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REPORT ON THE WHO/FAO/IAEA CONSULTATION ON ENZYME IMMUNOASSAY (EIA) FOR BRUCELLOSIS DIAGNOSIS AND RESEARCH

Vienna, 23-24 May 1991

CONTENTS

INTRODUCTION

Dr T. Fujikura, Scientist, Veterinary Public Health, Division of Communicable Diseases, World Health Organization (WHO) thanked all the participants (Annex I) for their continued collaboration. He also expressed his thanks to Dr J.D. Dargie, Head of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Agency (IAEA), Dr H. Jeggo, Programme Coordinator of the Division, and Dr P. Wright, Head of the Animal Health Unit, IAEA's Laboratory, Seibersdorf (near Vienna) for hosting and providing financial support for the meeting. Dr Fujikura, on behalf of Dr Hiroshi Nakajima, Director General of WHO, opened the meeting, and explained its purpose and scope:

(1) to review activities of the EIA working group since their 1990 meeting;

(2) to discuss improvements in EIA methods, with special reference to the new development of antigens and antigen production;

(3) to discuss the present techniques and usefulness of EIA for application to brucellosis diagnosis and research, with special attention to the FAO/IAEA/WHO diagnostic kit and further distribution and dissemination of this kit to interested countries;

(4) to discuss plans for further studies and international cooperation.

Dr C. Dubray was elected Chairman, Dr G. Wilke Vice-chairman, and Mr A.P. MacMillan served as rapporteur.

1. REVIEW OF COLLABORATIVE WORK (1990-1991)

(1) A review of the WHO working group's activities revealed that planned schedules for quality assurance testing of antigen preparations were well advanced. These comprised seven batches of smooth-lipopolysaccharide (S-LPS) antigens produced at the FAO/WHO Collaborating Centre for Reference and Research in Brucellosis, Central Veterinary Laboratory, Weybridge, UK. They were (i) B09 and B10 prepared by hot phenol water extraction method, (ii) PK1/A, PK2/AL AND PK1/K antigens prepared by proteinase K digestion, and (iii) AS1 and AS2 prepared from simple saline supernatant of brucella suspension. In addition, CAD antigens prepared by a method similar to (i) above was supplied by the Animal Diseases Research Institute, Nepean, Canada.

(2) The batches were examined for protein content and by polyacrylamide gel electrophoresis (PAGE). The protein content of the antigens at their working strength varied from about 40ng/100μl for the supernatant batches to less than 10ng/100μl for one of the proteinase K batches. Protein bands were observed in all batches, particularly in the range less than 35 kDa. The PK batches displayed only one band at about 25 kDa. The length of the 'O' chain appeared identical for all preparations.

Functional aspects of the antigen preparations were examined by titration and by EIA, using a range of sera from various sources.

Both supernatant batches showed an unusual titration curve with the weak positive serum, with optical densities rising to a peak at an antigen dilution of 1/1000 before falling away.

The most marked differences in functional activity were between the binding of serum from vaccinated animals to the phenol extracts and the binding to the PK batches. There was significantly less binding to the latter.

However, it was decided that as more information was available on the phenol water extraction procedure, this method would be used for the preparation of an antigen batch for further WHO trials. These initial results, whereby antigens made using the proteinase K procedure appear to be able to differentiate vaccinal reactions, should be confirmed by further research.

Note should be taken that biochemical assay of antigens above may not be sufficient to detect differences between different batches, and that some functional assessment should also be made.

S-LPS fractions were analyzed by SDS-PAGE at the concentration of 1 mg/ml. There was little material in the PK1/A and PK1/K fractions. Protein bands were not easily seen. The 25 kDa was present in the AS1 fraction and in the S-LPS PK99 and S-LPS PK B19 fractions with a lower molecular mass due to proteinase K digestion. The 36-38 kDa was also present in the two last fractions.

High molecular mass molecule of S-LPS were observed between the front migration to 40 kDa (protein markers) for AS1, AS2, B9 and B10. For fractions CDA, S-LPS PK B19 and S-LPS PK 99, the range of high molecular mass was between 21 to 40 kDa (protein markers).

The proteins present in the fractions and the differences in the distribution of length of S-LPS molecules may affect the antigenic activity of these fractions. For example, it was demonstrated that a monoclonal antibody against the 25 kDa was able to bind on S-LPS vesicles as seen by electron microscopy and bind on the same antigen used in ELISA (G. Dubray, data not published).

Detailed research information is given in Table 1, and Figures 1 and 2.

(3) As far as polyclonal conjugates available from various commercial sources is concerned, there were significant discrepancies in the potency of seven products used for internal quality control testing. This indicates that it is necessary to select the best brand/batch prior to running a number of samples for assessment of clinical and epidemiological situations (Figures 3 and 4).

Cross-reactivity has also been compared amongst 23 animal species with Canadian-produced monoclonal antibodies produced for respective immunoglobulins. In comparisons with 23 species, sheep had strong cross-reactivity with bovines in IgM and IgA in 100%, IgG1 in 60%, and IgG2 in 40%; in goats, less than 50% cross-reactivity occurred for all the bovine Ig isotypes (Figure 5). The above results indicate that monoclonal antibodies prepared for bovine immunoglobulin classes may not be useful for the testing of other species. Conjugate should be titrated before use in diagnostic testing with the reference antigen and positive/weak, positive/negative reference sera.

(4) In the meantime it was agreed to distribute new B. abortus diagnostic kits by September 1991 to 30 institutions. Comparative data of the EIA and conventional diagnostic tests should be fed back from these participating institutions.

2. DISCUSSION ON ACHIEVEMENTS 1990-1991

2.1 EIA antigen and enzyme conjugate

Bovine brucellosis EIA - a model for the development and transfer of the enzyme-linked immunosorbent assay methodology.

(1) Research and development. The Animal Production Unit, IAEA Agriculture Laboratory, Seibersdorf, is seeking to establish specific and sensitive EIA's that will function under sub-optimal conditions which may be present in some laboratories in developing countries.

In close collaboration with scientists in Weybridge, UK and Nepean, Canada, a robust FAO/IAEA/WHO EIA kit has been developed for the detection of antibodies against B. abortus in cattle. This kit is based on a simple indirect ELISA developed by the Animal Diseases Research Institute (ADRI), Nepean, Canada using an S-LPS antigen produced by the Central Veterinary Laboratory (CVL), Weybridge, UK.

(2) Comparison of different methods of antigen preparation

Seven batches of antigen for the IAEA were prepared by three different methods. Batches AS1 and AS2 were simply the supernatant of B. abortus cells in 0.5% phenol saline. Batches B9 and B10 were prepared by hot phenol/water extraction. Batches PK1/A, PK1/K and PK2/AL were prepared by proteinase K digestion.

(3) Panel of sera

A panel of sera consists of samples from bovine brucella infected or vaccinated animals, and some from miscellaneous sources. When the optical density (OD) of each sample is expressed as a percentage of the OD of the international standard serum, the results given by samples from infected animals varied very little between the different antigens. Samples from vaccinated animals, particularly the complement fixation test (CFT) series from animals vaccinated seven weeks previously, showed a marked difference between the results given by the AS- and B-batches and the PK-batches. These samples tended to give relatively lower OD's with antigens prepared by proteinase K digestion.

The latest batch, PK2/AL was prepared from 1 kg wet weight of S99 cells. The yield was good and sufficient reagent to test 75 million samples was obtained.

Detailed research information is shown in Table 2 and Figures 6 (1-3), 7 and 8 (1-7).

(4) Competition EIA and indirect EIA

Research on Enzyme Immunoassay for Brucellosis Diagnosis has been progressing on a number of fronts since the last group meeting in April 1990, particularly on further evaluation of a competition EIA (cEIA) using an anti-M monoclonal and indirect EIA (iEIA) with samples from pigs infected with Yersinia enterocolitica 0:9, goats infected with B. melitensis, and cattle vaccinated as calves with strain 19 vaccine. In collaboration with the IAEA and INRA (Institut national de la Recherche agronomique, Nouzilly, France) laboratories, the effect on its performance of the method of LPS antigen purification has been studied.

Goats infected with B. melitensis. Sera from 19 goats from a flock in Malta from which B. melitensis had been isolated were tested by the conventional tests for brucellosis and also cEIA and iEIA. Twelve (63%) were positive to the serum agglutination test (>60iu), 17 (89%) were positive to the complement fixation test (CFT) (>20icftu), 17 (89%) were positive to the cEIA (>50% inhibition at 1/2 dilution), and 13 (68%) were positive to the iEIA (>10% OD international standard serum).

Pigs infected with Yersinia enterocolitica 0:9. The UK is currently experiencing a severe problem in pigs shown to be infected with Yersinia enterocolitica 0:9. Two hundred pigs from herds from which the organism had been isolated were tested by CFT, cEIA and iEIA. Seventy (35%) were positive to the CFT, 140 (70%) were positive to the iEIA, and 165 (82.5%) were positive to the cEIA with brucella antigen.

Calves vaccinated with strain 19 vaccine. Six calves of 6 months of age were vaccinated with the standard dose of strain 19. Serial blood samples were taken and tested by the conventional tests and cEIA and iEIA. All calves became positive to the cEIA on the third day following vaccination, but not until day 14 in the iEIA. One calf had become negative to the iEIA and cEIA by day 79, although the others have remained positive.

2.2 Internal and external quality assurance

(1) External quality assurance. The Animal Production Unit of the IAEA is responsible for compiling a reference bank of characterized sera and implementing an external quality assurance programme for participating laboratories throughout the world.

A reference serum bank for *B. abortus* has been established in collaboration with the Central Veterinary Laboratory, Weybridge, the Animal Diseases Research Institute, Nepean, and the Department of Agriculture, Perth, Australia.

(2) Assay standardization and internal quality assurance. International acceptance of diagnostic test systems is a prerequisite for the international movement of animals and their products. Therefore the Animal Production Unit of IAEA has established EIA kits and protocols which are fully standardized and has implemented an internal quality assurance programme to monitor and guarantee both the quality of biological and chemical reagents and performance of the test.

South-east Asia and Latin America will be undertaking collaborative studies on the standardization of enzyme immunoassay reagents, under the auspices of WHO, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, and research institutes in Europe, Australia, Canada, and the USA. This work will focus for 1991 on the S-LPS antigen, a mouse monoclonal anti-bovine IgG_1 conjugate and a bovine IgG_1 anti-brucella S-LPS.

