test method
CFT: complement fixation test
cELISA: competitive enzyme-linked immunosorbent assay
FPA: fluorescent polarisation assay
iELISA: indirect enzyme-linked immunosorbent assay
RBT: Rose-Bengal test
SAT: serum agglutination test
An outlier is defined as a misclassification of the qualitative result for a sample where there is a positive or negative expectation

which there was an expected classification. There were 48 outliers from this subset, a rate of 2.58%.

Tables V, VI, VII, VIII, IX and X show, for each test (CFT, SAT, RBT, iELISA, cELISA, FPA respectively), totals of samples positive for each of the eight samples from infected animals, from the eight non-infected animals, and from the eight dilutions of the OIE standard sera. For this last group the individual qualitative results are tabulated for each lab and test. These tables show the results for the higher titre samples on the left hand side. Appropriate test cut-offs or requirements are indicated in the tables by vertical black dashed lines.

From the 18 laboratories which submitted CFT results 17 correctly classified the samples from infected and non-infected animals. The exception classified one positive as negative. Two laboratories reported negative results for a sample whose titre should have been 1/2 a dilution above the test cut-off. Four laboratories reported positive results for a sample that should have been 1/2 a dilution below the test cut-off. Only two laboratories reported qualitative results that were out of sequence with the titres of the samples. Twelve of the 18 sets of results submitted for the CFT correctly classified samples 1/2 a dilution either side of the cut-off. All correctly classified the samples containing 42.4 and 6.66 IUs.

From the 15 laboratories that submitted results for SAT all laboratories reported negative results for all the samples from non-infected animals. Three laboratories failed to correctly classify one or more of the eight positive samples from infected animals, and of these, two laboratories failed to correctly classify a sample 1/2 a dilution above the test cut-off. Three laboratories classified a sample that was nearly 1/2 a dilution below the test cut-off as positive. However, only one failed to correctly classify the next lowest sample and this laboratory did correctly classify a sample of 14.1 IUs as negative. For the SAT, 10 of the 15 participating laboratories correctly classified samples 1/2 a dilution either side of the cut-off.

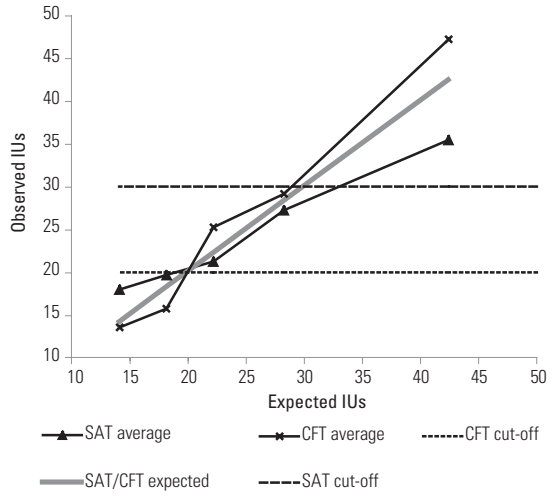
Due to the quantifiable nature of the CFT and SAT tests it was possible to depict these results graphically for each laboratory. These graphs are available on the VLA website. Figure 1 shows the average trial results for each sample plotted against the expected titre. This shows that the averages are close to the expectations.

Table V
Summary of qualitative complement fixation test (CFT) results from the 2003 ring trial
The dashed line represents the tests positive/negative threshold (20 IUs)

Lab ID	Sample description			Dilutions of standard sera							
	Number of positive classifications			OIEISS dilutions expressed as IUs						$OIEELISA_{SP}SS$	
	Infected	Non-infected	Standard sera	42.4	28.3	(1/45) 22.2	(1/55) 18.2	14.1	(1/150) 6.66	1/16	1/64
1	8	0	5	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg
2	8	0	3	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg
3	8	0	2	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg
6	8	0	5	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg
7	8	0	2	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg
8	7	0	3	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg
9	8	0	1	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg
10	8	0	4	Pos	Pos	Pos	Pos	Neg	Neg	Neg	Neg
11	8	0	3	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg
12	8	0	3	Pos	Pos	Neg	Pos	Neg	Neg	Neg	Neg
13	7*	0	3	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg
14	8	0	5	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg
15	8	0	4	Pos	Pos	Neg	Pos	Pos	Neg	Neg	Neg
16	8	0	4	Pos	Pos	Pos	Pos	Neg	Neg	Neg	Neg
17	8	0	2	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg
18	8	0	3	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg
19	8	0	1	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg
20	8	0	4	Pos	Pos	Pos	Pos	Neg	Neg	Neg	Neg

