

**SUMMARY OF FIELD TRIALS USING THE INDIRECT AND  
COMPETITIVE ENZYME IMMUNOASSAYS FOR  
DETECTION OF ANTIBODY TO *BRUCELLA ABORTUS***



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**Abstract**

SUMMARY OF FIELD TRIALS USING THE INDIRECT AND COMPETITIVE ENZYME IMMUNOASSAYS FOR DETECTION OF ANTIBODY TO *BRUCELLA ABORTUS*.

Two indirect and two competitive enzyme immunoassays for detection of antibody to *Brucella abortus*, validated elsewhere, were field tested in five different Latin American laboratories. Testing was performed according to standardised protocols using sera obtained in each area. Sera from *B. abortus* infected herds, from vaccinated (but serologically negative in a screening test) and non-vaccinated cattle were tested in each assay and compared to the results obtained with conventional diagnostic tests used for diagnosis of brucellosis in each country. Relative sensitivity and specificity values were calculated for each country as well as a weighted summary combining the data from all the participating laboratories. The results demonstrate that all ELISAs performed as well as, or better than, the conventional serological tests. Given the inherent errors in the use of the latter in the diagnosis of brucellosis, it is recommended that the ELISAs described here be considered as replacements for the conventional tests. The CELISA using the lipopolysaccharide antigen with the competing monoclonal antibody M84, should be considered as the most useful because of cross-species and vaccination considerations.

1. INTRODUCTION

Indirect enzyme immunoassays (IELISA) were developed to increase diagnostic sensitivity, diagnostic specificity and accuracy of diagnostic tests in use for detection of antibody to *Brucella abortus*. However, because of the increase in sensitivity of the IELISA there was a small decrease in assay specificity. In addition, it became apparent that antibody resulting from vaccination of cattle with *B. abortus* strain 19 sometimes contributed to the decrease in specificity. The specificity of the IELISA was markedly enhanced in early trial incorporation divalent cation chelating agents into the serum incubation step [2]; however, the assay could still not differentiate some cases of vaccinal antibody from antibody resulting from infection. Competitive enzyme immunoassays (CELISA) were developed because the affinity of the competing monoclonal antibody could be selected to exclude residual vaccinal antibody thus creating assays that could distinguish field infection from vaccination [1,3]. An additional advantage of the CELISA is its use for detection of antibody in various species. Unfortunately the I- and CELISAs were developed and validated under conditions where no bovine brucellosis was in evidence and where vaccination with strain 19 had not been practised for a number of years. All sera from infected and vaccinated cattle used in the initial studies were derived from banks of frozen serum. Because of this, it was decided that an extensive trial of these assays should be undertaken in countries where various levels of brucellosis were present in the bovine population and where vaccination with strain 19 was practised. This decision resulted in Research Contracts between five Latin American laboratories and the Joint FAO/IAEA Division of the International Atomic Energy Agency. These contracts allowed for the transfer of technologies to the laboratories, a supply of all necessary biological reagents from the Animal Diseases Research Institute (ADRI), a research grant and advice and backstopping by ADRI personnel.

## 2. MATERIALS AND METHODS

### 2.1. Participating laboratories

Dr. L.E. Samartino  
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Programa Investigación de Enfermedades  
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Dra. B.E. Perez  
Servicio Agrícola y Ganadero  
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CHILE

Dr. C. Peraza  
Instituto Medicina Veterinaria  
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Ciudad de Habana  
CUBA

Dra. O.C. Mariño Jannaut  
Instituto Colombiano Agropecuario  
ICA-CORPOICA  
Avenida El Dorado 42  
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### 2.2. Serological tests

The protocols for performing the Rose Bengal (RBT), buffered plate antigen (BPAT), confirmatory test and the ELISAs (the Joint FAO/IAEA Division International Atomic Energy Agency, OIE approved) version of the IELISA (ELISA1), a modified screening version of the IELISA (ELISA2), a CELISA using O-polysaccharide as the antigen (ELISA3) and a CELISA using smooth lipopolysaccharide (SLPS) as the antigen (ELISA4) are described elsewhere in this report. In each case, sera were tested by the RBT using antigen purchased from Mérieux; BPAT with antigen purchased from USDA; the regularly used confirmatory test of each country; the OIE approved IAEA IELISA kit; the ADRI modified IELISA; the CELISA using O-polysaccharide antigen and by the CELISA using SLPS antigen.