2.3 EIA diagnostic kit and distributors

(1) Kit production. In the period 1986-1990, *B. abortus* EIA assay kits able to test 20 000 sera were sent by the Joint FAO/IAEA Division in Vienna to laboratories in Latin America, Africa and South-east Asia.

(2) Technology transfer methods. The transfer of EIA methodology to laboratories of project counterparts and the training of scientists and technicians are major objectives. Training is provided during:

- interregional training courses;
- regional training courses;
- national training courses; and
- fellowship training programmes.

Using the *B. abortus* EIA test as a model for methodology transfer, EIA technology has been introduced in 26 laboratories throughout Latin America, Africa and South-east Asia; 113 people have been trained over the period 1986-1990.

(3) Technical support. Once the EIA methodology is transferred, on-site technical support may be required by regional experts, technical officers or participating research institutes. Technical support missions are undertaken to resolve specific problems, to adapt existing EIA techniques, or to introduce new developments. Between 1986 and 1990, 54 technical support missions have been made to several laboratories using the *B. abortus* EIA.

2.4 Data collection and analysis

(1) Diagnostic validation. An assessment of the diagnostic performance of the enzyme immunoassay in terms of diagnostic sensitivity and specificity is essential to define its diagnostic usefulness.

For the B. abortus EIA test:

- Sera from cattle with known histories (experimentally infected, bacteriologically defined, vaccinated and disease-free animals) provided by the Department of Agriculture, Perth, Australia and the Central Veterinary Laboratory, Weybridge, UK, were tested using the EIA and compared with the CFT.

- Sera from cattle populations from areas of natural infection were tested by research institutes in Latin America, Africa and South-east Asia, comparing the EIA results with those from other serological tests, including CFT, the acidified plate tests and the standard tube agglutination test (STAT). The results of these studies indicated that the diagnostic performance of the B. abortus EIA test was comparable, if not superior to the serological tests used as confirmatory tests in control and eradication programmes.

3. WORK PLANS FOR 1991-1992

The group agreed on the following plans for further collaboration:

3.1 The EIA kit for B. abortus diagnosis will be despatched by September 1991 to 30 institutions worldwide with the request that they report back to the Joint FAO/IAEA Division, Vienna, and to the Veterinary Public Health unit of WHO by end 1991. The kit should be accompanied by strong/weak positive and negative reference sera, including the international standard anti-brucella serum (ISABS). For testing the samples, the institutions can be requested to include sera from culture positive animals for EIA testing and the data of RBT, CFT, mercaptoeshanol (ME) and Rivanol tests. Each kit should be able to test 4800 serum samples.

3.2 For B. melitensis diagnosis, collaborative work was also urged for (a) antigen production following the three procedures in 1 (1) at Weybridge by August 1991, (b) monoclonal antibody conjugate at INRA in Nouzilly by August 1991, and (c) A and M antigen in Hanover by September-October 1991. Preliminary tests could be performed at the IAEA Seibersdorf Laboratory in December 1991.

3.3 For competitive EIA, antigens will be produced with strain 99 and strain 16M (Weybridge). The procedures for antigen production will be similar to those used for the present study, namely (a) phenol water extraction, (b) proteinase K digestion, and (c) Nielsen's method. Anti-globulin conjugated monoclonals will also be prepared from YST9 (Nepean), BM 38 and BM 40 (Weybridge), and AMC hybrydomas (Nouzilly).

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 The working group agreed work plans and responsibility for division of collaborative studies within the time frame 1991-1992. To facilitate smooth exchange of the research material amongst working group members, recent regulations and rules for international transfer of biological materials should be carefully observed.

4.2 To facilitate prompt exchange of data related to the collaborative studies, a computer network should be developed quickly for smooth transfer of data and information between institutions in Europe, USA and Canada in particular.

4.3 To assist brucellosis scientists working in public health, clinical medicine and animal health in regions/countries/groups, those institutions worldwide interested in the working group's activities should be identified, particularly in the Eastern Mediterranean and South-east Asia regions. Brucellosis diagnosis in sheep and goats as well as in man should be improved by use of the EIA method, especially in these regions.

4.4 The next meeting of the working group will be held in Geneva in April 1992 for review and discussion of the outcome of collaborative research agreed upon at the Vienna 1991 meeting.

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Acknowledgements

The participants wished to acknowledge the following scientists of the Agency's Laboratory, Animal Production Unit, Seibersdorf, who joined the meeting and contributed to lively discussions:

- Mr Ernse Nilsson, APO, Sweden;
  Dr Eugine van Roog, APO, Netherlands;
- Mr Lelenta Mamadou, Chief Technician.

TABLE 1. PROTEIN CONTENT OF S-LPS FRACTIONS

Batches	LOWRY %	BCA*
AS1	28.8	31.8
AS2	26.8	28
PK1/A	24.8	21.7
PK1/K	6.1	18.8
B 9 (87)	ND	19.7
B 10 (89)	ND	28.8

* BCA : bicinchoninic acid method

** ND : not done

TABLE 2. **Serological Results (Standard Weybridge Method) Expressed as % of ISABS Control**

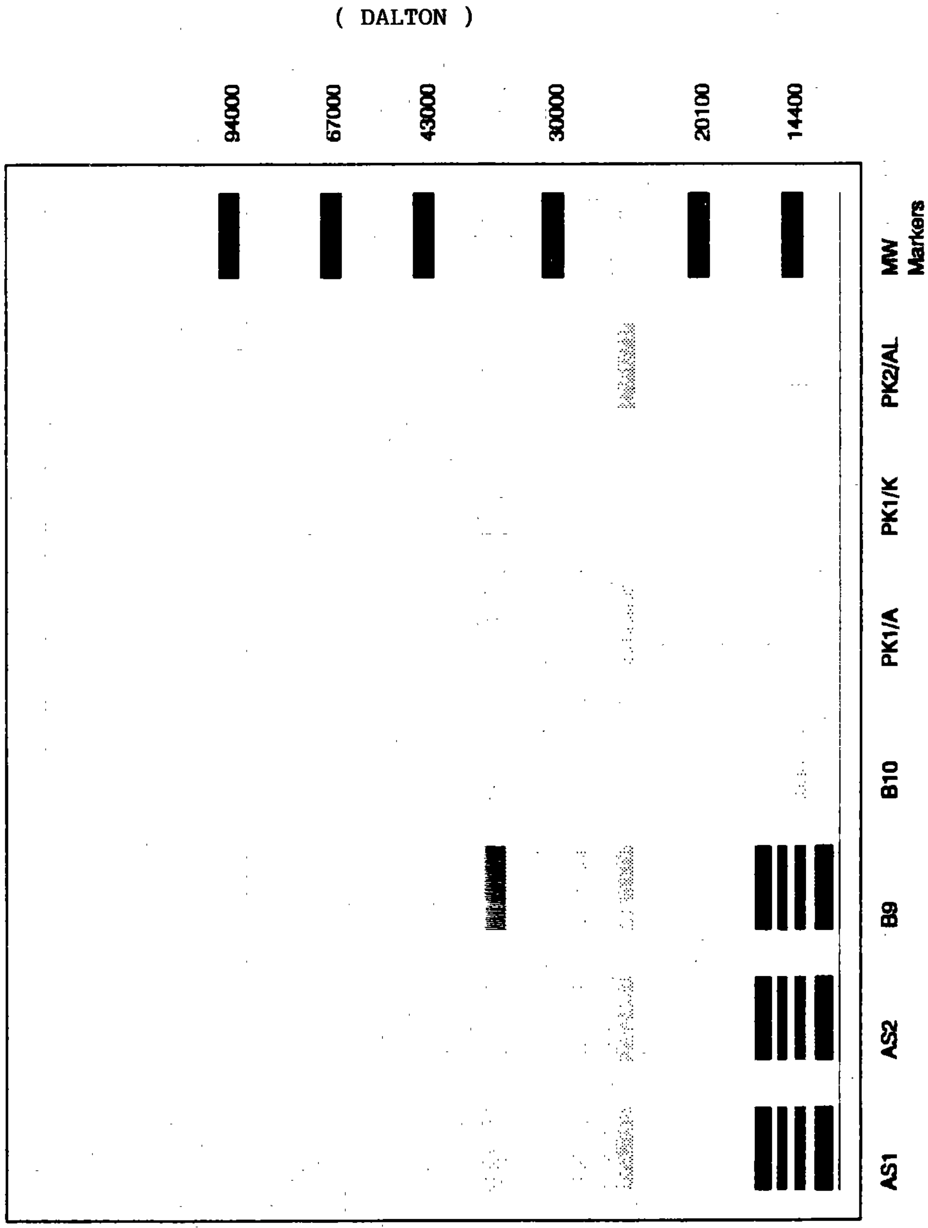
	STATUS	AS1	AS2	B9	B10	PK1/A	PK1/K	PK2/AL
DOB1	Vaccinated	27.9	34.2	39.9	25.6	10.4	5.9	6.2
DOB2	Vaccinated	72.7	80.6	81.3	68.4	31.9	18.9	19.2
DOB3	Vaccinated	9.0	7.1	12.4	3.0	6.8	3.3	3.8
DOB4	Vaccinated	39.6	51.4	53.2	35.6	21.6	18.2	16.8
DOB5	Vaccinated	44.1	53.8	54.5	43.3	39.6	43.6	32.4
DOB6	Vaccinated	79.8	77.8	78.9	70.7	48.8	97.5	56.6
3900	Infected	106.5	103.4	99.8	108.6	125.8	137.5	127.6
4448	Infected	42.5	49.4	50.9	38.5	21.8	21.3	15.2
5570	Infected	62.0	60.4	52.4	45.4	34.4	45.2	36.7
5706	Infected	103.8	99.9	102.1	101.1	109.3	128.6	118.3
CP88	Infected	88.1	89.0	90.3	80.1	73.9	83.4	73.9
PM238	Infected	109.7	102.0	106.5	104.2	102.7	106.1	106.0
PM248	Infected	101.9	99.7	102.7	99.6	112.6	113.9	109.4
C8046	Infected	102.3	102.5	102.8	101.8	90.8	104.0	94.9
WWS-4	Infected	100.4	102.0	102.7	99.4	91.9	95.6	97.1
WWS-HT	Infected	104.1	103.1	105.4	103.4	98.0	113.2	101.9
PE22	Yersinia Pig	9.2	6.4	7.7	6.3	5.9	4.8	3.5
PE25	Yersinia Pig	6.9	5.7	5.4	4.5	4.9	2.1	2.0
2788	B. ovis Ram	36.7	33.4	35.3	30.7	15.4	22.3	18.2
CF45	Vaccinated	31.8	33.7	44.0	15.7	6.9	4.2	4.2
CF46	Vaccinated	45.9	59.8	66.3	31.9	16.1	6.0	7.0
CF47	Vaccinated	46.9	52.3	60.0	32.2	15.6	5.5	7.2
CF48	Vaccinated	40.3	54.6	60.1	31.3	7.8	2.2	4.1
CF49	Vaccinated	35.0	44.3	50.9	23.5	7.7	2.5	3.1
CF50	Vaccinated	44.2	52.0	56.0	32.3	10.4	3.9	3.9
Bovine -ve	Negative	3.0	1.3	1.0	0.3	2.0	1.0	3.1
PM249	Negative	3.5	2.1	1.5	1.7	4.1	1.5	1.0
ISABS +ve Control		1.811	1.910	1.891	1.772	1.342	1.082	1.199
-ve Control		0.037	0.036	0.029	-0.012	0.059	0.015	0.027
Binding Ratio		49	53	65		23	72	44
Blank		0.048	0.047	0.050	0.128	0.056	0.053	0.048

Protein Content (Pierce BCA Assay)

	AS1	AS2	B9	B10	PK1/A	PK1/K	PK2/AL
Concentration mg/ml	0.82	0.73	0.9	0.2	0.1	0.07	0.37
Working Concentration ng/100ul	41	36.5	30	12.5	67	12	7.4

Figure 1.