* Laboratory 13 only received seven of the eight samples from the infected animals
OIEISS: OIE International Standard (anti-*Brucella*) Serum
$OIEELISA_{SP}SS$: strong positive OIE enzyme-linked immunosorbent assay standard serum
IU: international unit

For the SAT and CFT combined, five samples from infected animals from a total of 262 were classified as negative and no samples from non-infected animals from a total of 264 were classified as positive.



IU: international units

Fig. 1

Average observed complement fixation test (CFT) and serum agglutination test (SAT) results for samples prepared from the World Organisation for Animal Health international standard serum against expected results for the 2003 ring trial

From the 19 laboratories that submitted results for the RBT all correctly classified the samples from the non-infected animals. All the samples from infected animals were correctly classified except for in three laboratories which each misclassified one sample. Only six from the 22 sets of data received classified the 1/45 (22.5 IUs) and 1/55 (18.2 IUs) dilutions of the OIEISS as positive and negative respectively. Ten laboratories were too sensitive, two of these classified samples of 6.66 IUs as positive (a 1/150 dilution of the OIEISS). All laboratories classified the sample containing 42.4 IUs as positive.

All but one of the 20 participating laboratories tested the ring trial sera by iELISA. Several laboratories used more than one method, reflecting the choice of commercial kits and 'in house' production that is available. Two laboratories failed to correctly classify all samples from infected animals. One classified two of these samples as negative, and the other classified a positive sample as inconclusive in accordance with the kit instructions. All laboratories correctly classified the samples from non-infected animals. Five of the 23 sets of data received did not meet the requirements for classification of the OIE standards that are referred to in EU Directive 64/432/EEC. The EU requirement is that one or both of the samples between the dashed lines in Table VIII (OIEISS at 1/150 and OIEELISA$_{SP}$SS at 1/16) is positive and that the sample to the right of both lines (OIEELISA$_{SP}$SS at 1/64) is negative. On three occasions all the relevant standards included were negative. One laboratory classified all three of these

Table VI

Summary of qualitative serum agglutination test results from the 2003 ring trial

The dashed line represents the tests positive/negative threshold (30 IUs)

Lab ID	Sample description: Number of positive classifications			Dilutions of standard sera: OIEISS dilutions expressed as IUs						OIEELISA$_{SP}$SS	
	Infected	Non-infected	Standard sera	42.4	28.3	(1/45) 22.2	(1/55) 18.2	14.1	(1/150) 6.66	1/16	1/64
2	8	0	3	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg
3	8	0	4	Pos	Pos	Pos	Pos	Neg	Neg	Neg	Neg
5	7	0	1	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg
7	8	0	2	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg
8	7	0	0	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
9	8	0	1	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg
10	8	0	3	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg
11	8	0	1	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg
13	7*	0	1	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg
15	6	0	0	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
16	8	0	2	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg
17	8	0	3	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg
18	8	0	2	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg
19	8	0	1	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg
20	8	0	2	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg

* Laboratory 13 only received seven of the eight samples from the infected animals

OIEISS: OIE International Standard (anti-*Brucella*) Serum

OIEELISA$_{SP}$SS: strong positive OIE enzyme-linked immunosorbent assay standard serum

IU: international unit

Table VII
Summary of Rose-Bengal test results from the 2003 ring trial
The dashed line represents the tests positive/negative threshold