### 2.3. Sera

Sera used for this study are described in the contributions by each individual laboratory. The initial premises of the field trials were that each laboratory would test a minimum of 1000 serum samples, from serum banks or obtained from diagnostic services, of the following groups:

2.3.1. *Sera from non-vaccinated, non-exposed cattle*

All sera were BRBT and CFT negative.

2.3.2. *Sera from cattle receiving an approved schedule of B. abortus strain 19 vaccine:*

obtained at the normal testing interval. All sera were RBT and CFT negative.

2.3.3. *Sera from herds with proven B. abortus infection:*

at a prevalence of not less than 5% of the animals. Sera selected were RBT and CFT positive.

### 2.4. Data

The data from each laboratory is presented in the individual reports.

### 2.5. Statistical analysis

Where possible, each group of cattle was treated separately. Frequency distributions and receiver operator characteristic (ROC) analysis were used to determine the most suitable cut-off values

between negative and positive results [4]. From these data, relative diagnostic sensitivity and specificity values were calculated for each country.

### 3. RESULTS

The in-use diagnostic test combination for each country was used as the criteria to establish whether individual serum samples were positive or negative. These data were compared to the values obtained with each of the ELISA tests to determine the most appropriate cut-off value (Table I) and the relative diagnostic sensitivity and specificity for each ELISA for each country was calculated. These calculations are presented in Tables II (sensitivity) III and IV (specificity for vaccinated and non-vaccinated cattle). For comparison, values, where available, for Canada were included [3]. The overall relative diagnostic sensitivity and specificity values were calculated using analysis weighted for the number of samples tested in each location. These data are presented in Table V.

TABLE I. CUT-OFF VALUES DETERMINED BY FREQUENCY DISTRIBUTIONS AND ROC ANALYSIS OF THE DATA FROM EACH LABORATORY OF THE FOUR ELISAS FOR DETECTION OF ANTIBODY TO *BRUCELLA ABORTUS*

	ELISA1 %P	ELISA2 %P	ELISA3 %I	ELISA4 %I
Argentina	40	67	35	44
Chile	16	21	18	27
Colombia	14	40	30	29
Costa Rica	70	73	20	18
Cuba	38	38	28	21
Canada	na	46	30	30

na - data not available.

TABLE II. RELATIVE SENSITIVITY VALUES CALCULATED FOR EACH COUNTRY BASED ON CONVENTIONAL SEROLOGICAL TEST RESULTS ON SERA FROM HERDS INFECTED WITH *BRUCELLA ABORTUS*

	ELISA1 %	ELISA2 %	ELISA3 %	ELISA4 %
Argentina	97.9	92.7	96.9	97.7
Chile	98.9	97.1	98.9	100
Colombia	100	100	99.3	98.2
Costa Rica	100	98.4	92.1	93.2
Cuba	89.7	94.9	94.9	71.8
Canada	na	100	na	100

TABLE III. RELATIVE SPECIFICITY OF TESTS USING CATTLE VACCINATED WITH *BRUCELLA ABORTUS* STRAIN 19 BUT SEROLOGICALLY NEGATIVE BY CONVENTIONAL TESTS

	ELISA1 %	ELISA2 %	ELISA3 %	ELISA4 %
Argentina	98.1	96.3	98.1	98.1
Chile	96.9	78.8	94.4	100
Colombia	86.8	87.6	95.6	92.3
Costa Rica	91.8	94.6	93.8	96.0
Cuba	90.1	94.1	99.6	100
Canada	na	94.8	na	97.7

TABLE IV. RELATIVE SPECIFICITY OF REACTIONS OF SERA FROM NON-VACCINATED CATTLE BASED ON REACTIVITY IN CONVENTIONAL SEROLOGICAL TESTS