SDS-Page Electrophoreisis of LPS Antigens
(PHAST System)



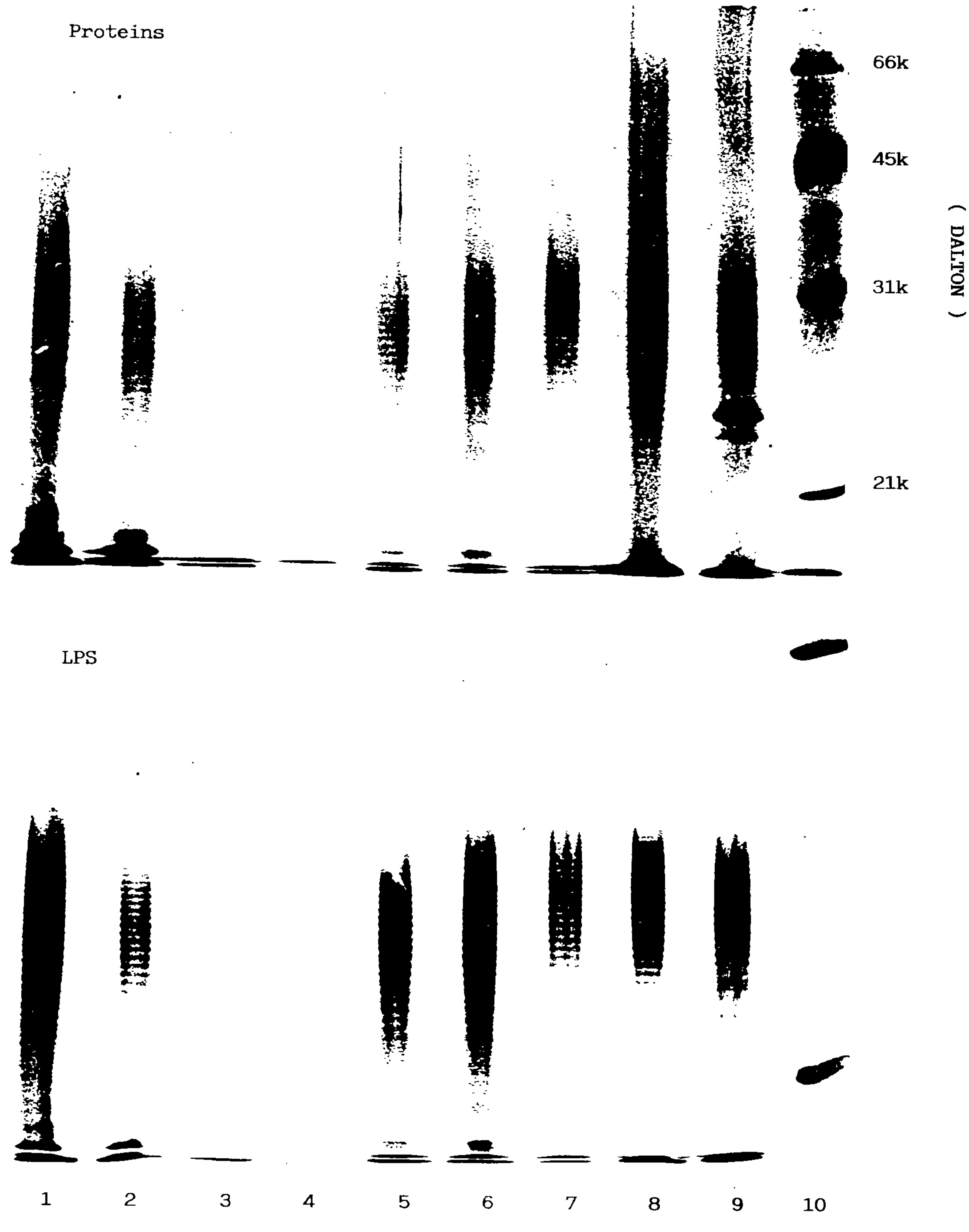
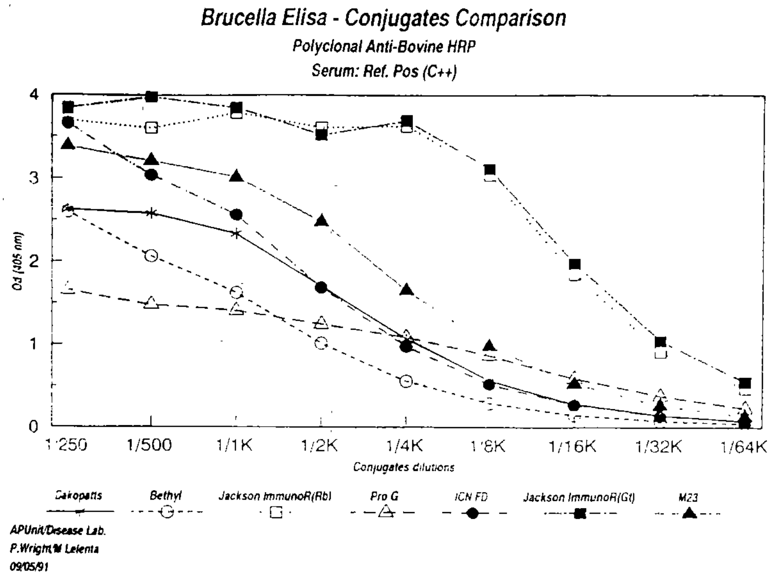


Figure 2 : **SDS Page analysis of S-LPS fractions of Brucella**

AS1 : lane 1, AS2 : Lane 2, PK1/A : lane 3, PK1:K : lane 4, B 10 (89) : lane 5, B 9 (87) : lane 6, CDA : lane 7, S-LPS PK B19 : lane 8, S-LPS PK 99 : lane 9 and protein markers kit : lane 10.

A : protein staining

B : carbohydrate staining


Brucella Elisa - Conjugates Comparison
Polyclonal Anti-Bovine HRP
Serum: Ref. Pos (C++)
Od (405 nm)
0
1
2
3
4
1/250
1/500
1/1K
1/2K
1/4K
1/8K
1/16K
1/32K
1/64K
Conjugates dilutions
Dakopatts
Bethyl
Jackson ImmunoR(Rb)
Pro G
ICN FD
Jackson ImmunoR(Gt)
M23
APUnit/Disease Lab.
P.Wright/M Lelenta
09/05/91

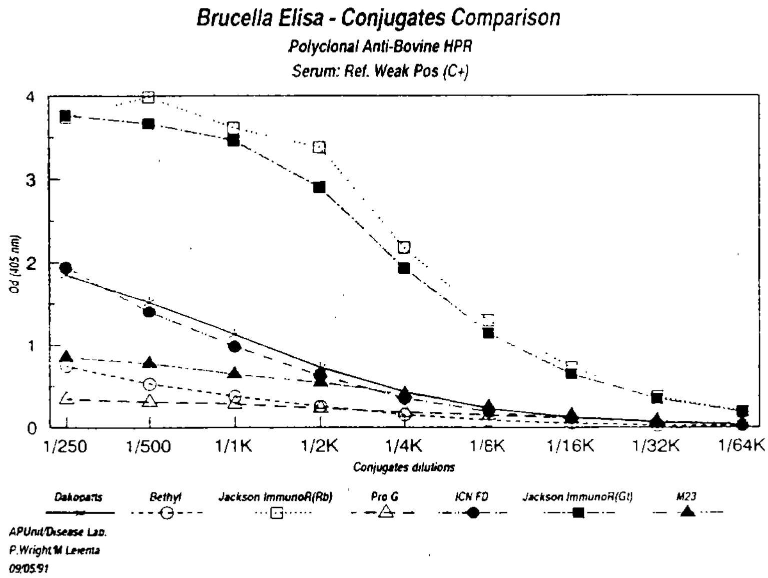
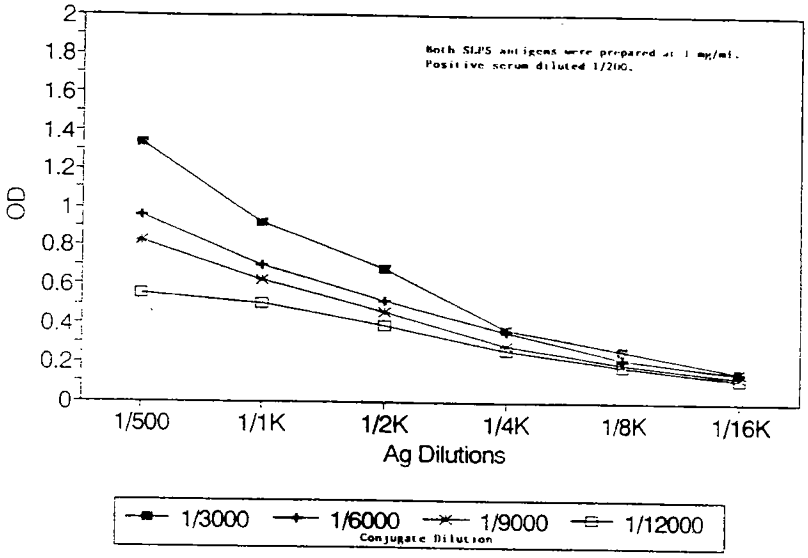