Lab ID	Sample description Number of positive classifications			Dilutions of standard sera OIEISS dilutions expressed as IUs						OIEELISA$_{SP}$SS	
	Infected	Non-infected	Standard sera	42.4	28.3	(1/45) 22.2	(1/55) 18.2	14.1	(1/150) 6.66	1/16	1/64
1a[a]	8	0	5	Pos	Pos	Pos	Pos	Pos	Neg	**IC**	Neg
1b	8	0	5	Pos	Pos	Pos	Pos	Pos	Neg	**IC**	Neg
1c	8	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
2	7	0	2	Pos	Neg	Neg	Neg	Pos	Neg	Neg	Neg
3	8	0	2	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg
5	7	0	3	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg
6	8	0	3	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg
7	8	0	3	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg
8	8	0	3	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg
9	8	0	3	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg
10	8	0	5	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg
11a	8	0	4	Pos	Pos	Pos	Pos	Neg	Neg	Neg	Neg
11b	8	0	4	Pos	Pos	Pos	Pos	Neg	Neg	Neg	Neg
12	8	0	6	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
13	7*	0	3	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg
14	8	0	5	Pos	Pos	Pos	Pos	Pos	Neg	**IC**	Neg
15	8	0	2	Pos	Pos	**IC**	Neg	**IC**	Neg	Neg	Neg
16	8	0	4	Pos	Pos	Pos	Pos	Neg	Neg	Neg	Neg
17	7	0	1	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg
18	8	0	1	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg
19	8	0	2	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg
20	8	0	4	Pos	Pos	Pos	Pos	Neg	Neg	Neg	Neg

a) Where a laboratory number has been listed more than once this is due to multiple sets of results being returned from that laboratory, each representing a variation of test method
* Laboratory 13 only received seven of the eight samples from the infected animals
IC: the result was classified as 'inconclusive' by the returning laboratory
IU: international unit
OIEELISA$_{SP}$SS: strong positive OIE enzyme-linked immunosorbent assay standard serum
OIEISS: OIE International Standard (anti-*Brucella*) Serum

standards as positive and one laboratory classified the OIE ELISA$_{SP}$SS at a 1/64 dilution as inconclusive.

Ten of the participating laboratories submitted data for a cELISA as shown in Table IX. One laboratory failed to correctly classify two of the samples from infected animals. This laboratory also failed to classify any of the dilutions of the standards as positive. All laboratories correctly classified the negative samples. There was considerable variation in the pattern of classification between laboratories. Only one laboratory submitted results that complied with the requirements for the classification of the OIE standards.

Five of the participating laboratories reported results for the FPA test as shown in Table X. All but one of the samples from infected animals was correctly classified. All the samples from non-infected animals were correctly classified. There was a large variation between laboratories in the classification of the dilutions of the OIE standards. Only one laboratory produced results that would have been within the requirements for the classification of the OIE standards for ELISA.

Discussion

The SAT and CFT are standardised against the OIEISS. Because they are quantitative tests any dilution of the OIEISS could be used to assess their standardisation. The SAT and CFT are also qualitative, with positive/negative thresholds of 30 IUs and 20 IUs respectively. It was important to measure qualitative as well as quantitative performance because not all laboratories submitted full qualitative results for all samples (despite being requested to do so). The *Terrestrial Manual* indicates that the standardisation of the CFT and SAT is performed using the OIEISS diluted in test buffer rather than negative sera. Tests at the co-ordinating laboratory showed that there was no significant effect on the results between each dilution method.

The 1/45 and 1/55 dilutions of the OIEISS were included in 2002 and 2003 to particularly assess the standardisation of the RBT. These were prepared by dilution in negative serum and not, as is stated in the *Terrestrial Manual*, in 0.5% phenol saline, although using negative serum to

Table VIII

Summary of qualitative indirect enzyme-linked immunosorbent assay (iELISA) results from the 2003 ring trial

The European Union requirement is that one or both of the samples between the dashed lines (OIEISS at 1/150 and OIEELISA$_{SP}$SS at 1/16) is positive and the sample to the right of both lines (OIEELISA$_{SP}$SS at 1/64) is negative