	ELISA1 %	ELISA2 %	ELISA3 %	ELISA4 %
Argentina	na	na	na	na
Chile	99.3	99.6	99.7	99.6
Colombia	99.8	99.8	99.8	97.1
Costa Rica	95.8	95.0	93.4	95.8
Cuba	99.7	99.7	100	100
Canada	na	99.4	na	99.9

TABLE V. MEANS (WEIGHTED BASED ON SAMPLE NUMBERS) OF RELATIVE SENSITIVITIES AND SPECIFICITIES OF THE ENZYME IMMUNOASSAYS

	ELISA1	ELISA2	ELISA3	ELISA4
Cut-off value	41%P	41%P	26%I	29%I
Sensitivity %	96.3	96.7	96.9	97.3
Specificity %	97.0	93.8	98.4	98.8

#### 4. DISCUSSION

As the enzyme immunoassays are currently being considered for use as confirmatory tests, all the data were calculated to try to maximise diagnostic specificity. In most of the countries involved in this study, vaccination with *B. abortus* strain 19 is widely practised in both calves and adult cattle. It is therefore debatable if the data for diagnostic specificity obtained with non-vaccinated cattle is of current interest. In other circumstances, such as areas where brucellosis has been eradicated and vaccination is decreased or stopped, this data will be more useful. All enzyme immunoassay data are based on the premise that the screening test followed by the confirmatory test for antibody are absolute. That is, if the screening and confirmatory tests are positive, it was assumed that the serum originated from an infected animal. Similarly, if both tests were negative, the serum was considered as originating from a non-exposed animal. If the screening or the confirmatory tests did not agree, the data was not considered. Because of these selection parameters, the ELISAs will not appear to function as well as the conventional tests. For instance, if a vaccinated animal is positive in the screening and confirmatory tests but negative in one or more of the ELISAs, it is assumed that the ELISAs failed to diagnose brucellosis. This reaction could equally well be due to residual antibody from *B. abortus* strain 19 vaccination. Alternately, if an animal from an infected herd gave a negative result in the conventional tests but was positive in one or more ELISA, it was assumed to be a false positive reaction where in fact it may be brucellosis in early stages of incubation, detected by the ELISAs due to their higher analytical sensitivities. It is of interest to note the considerable variation in the cut-off values established for the IELISAs (ELISA1 and ELISA2) while there were only minor variations in the cut-off values for the CELISAs (ELISA3 and ELISA4). These data are presented in Table 1. The reasons for the variations in the IELISA are not understood but may be a result of cross-reacting antigens present in localised cattle populations. Alternately, vaccination status of animals may be incorrect, especially where adult vaccination is practised. From Table 2, it appears that ELISA4, the CELISA that uses SLPS antigen, is marginally more sensitive than the other three ELISAs, all of which have nearly identical sensitivities, relative to the diagnostic serology tests used in each laboratory. The one aberrant value for the ELISA4 sensitivity is due to testing of sera grossly contaminated with bacteria, a factor known to interfere with M84 monoclonal antibody binding to the SLPS. This data should therefore be omitted in the analysis. The relative specificity in non-vaccinated or vaccinated but diagnostically negative cattle are very similar for all the ELISAs (Tables 3 and 4). It is of interest to note that using these samples, the

IELISAs performed nearly as well as the CELISAs. This is not surprising as the samples were selected based on negativity or lack of exposure to *B. abortus*. From the weighted summary (Table 5), ELISA4 slightly outperforms the other ELISAs for relative sensitivity while its relative specificity is very similar to those of the other ELISAs. From the data presented, the ELISAs perform as well or better than the combination of conventional serological tests used for diagnosis of brucellosis. Because of the inherent advantages of primary binding assay and in particular, the ability to perform assay quality control on an international scale and the ability of the CELISAs to eliminate residual antibody from *B. abortus* strain 19 vaccination, it is envisaged that these assay will replace the in-use serological tests.

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