Brucella Elisa - Conjugates Comparison
Polyclonal Anti-Bovine HPR
Serum: Ref. Weak Pos (C+)
Od (405 nm)
0
1
2
3
4
1/250
1/500
1/1K
1/2K
1/4K
1/8K
1/16K
1/32K
1/64K
Conjugates dilutions
Dakopatts
Bethyl
Jackson ImmunoR(Rb)
Pro G
ICN FD
Jackson ImmunoR(Gt)
M23
APUnit/Disease Lab.
P.Wright/M Lerenta
09/05/91

Figure 3

CHECKERBOARD TITRATION

- Ag: Fr - Serum Pos.- Conj.: M23 -



CHECKERBOARD TITRATION

Ag: CDA-Plate 1-Serum Pos.-Conj.: M23

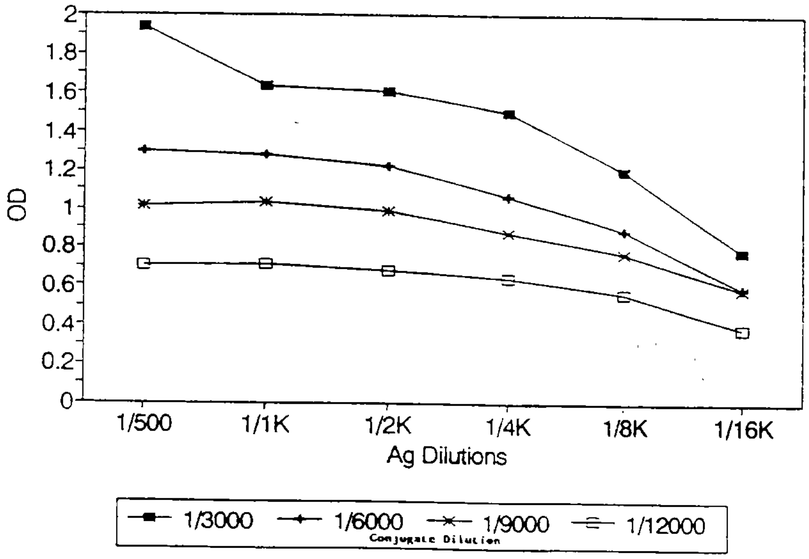


Figure 4

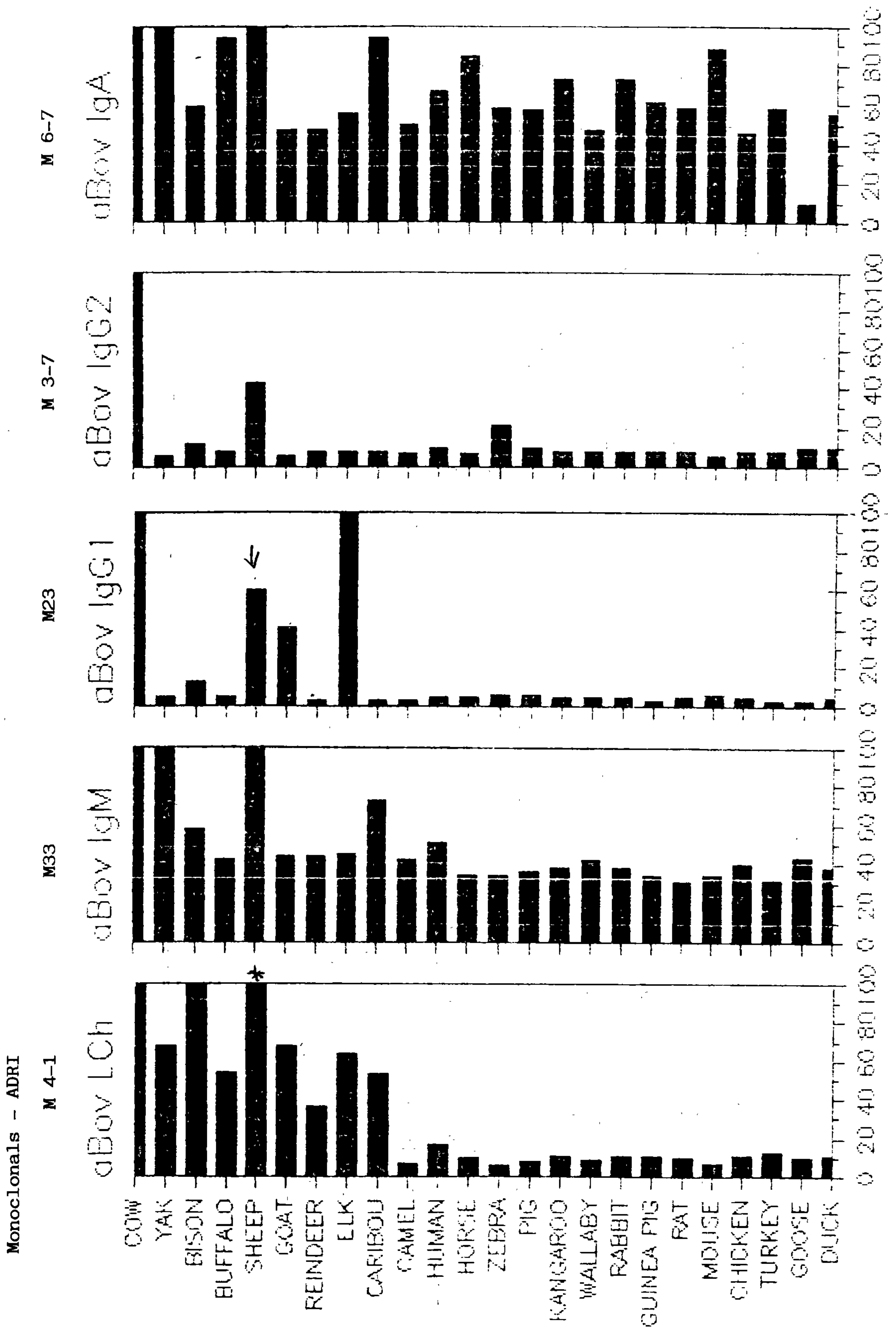


Figure 5 : % Cross-reactivity of monoclonal antibodies to bovine immunoglobulins with different animal species

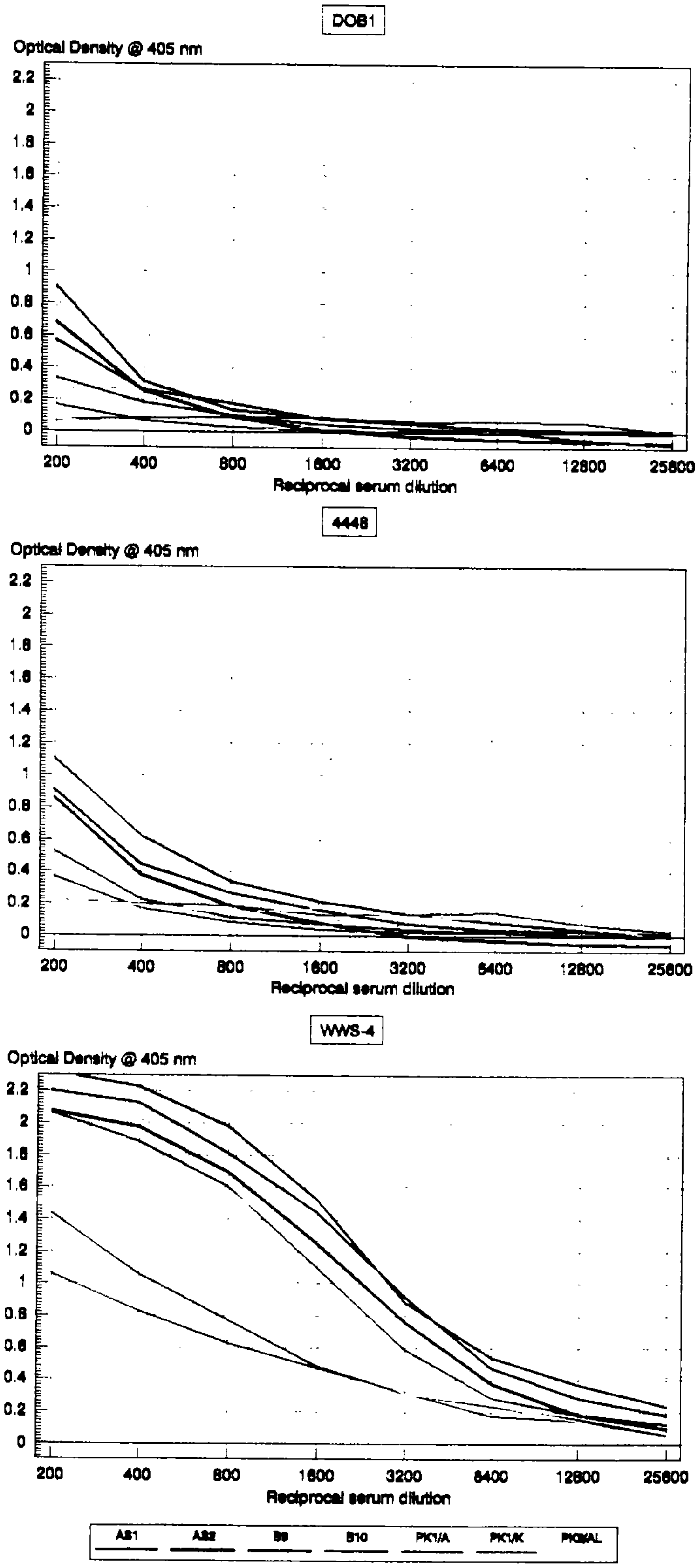


Figure 6 : Kinetics of various brucella EIA antigens Fig.6(1)