Lab ID	Kit used	Sample description: Number of positive classifications			Dilutions of standard sera: OIEISS dilutions expressed as IUs						OIEELISA$_{SP}$SS	
		Infected	Non-infected	Standard sera	42.4	28.3	(1/45) 22.2	(1/55) 18.2	14.1	(1/150) 6.66	1/16	1/64
1	A	8	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
2	?	8	0	6	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg
3	?	8	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
4	B	8	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
5	C	8	0	8	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
6	D	8	0	5	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg
7	B	8	0	6	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg
8	E	8	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
10	F	6	0	6	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg
11a[a]	B (pool)	8	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
11b	B	8	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
11c	E (pool)	8	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	**IC**
11d	E	8	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
12	G	8	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
13	C	7*	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
14	C	8	0	6	Pos	Pos	Pos	Pos	Pos	Neg	Pos	Neg
15	D	8	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
16	H	8	0	5	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg
17	E	8	0	6	Pos	Pos	Pos	Pos	Pos	Pos	**IC**	Neg
18a	I	8	0	6	Pos	Pos	Pos	Pos	Pos	Neg	Pos	Neg
18b	E	8	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
19	E	7	0	4	Pos	Pos	Pos	Pos	**IC**	Neg	Neg	Neg
20	A	8	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg

a) Where a laboratory number has been listed more than once this is due to multiple sets of results being returned from that laboratory, each representing a variation of test method or kit as shown in the second column

* laboratory 13 only received seven of the eight samples from the infected animals

IC: The result was classified as 'inconclusive' by the returning laboratory

IU: international unit

OIEISS: OIE International Standard (anti-*Brucella*) Serum

OIEELISA$_{SP}$SS: strong positive OIE enzyme-linked immunosorbent assay standard serum

dilute the sample helped to preserve the titre during preservation and storage. Tests at the co-ordinating laboratory showed that there was no significant effect on the results between each dilution method.

The standard reference sera for ELISAs were not all included in the third ring trial panel. The OIEELISA$_{WP}$SS and OIEELISA$_{N}$SS were both omitted. The former because it is a 1/8 dilution of the OIEELISA$_{SP}$SS and the latter because there were already enough samples from non-infected animals.

Performance standards for the FPA are currently not legislated for within the EU or defined by the OIE, despite several publications validating its use to diagnose bovine brucellosis (4). Therefore, it is only possible to assess the FPA in terms of its precision between laboratories rather than its adherence to any standard.

Comparison between the classification of the samples and their expected results formed the basis of the analysis. The expected results for each sample derived from an OIE standard, depended upon its titre relative to the positive/negative threshold for each test. Expected qualitative results were only defined where the expectation was unequivocal. If the expected titres were close to a test positive/negative threshold the sample could be defined as 'borderline' in circumstances where an acceptable level of variation could cause the results to be classified as either positive or negative. This was the case for the SAT and CFT where the expected titres were within 1/2 a dilution from the positive/negative threshold.

Table IX

Summary of qualitative competitive enzyme-linked immunosorbent assay (cELISA) results from the 2003 ring trial

The European Union requirement is that one or both or the samples between the dashed lines (OIEISS at 1/150 and OIEELISA$_{SP}$SS at 1/16) is positive and the sample to the right of both lines (OIEELISA$_{SP}$SS at 1/64) is negative

Lab ID	Kit used	Sample description: Number of positive classifications			Dilutions of standard sera: OIEISS dilutions expressed as IUs						OIEELISA$_{SP}$SS	
		Infected	Non-infected	Standard sera	42.4	28.3	(1/45) 22.2	(1/55) 18.2	14.1	(1/150) 6.66	1/16	1/64
1	A	8	0	4	Pos	Pos	Pos	Pos	Neg	Neg	Neg	Neg
8	A	8	0	5	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg
11a[a]	B	8	0	2	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg
11b	A	8	0	5	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg
12	C	8	0	7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg
14	A	8	0	4	Pos	Pos	Pos	Pos	**IC**	Neg	Neg	Neg
16	B	8	0	5	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg
17	B	6	0	0	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
18	A	8	0	4	Pos	Pos	Pos	Pos	Neg	Neg	Neg	Neg
19	A	8	0	5	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg
20	B	8	0	2	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg

a) Where a laboratory number has been listed more than once this is due to multiple sets of results being returned from that laboratory, each representing a variation of test method or kit as shown in the second column

IC: the result was classified as 'inconclusive' by the returning laboratory
IU: international unit
OIEISS: OIE International Standard (anti-*Brucella*) Serum
OIEELISA$_{SP}$SS: strong positive OIE enzyme-linked immunosorbent assay standard serum

Table X

Summary of qualitative fluorescent polarisation assay (FPA) results: from the 2003 ring trial

There are currently no European Union requirements that relate to the performance of the FPA against specific international samples

Lab ID	Sample description: Number of positive classifications			Dilutions of standard sera: OIEISS dilutions expressed as IUs						OIEELISA$_{SP}$SS	
	Infected	Non-infected	Standard sera	42.4	28.3	(1/45) 22.2	(1/55) 18.2	14.1	(1/150) 6.66	1/16	1/64
1	8	0	3	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg
7	7	0	0	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
14	8	0	6	Pos	Pos	Pos	Pos	Pos	Neg	Pos	Neg
16	8	0	5	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg
20	8	0	3	Pos	Pos	Neg	Neg	Pos	Neg	Neg	Neg

IU: international unit
OIEISS: OIE International Standard (anti-*Brucella*) Serum
OIEELISA$_{SP}$SS: strong positive OIE enzyme-linked immunosorbent assay standard serum

The results from the 2000 ring trial showed an excellent overall classification of the samples categorised as 'positive' and 'negative' for all the tests and identified the laboratories where errors were made. Analysing the CFT and SAT results by the $\log_2$ results demonstrated that most laboratories were within one dilution of the expected titre for samples prepared from the OIEISS. Less could be concluded about the RBT due to the lack of inclusion of the appropriate OIEISS dilutions, and at the time standardisation for the iELISA was not well defined.

The results from the 2002 trial showed that the laboratories were similarly harmonised for CFT and SAT although the difference from the expected titres was greater. The reduced level of accuracy has to be tempered against the question marks that arose over the stability of the titres of the samples in the ring trial panel post freeze-drying. Two laboratories were identified as poor performers for the CFT on the samples from the infected and non-infected animals, but all performed SAT to an acceptable standard.

The introduction into the panel of specific samples to assess the standardisation of the RBT and iELISA provided a useful tool. Only three of 15 laboratories successfully classified the standards related to the RBT, yet only one

laboratory returned data for the samples from the infected and non-infected animals that were considered unacceptable due to poor sensitivity. For the iELISA, 11 out of 15 laboratories returned wholly satisfactory results for sensitivity and specificity and demonstrated good harmonisation. The results identified one laboratory in particular as being under-sensitive. This laboratory took immediate action to remedy this situation.

The underperformance of the cELISA against the OIEELISA standards included gave cause for concern. For at least one of the included methods the test is known to be validated for use in bovines (4) but its failure here raises some doubts as to its use and the appropriateness of the OIEELISA standards for cELISAs. The current requirements for standardisation of the FPA states that the $OIEELISA_{SP}SS$ and $OIEELISA_{WP}SS$ should both give positive results when undiluted, but there is no requirement for a particular sample, or dilution of a sample, to give negative results (9). On the basis of this trial, the FPA does not fit into the criteria defined for the ELISAs. Furthermore, the variability between results (Table X) suggests that a tighter criteria for standardising this test is desirable.

The overall number of outliers (defined as samples not classified in line with expectations) in the 2003 ring trial was 48 out of a possible total of 1898 (2.58%). Although higher than the total from the 2000 ring trial, the 2003 figures included some of the dilutions of the OIE standard sera which were not used in 2000. Excluding these samples the 2003 outlier rate was 1.61%. In 2000 the rate was 0.61%. Figures for 2002 were not calculated due to the question marks over sample stability.

Tables V, VI, VII, VIII, IX and X show summary results from the 2003 trial for each individual test. The positioning of the dashed lines indicates which samples should be positive and which negative. The ordering of the samples created from dilutions of OIE standard sera from left to right enables some assessment of laboratory precision. There should be no overlap between positive and negative samples. Quantitative results for the CFT and SAT are not shown due to the number of laboratories that did not include a titre for negative samples.

The results for the CFT and SAT show that the majority of laboratories correctly classified samples 1/2 a dilution from the test cut-off, and few had any difficulty with the classification of the samples from infected and non-infected animals. Overall this represents a good performance. Figure 1 confirms this by showing how the qualitative results that were included were close to those expected.