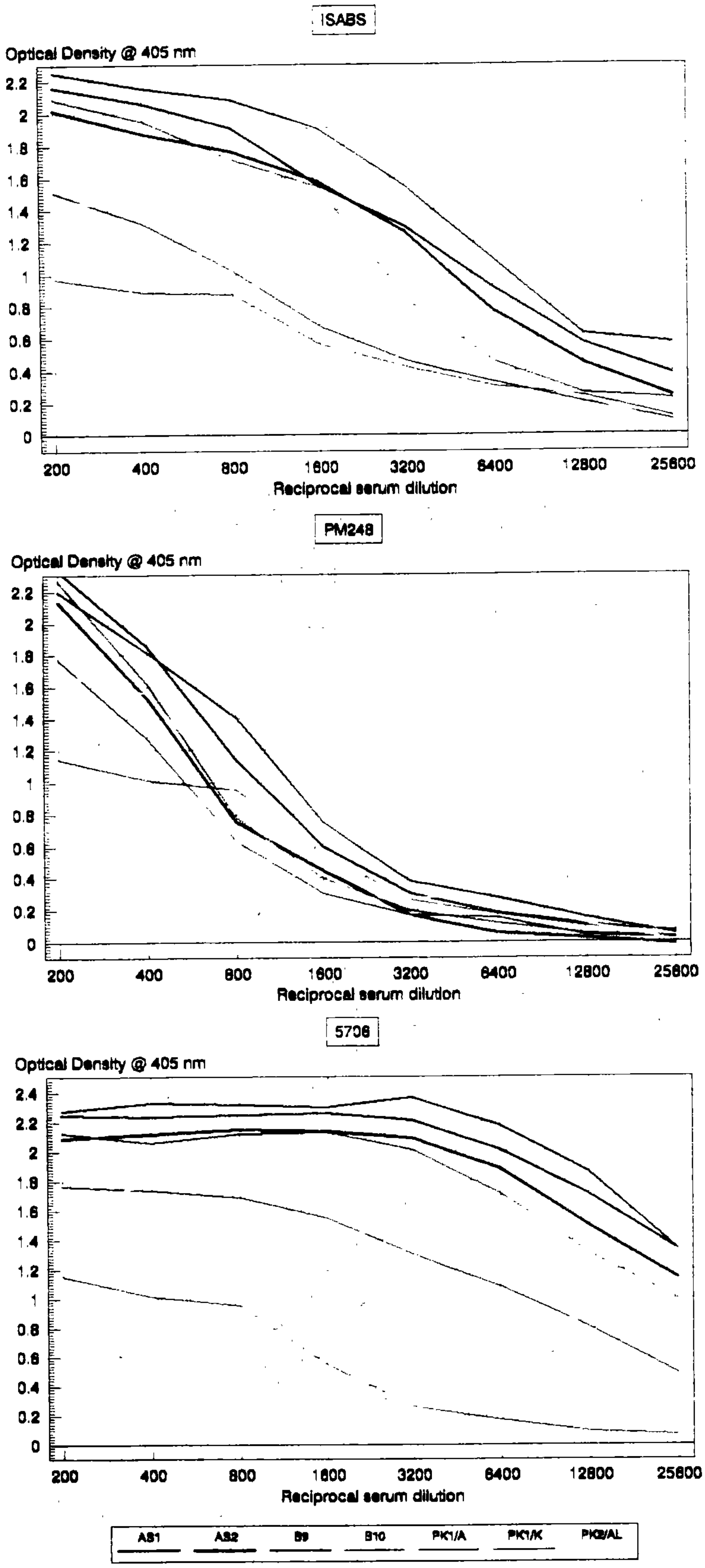

ISABS
Optical Density @ 405 nm
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
200
400
800
1600
3200
6400
12800
25600
Reciprocal serum dilution
PM248
Optical Density @ 405 nm
Reciprocal serum dilution
5706
Optical Density @ 405 nm
2.4
Reciprocal serum dilution
AS1
AS2
B9
B10
PK1/A
PK1/K
PK8/AL

Fig.6(2)

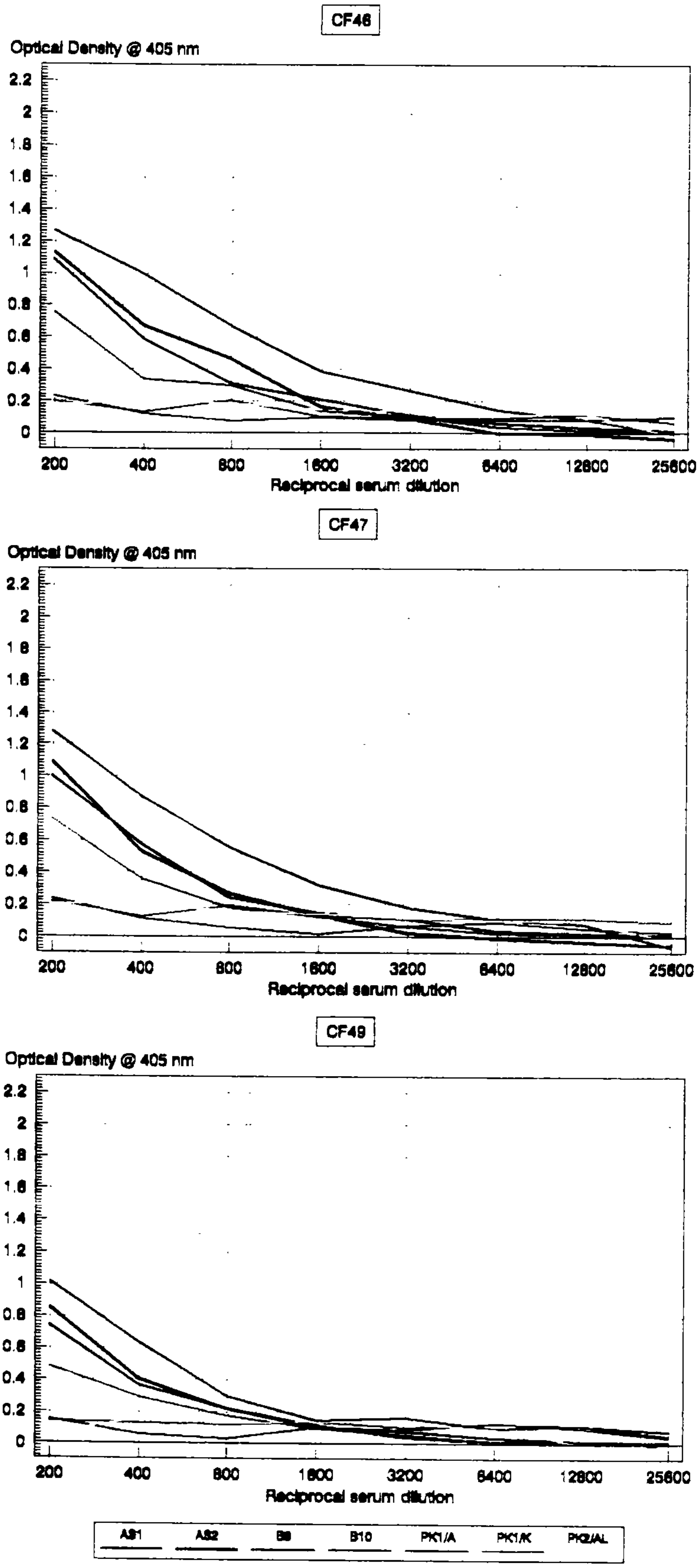
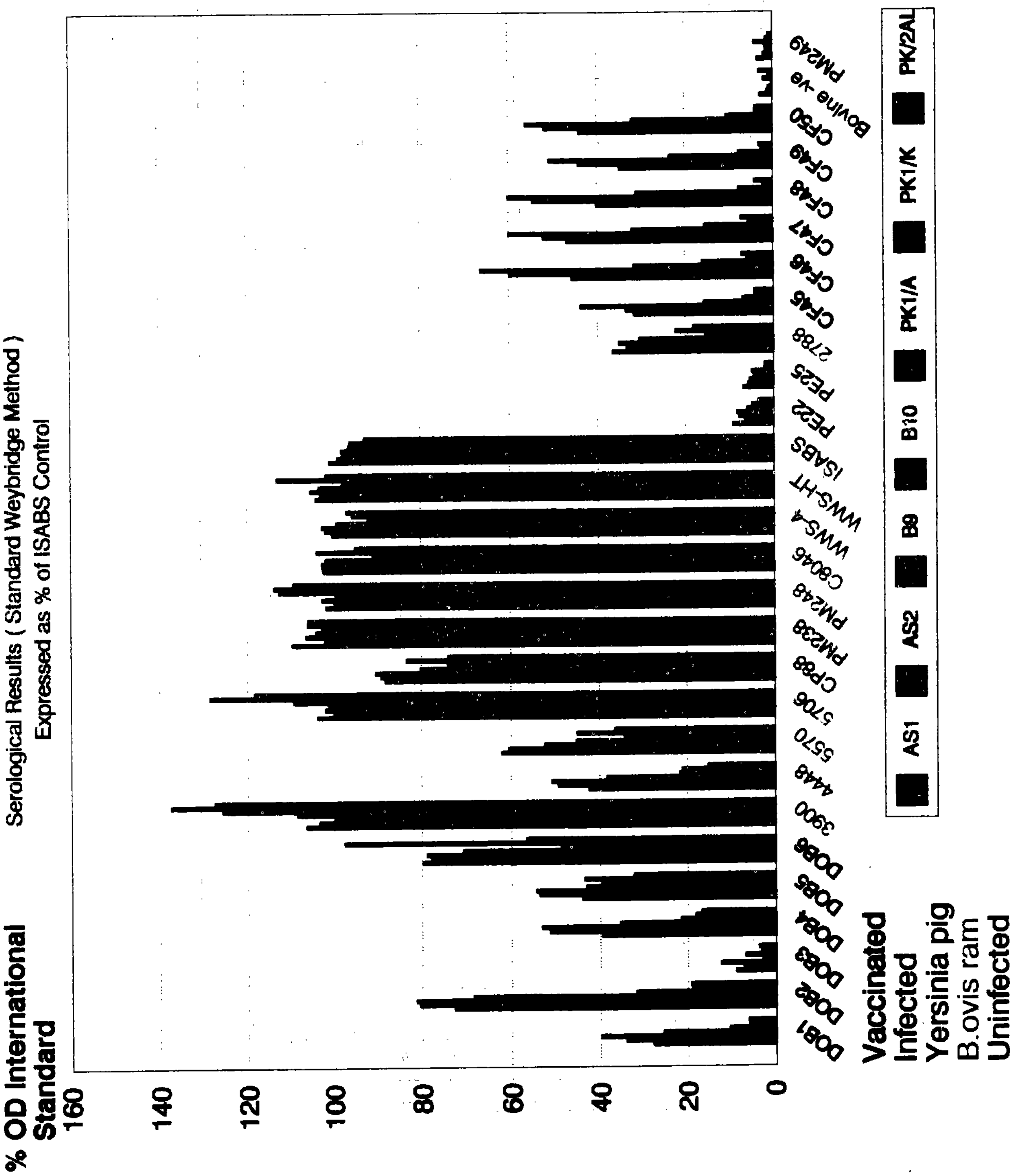
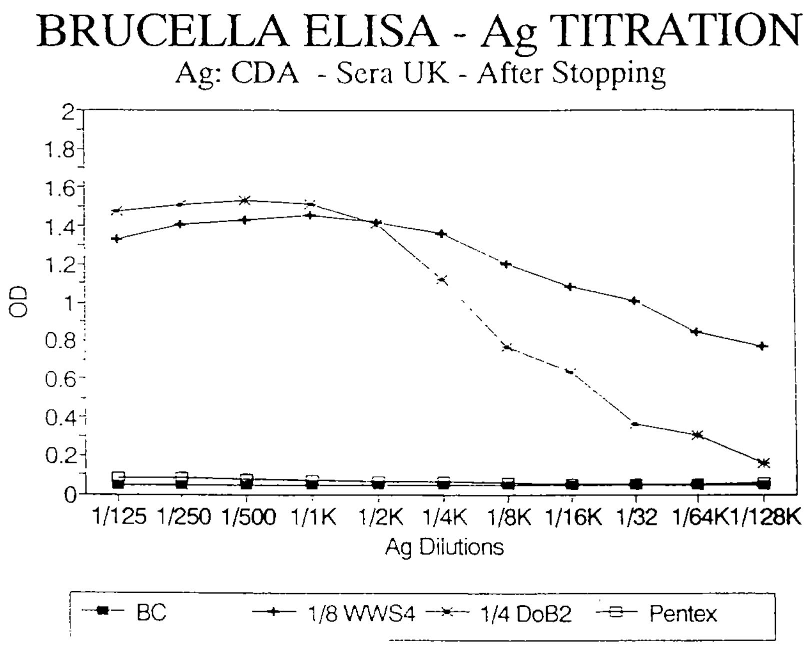

CF46
Optical Density @ 405 nm
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
200
400
600
1600
3200
6400
12800
25600
Reciprocal serum dilution
CF47
Optical Density @ 405 nm
CF49
Optical Density @ 405 nm
Reciprocal serum dilution
AS1
AS2
B10
PK1/A
PK1/K
PK2/AL

Fig.6(3)

Figure 7


Serological Results (Standard Weybridge Method)
Expressed as % of ISABS Control
% OD International Standard
160
140
120
100
80
60
40
20
0
DOB1
DOB2
DOB3
DOB4
DOB5
DOB6
3900
4448
5570
5706
CP88
PM238
PM248
C8046
WWS-4
WWS-HT
ISABS
PE22
PE25
2788
CF45
CF46
CF47
CF48
CF49
CF50
Bovine -ve
PM249
AS1
AS2
B9
B10
PK1/A
PK1/K
PK/2AL
Vaccinated
Infected
Yersinia pig
B.ovis ram
Uninfected


BRUCELLA ELISA - Ag TITRATION
Ag: CDA - Sera UK - After Stopping
OD
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1/125 1/250 1/500 1/1K 1/2K 1/4K 1/8K 1/16K 1/32 1/64K 1/128K
Ag Dilutions
BC
1/8 WWS4
1/4 DoB2
Pentex

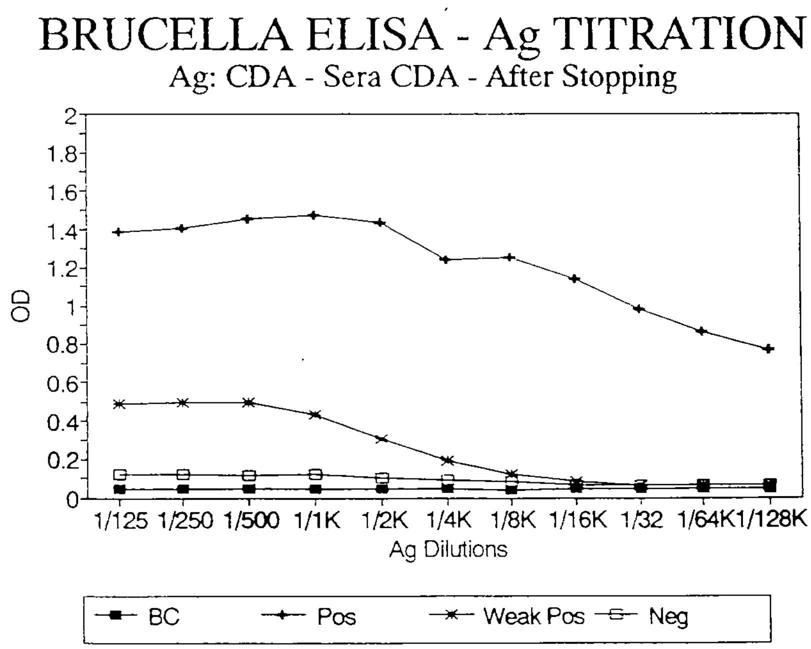
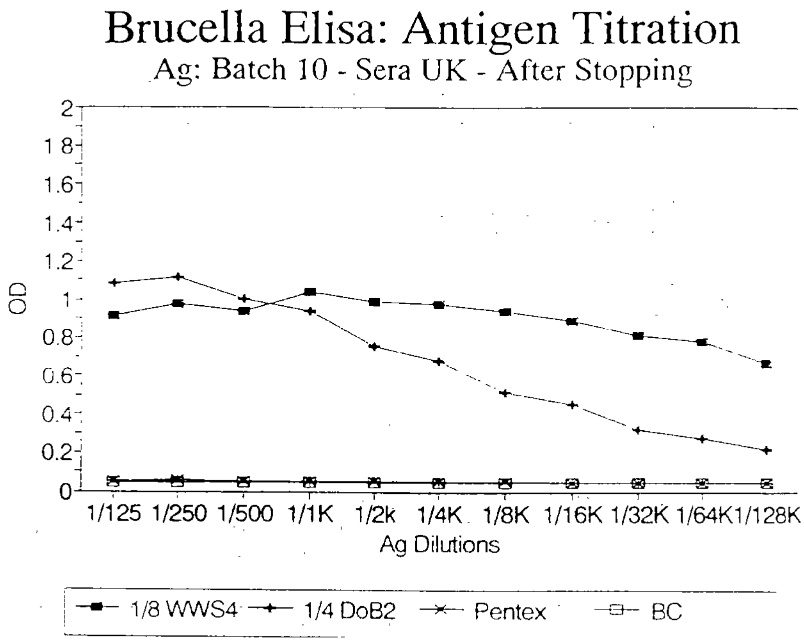

BRUCELLA ELISA - Ag TITRATION
Ag: CDA - Sera CDA - After Stopping
OD
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1/125 1/250 1/500 1/1K 1/2K 1/4K 1/8K 1/16K 1/32 1/64K 1/128K
Ag Dilutions
BC
Pos
Weak Pos
Neg

Figure 8 (1)


Brucella Elisa: Antigen Titration
Ag: Batch 10 - Sera UK - After Stopping
OD
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
1/125 1/250 1/500 1/1K 1/2k 1/4K 1/8K 1/16K 1/32K 1/64K 1/128K
Ag Dilutions
1/8 WWS4
1/4 DoB2
Pentex
BC

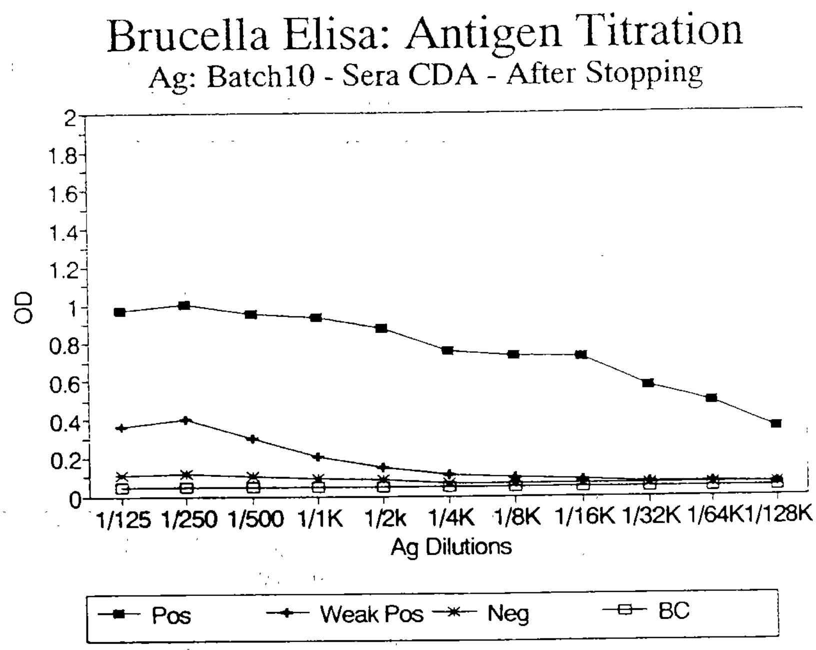
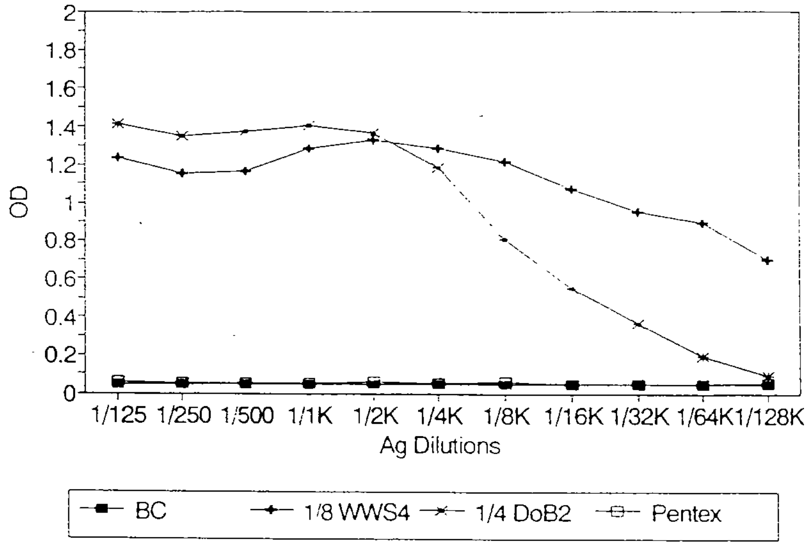