The results for the RBT show that the majority of the laboratories did not correctly classify the 1/45 and 1/55 dilutions of the OIEISS. This demonstrates widespread difficulty in reading this subjective test to the levels of accuracy required for borderline samples. This may include difficulties with the standardisation of RBT antigens. Few laboratories reported the source and batch details of the antigens that they used. This made it impossible to determine whether or not a particular antigen contributed to the disparity of the results. It is possibly too much to expect the laboratories to each achieve this accuracy given that the CFT (which gave good results overall) was just as poor at reliably discriminating correctly between samples of these titres. However, the interpretation of the samples from infected and non-infected animals was good overall.

Most of the laboratories participating in the 2003 trial reported results for iELISA, thus demonstrating that its use is routine across Europe. The level of conformity with the requirements of the EU was good with only four laboratories failing to reach this standard. The general level of harmonisation between the laboratories was also good with none of the laboratories reporting a negative result for the dilutions of the OIEISS between 42.4 and 14.1 IUs, and only one laboratory reporting a negative result for a sample from an infected animal. All laboratories reported the iELISA kit that they used for testing. The results did not show that any particular kit type was a particularly strong or poor performer. In most instances where requirements were not met with one kit type, another laboratory met these using the same kit.

The pattern of results for the cELISA in 2003 were similar to those found in 2002. Only one of the 11 sets of data returned for the cELISA conformed to the standards for ELISAs set by the EU. This laboratory used a different kit from all the rest. One laboratory classified all the dilutions of the OIE standard sera as negative. Harmonisation for this test was poor and the data shows that the different kits in use may be a factor in this.

The FPA results showed inconsistency between laboratories and in one case a clear inconsistency within the laboratory. The introduction of standards which these tests can work to would in all probability help to harmonise their performance.

There have been a variety of samples used for the three ring trials and a variety of methods of analysis performed. The next ring trial will use the same samples (but renumbered) as used in 2003 and 2005, which will provide a level of consistency in the analysis and allow a more direct method of comparison between the data from each trial. To further improve the analysis for the next round it would be desirable for the participating laboratories to submit complete data for all their tests, especially for the SAT and CFT. The next ring trial could also provide useful

information to be used in the preparation of common standards to help harmonise the cELISA and FPA.

Conclusions

Each successive trial produced data of increasing value and reliability and experience from each trial was used to improve the subsequent one. The selection of the serum panel was improved to reflect the legislated use of the OIE standards and provide a better mix of samples from field-infected and non-infected bovines. Each ring trial has included more laboratories than its predecessor, reflecting the growth in the EU and the increasing importance of quality assurance (QA) in diagnostic laboratories. On each occasion, the number of laboratories using more recently developed tests such as the cELISA and FPA has increased. The quality of the serum panel has also improved due to a greater focus on the timely delivery of samples to the participating laboratories and a quality controlled preservation process.

The overall level of harmonisation for the SAT, CFT, and iELISA appears to be at a satisfactory level. In particular the OIEELISA standards for iELISA have been effective in demonstrating this harmony. Problems with the RBT appear to stem from the tight requirements for standardisation (when compared to the SAT and CFT) and the subjectivity of the test, although the number of different antigen batches from different suppliers may also be a contributory factor. The level of harmonisation for the cELISA and FPA is lower and new standards for these should be introduced if there is a desire to use these tests for trade and eradication.

The trial was conducted in a manner that protected the confidentiality of each participating laboratory and their codes of identity differed in each ring trial. Each laboratory was informed of their own identity so that they could take the necessary measures to improve their performance if required and so that they could include the results in their own QA system (participation in such ring trials is encouraged in ISO:17025). This was agreed by the representatives of each participating NRL prior to the ring trials with the understanding that laboratories that were under-performing, by their own judgement, should seek improvement. Each trial was successful in providing evidence to laboratories to assist them in monitoring their own performance.

Ring trials assessing serological diagnosis have been performed before (6) but not on this many tests with this many international participants, most are conducted within countries. For the benefits of eradication and trade, harmonisation of results between testing laboratories is of paramount importance. The performance attributes of specific tests may be well known through validation and historical studies, but unless adopted practices of testing are up to the required standards these attributes cannot be extrapolated to each laboratory. It is therefore in the interests of trading partners to firstly investigate the degree of their harmonisation and secondly to act upon the results if necessary.

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Harmonisation des tests européens pour le diagnostic sérologique de l'infection à *Brucella* chez les bovins

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Résumé

Les principales méthodes de diagnostic sérologique de la brucellose bovine sont l'épreuve de fixation du complément (FC), le titrage des anticorps par séroagglutination (SAT), l'épreuve au rose Bengale (RBT), la méthode immuno-enzymatique indirecte (iELISA) et plus récemment la technique ELISA de compétition (cELISA) ainsi que l'épreuve de polarisation en fluorescence (FPA). Les Lignes directrices établies par l'Organisation mondiale de la santé animale

(OIE) décrivent les méthodes et les seuils diagnostiques pour chacune de ces épreuves. De nombreux pays ont adopté ces méthodes à des fins d'éradication de la brucellose et ont prévu l'utilisation de ces épreuves (la FC et le SAT en particulier) pour la prévention de la propagation de la maladie par l'intermédiaire du commerce international. Au sein de l'Union européenne (UE), chaque État membre dispose d'un Laboratoire national de référence qui fixe des règles concernant la qualité du diagnostic de la brucellose et des travaux qui s'y rapportent selon les recommandations établies par l'OIE. Le présent article décrit les résultats issus des trois premiers essais interlaboratoires de l'UE permettant d'évaluer le degré d'harmonisation des tests de diagnostic entre chaque État membre. Le niveau général d'harmonisation pour le SAT, la FC et l'iELISA a été jugé satisfaisant, mais les problèmes de standardisation du RBT, de cELISA et de la FPA demeurent. L'épreuve cELISA et la FPA en particulier doivent faire l'objet de travaux plus poussés pour parvenir à une harmonisation européenne. Les essais interlaboratoires ont également permis d'apporter la preuve de l'existence de performances insuffisantes dans certains domaines. Il appartient à chaque laboratoire concerné de décider ou pas de prendre des mesures sur la base de ces résultats. L'augmentation, au fil du temps, du nombre de participants à ces tests témoigne de l'élargissement de l'UE et accentue la nécessité d'une assurance qualité.

Mots-clés
Brucella – Épreuve de fixation du complément – Épreuve au rose Bengale – Essai interlaboratoire – Harmonisation – Méthode de dosage immuno-enzymatique – Titrage des anticorps par séroagglutination.

Armonización de las pruebas europeas para el diagnóstico serológico de la brucelosis en bovinos

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Resumen
Las principales técnicas para efectuar un diagnóstico serológico de la brucelosis bovina son: la de fijación del complemento (FC); la de seroaglutinación (SA); la de rosa de Bengala (RB); el ensayo inmunoezimático indirecto (ELISAi); y, en fechas más recientes, el ELISA de competición (ELISAc) y el ensayo de fluorescencia polarizada (FP). En las directrices elaboradas por la Organización Mundial de Sanidad Animal (OIE) se describen todas estas técnicas y sus correspondientes umbrales de diagnóstico. Muchos países las han adoptado con fines de erradicación de la brucelosis y han promulgado normas que regulan su utilización (en particular la de FC y de SA) para prevenir la diseminación de la enfermedad por el comercio internacional. En cada Estado Miembro de la Unión Europea (UE) hay un laboratorio de referencia nacional encargado de regular la calidad del diagnóstico de la brucelosis y aplicar las recomendaciones formuladas por la OIE. Los autores exponen los resultados de las tres primeras pruebas interlaboratorios realizadas en la UE para evaluar el

grado de armonización de las pruebas de diagnóstico entre los distintos Estados Miembros. En general se observó un buen nivel de armonización por lo que respecta a las pruebas de SA, FC y ELISAi, mientras que aún subsistían problemas en las de RB, ELISAc y FA. En el caso de las dos últimas, en particular, aún queda trabajo por delante para lograr la uniformidad a escala europea. Las pruebas interlaboratorios también resultaron útiles para poner de relieve el deficiente rendimiento de las pruebas de diagnóstico en determinadas zonas. Incumbía después a cada laboratorio decidir si adoptaba o no medidas a raíz de tales resultados. El progresivo aumento del número de participantes en esos ensayos evidencia el crecimiento de la UE y la mayor necesidad de disponer de una garantía de calidad.

Palabras clave
Armonización – Brucella – Ensayo inmunoenzimático – Prueba de fijación del complemento – Prueba de rosa de Bengala – Prueba de seroaglutinación – Prueba interlaboratorios.

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