Brucella Elisa: Antigen Titration
Ag: Batch10 - Sera CDA - After Stopping
OD
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
1/125 1/250 1/500 1/1K 1/2k 1/4K 1/8K 1/16K 1/32K 1/64K 1/128K
Ag Dilutions
Pos
Weak Pos
Neg
BC

Figure 8 (2)

BRUCELLA ELISA - Ag TITRATION
Ag: B09 - Sera: UK - After Stopping



BRUCELLA ELISA - Ag TITRATION
Ag: B09 - Sera: CDA - After Stopping

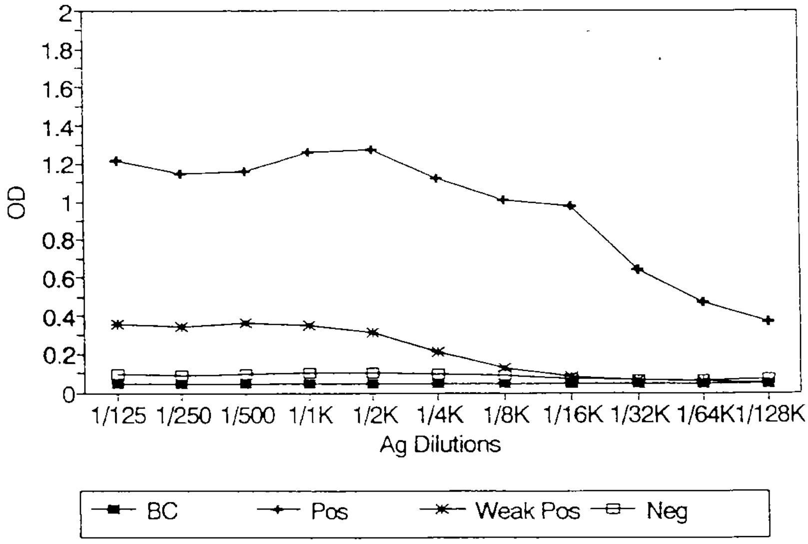
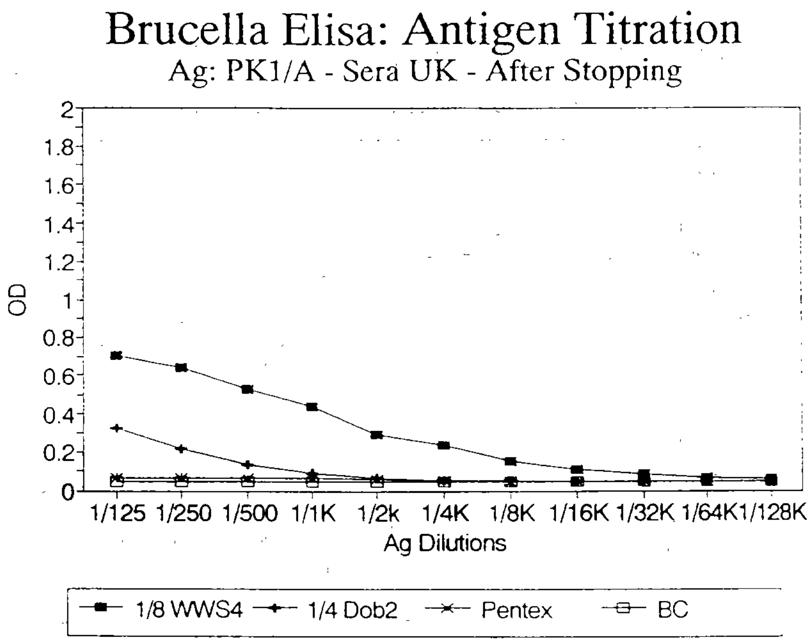


Figure 8 (3)


Brucella Elisa: Antigen Titration
Ag: PK1/A - Sera UK - After Stopping
OD
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1/125 1/250 1/500 1/1K 1/2k 1/4K 1/8K 1/16K 1/32K 1/64K 1/128K
Ag Dilutions
1/8 WWS4
1/4 Dob2
Pentex
BC

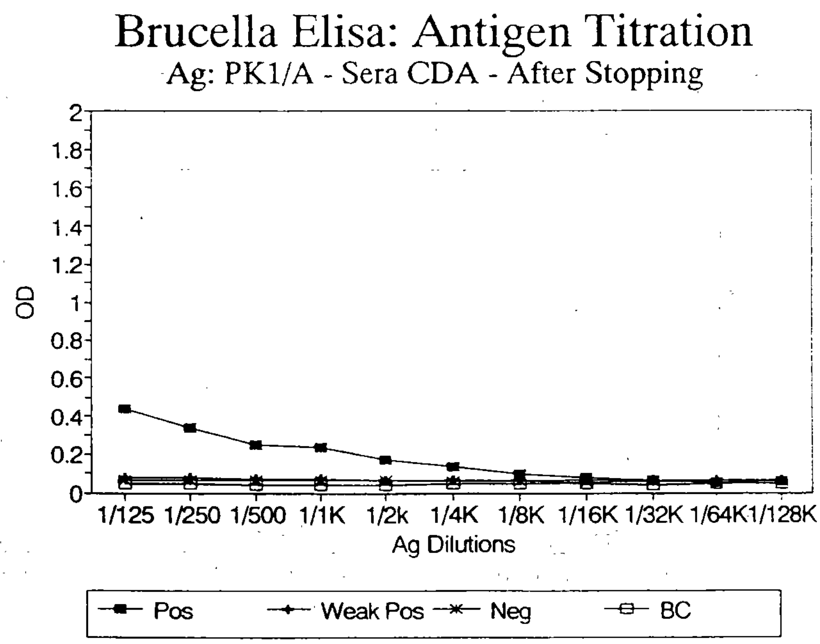
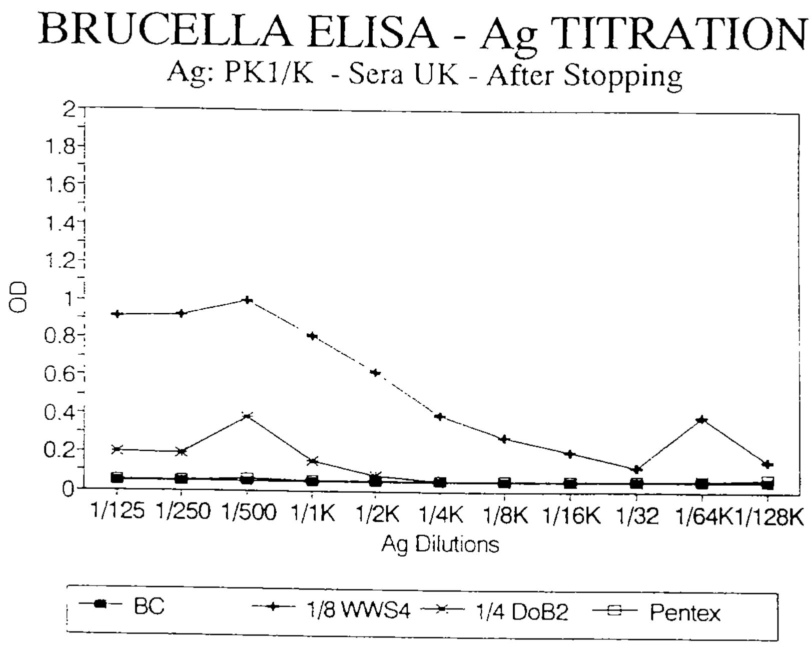

Brucella Elisa: Antigen Titration
Ag: PK1/A - Sera CDA - After Stopping
OD
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1/125 1/250 1/500 1/1K 1/2k 1/4K 1/8K 1/16K 1/32K 1/64K 1/128K
Ag Dilutions
Pos
Weak Pos
Neg
BC

Figure 8 (4)


BRUCELLA ELISA - Ag TITRATION
Ag: PK1/K - Sera UK - After Stopping
OD
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1/125 1/250 1/500 1/1K 1/2K 1/4K 1/8K 1/16K 1/32 1/64K 1/128K
Ag Dilutions
BC
1/8 WWS4
1/4 DoB2
Pentex

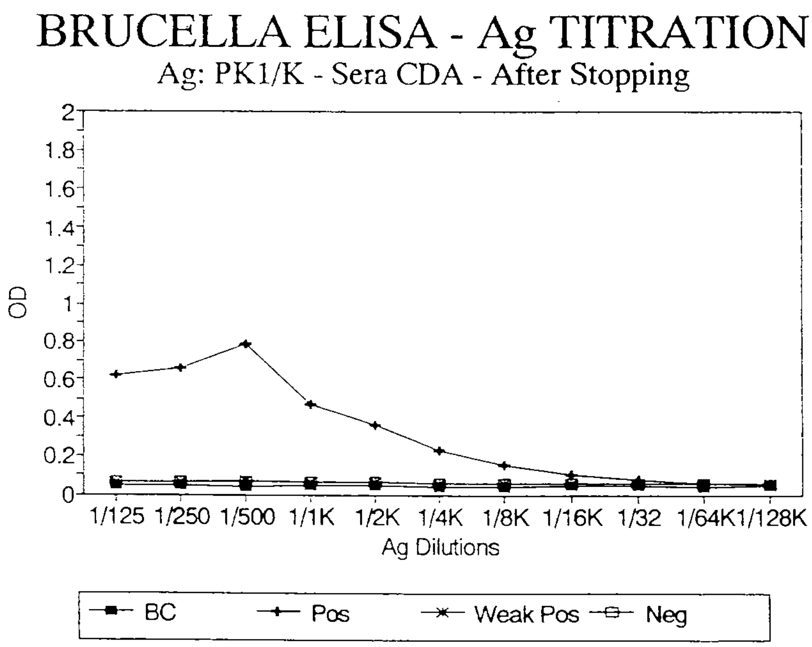
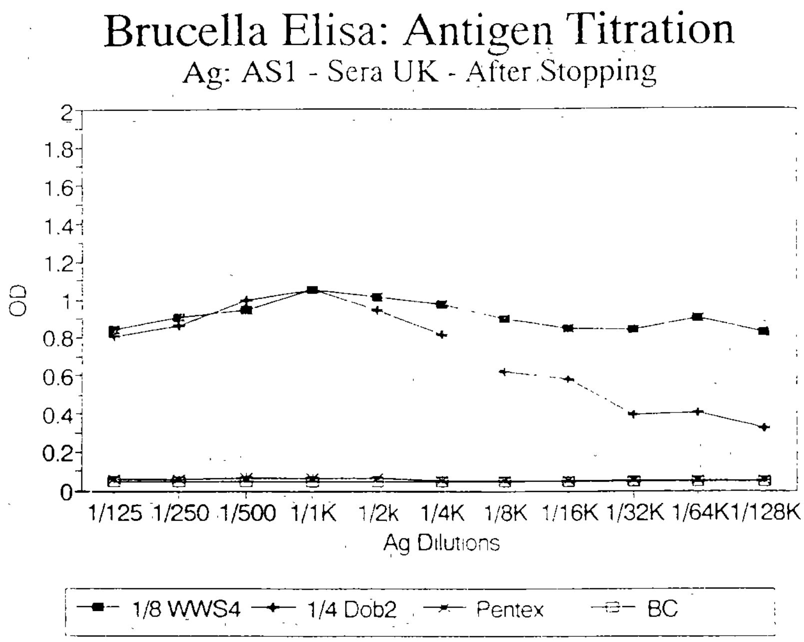

BRUCELLA ELISA - Ag TITRATION
Ag: PK1/K - Sera CDA - After Stopping
OD
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1/125 1/250 1/500 1/1K 1/2K 1/4K 1/8K 1/16K 1/32 1/64K 1/128K
Ag Dilutions
BC
Pos
Weak Pos
Neg

Figure 8 (5)


Brucella Elisa: Antigen Titration
Ag: AS1 - Sera UK - After Stopping
OD
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1/125 1/250 1/500 1/1K 1/2k 1/4K 1/8K 1/16K 1/32K 1/64K 1/128K
Ag Dilutions
1/8 WWS4
1/4 Dob2
Pentex
BC

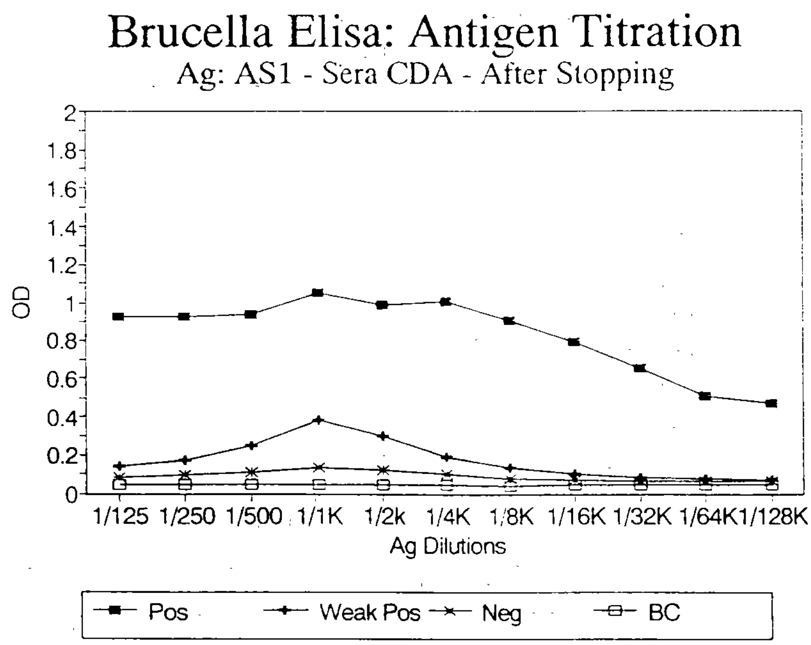
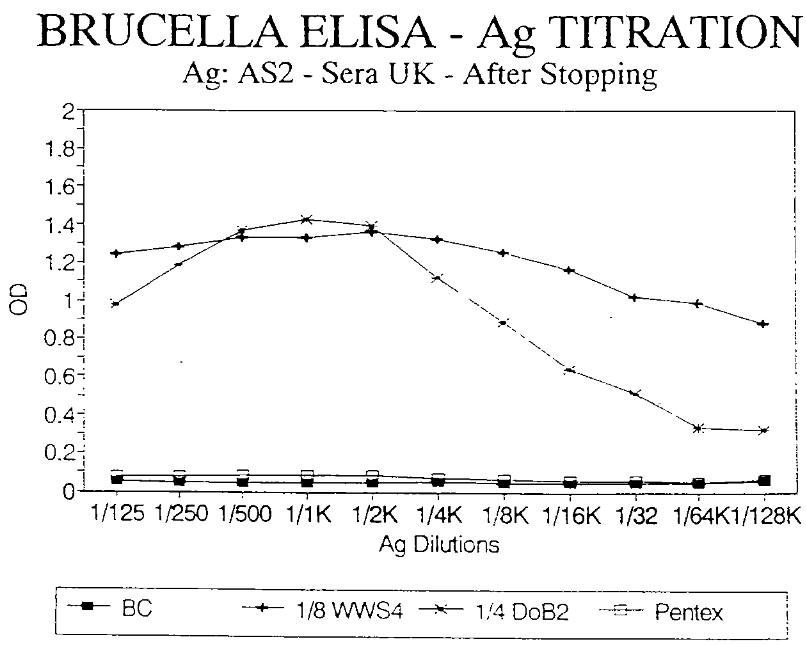

Brucella Elisa: Antigen Titration
Ag: AS1 - Sera CDA - After Stopping
OD
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1/125 1/250 1/500 1/1K 1/2k 1/4K 1/8K 1/16K 1/32K 1/64K 1/128K
Ag Dilutions
Pos
Weak Pos
Neg
BC

Figure 8 (6)


BRUCELLA ELISA - Ag TITRATION
Ag: AS2 - Sera UK - After Stopping
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD
1/125 1/250 1/500 1/1K 1/2K 1/4K 1/8K 1/16K 1/32 1/64K 1/128K
Ag Dilutions
BC
1/8 WWS4
1/4 DoB2
Pentex

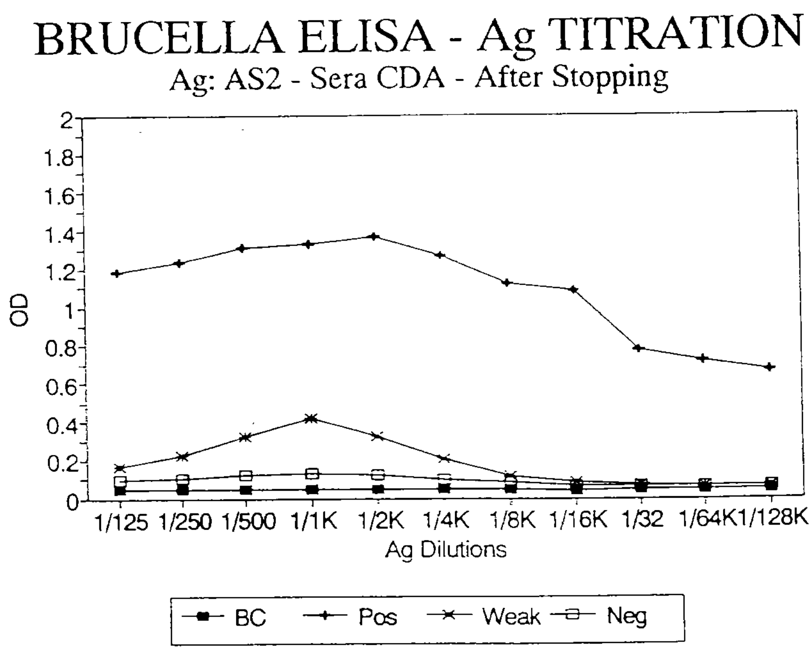

BRUCELLA ELISA - Ag TITRATION
Ag: AS2 - Sera CDA - After Stopping
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD
1/125 1/250 1/500 1/1K 1/2K 1/4K 1/8K 1/16K 1/32 1/64K 1/128K
Ag Dilutions
BC
Pos
Weak
Neg

Figure 8 (7)

ANNEX I

LIST OF PARTICIPANTS

Dr G. Dubray, Laboratoire de Pathologie et d'Immunologie, Unité Brucellose, Station de Pathologie de la Reproduction, Institut National de la Recherche Agronomique (INRA), F-37380 Nouzilly, France (Chairman)

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Secretariat

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Dr T. Fujikura, Veterinary Public Health, Division of Communicable Diseases, World Health Organization, 1211 Geneva 27, Switzerland (Co-Secretary)

Dr H. Jeggo, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, 1400 Vienna, Austria

Dr P. Wright, Animal Production Unit, Joint FAO/IAEA Programme, Agency's Laboratory, 2444 Seibersdorf, Vienna, Austria (Co-Secretary)

* invited but unable to attend.

ANNEX II

LIST OF WORKING PAPERS AND BACKGROUND DOCUMENTS

VPH/BRUC/EIA/WP/91.1 - Brucellosis immunoassay, A.P. MacMillan, Central Veterinary Laboratory, Weybridge, UK.

VPH/BRUC/EIA/WP/91.2 - Analysis of Brucella S-LPS antigens for ELISA, G. Dubray, INRA, Nouzilly, France.

VPH/BRUC/EIA/WP/91.3 - Bovine brucellosis ELISA, Animal Production Unit, Agency's Laboratory, Seibersdorf, Vienna, Austria.

VPH/BRUC/EIA/BD/91.1 - Bruno Garin-Bastuji, et al. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting analysis of smooth-lipopolysaccharide heterogeneity among Brucella biovars related to A and M specificities, J. of Clinical Microbiology, 28, 2169-2174 (1990).

VPH/BRUC/EIA/BD/91.2 - A. Cloeckaert, et al. Identification of seven surface-exposed Brucella outer membrane proteins by use of monoclonal antibodies: immunogold labelling for electron microscopy and enzyme-linked immunosorbent assay, Infection and Immunity, 58, 3980-3987 (1990).

VPH/BRUC/EIA/BD/91.3 - Michel S. Zygmunt, et al. Analysis of immune response: comparison of immunoblots after isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis using cytoplasmic protein extract from Brucella, FEMS, Microbiology Letters, 70, 263-268 (1990).

VPH/BRUC/EIA/BD/91.4 - A. Cloeckaert, et al. Protection conferred on mice by monoclonal antibodies directed against outer membrane protein antigens of Brucella, J. Med. Microbiol., 34, 175-180 (1991).

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