VALIDATION OF A FOOT-AND-MOUTH DISEASE ANTIBODY ELISA IN FIVE LATIN AMERICAN COUNTRIES

M.S. SONDAHL, M.Da PENHA DIAS GOMES,
M. AURNHEIMER MARTINS, J. WASHINGTON LOPEZ
Pan American Foot and Mouth Disease Center,
Rio de Janeiro, Brazil

Abstract

VALIDATION OF A FOOT-AND-MOUTH DISEASE ANTIBODY ELISA IN FIVE LATIN AMERICAN COUNTRIES.
The work plan consisted of using a liquid phase blocking ELISA test for the detection of antibodies to foot-and-mouth disease virus (FMDV) using the following categories of sera: (A) Spot test 120 non-infected/non-vaccinated bovine sera diluted 1:32; (B) Titration test: 120 bovine sera from animals vaccinated with trivalent oil vaccine, bled 30 days after vaccination; (C) Titration test with sera from non-infected/non-vaccinated bovines that presented titers >1:32 in the spot test. To detect FMD positive animals in the field, the spot test established with a cut-off of 1:32 demonstrated in this work a good specificity with the non-vaccinated group, where 3 animals out of 120 were considered positive. The antibody titration test is an excellent tool to determine the level of antibodies in cattle populations. The protocol indicates that positive sera from the spot test should be tested in the titration assay in a starting dilution of 1:32. We suggest to use a lower starting dilution (1:16) in order to start below the discriminative of positive spot test sera 1:32 for the titration assay procedures.

1. INTRODUCTION

A liquid phase blocking ELISA for detection of serum antibodies to FMDV [1,2] was used according to the protocol established in the first Research Coordinated Meeting FAO/IAEA/PAHO-PANAFTOSA [3,4] held in September, 1995 in Rio de Janeiro, Brazil. The biological reagents [5] were supplied by PANAFTOSA and the chemicals reagents by IAEA. PANAFTOSA made the necessary adjustments in the test and the determination of the upper and lower control limits which then were introduced in the FAO/IAEA ELISA software programme EDI.

The test is based upon specific blocking of liquid phase FMDV antigen [6] by antibodies in serum samples.

2. MATERIAL AND METHODS

2.1. Serum samples

2.1.1. Sera from vaccinated animals

One hundred and twenty sera from 18-24 months old animals, vaccinated with trivalent oil adjuvant vaccine and bled 30 days after vaccination.

2.1.2. Sera from non-infected animals

One hundred and twenty sera from 18-24 months old animals from selected herds without history of FMD, not vaccinated and tested previously to confirm that all were FMD antibody free.

Sera were kindly supplied by the Vaccine Control Laboratory of Brazil, Ministry of Agriculture (MAARA-LARA/RS) Rio Grande do Sul State, which carries out the official vaccine potency control for commercial FMD vaccines. Sera were used in the test without treatment or preservation procedures.

2.2. ELISA biologicals

2.2.1 Virus strains

For this work the reference strains O_1 Campos-Br.1/58, A_{24} Cruzeiro-Br.1/55 and C_3 Indaial-Br.1/71 were used. Colombia and Venezuela in view of being free of FMD virus strain C, only received O and A virus strains [7].

Viruses were obtained from BHK-21, C-13 (Baby Hamster Kidney) cell cultures, inactivated by Binary Ethylenimine (BEI), treated with 50% v/v with sterile glycerol and stored at -20°C [8,9].

2.2.2 Trapping antibodies

Hyperimmune sera were obtained by inoculations of rabbits with above mentioned virus strains, after cesium chloride gradient purification, and stored at -20°C.

2.2.3. Detecting antibodies

Hyperimmune antisera were produced in guinea pig [10] with the strains previously mentioned, adapted in this species and stored at -20°C.

2.2.4. Conjugate

The conjugate, IgG-anti-guinea pig labeled peroxidase, was produced at PANAFTOSA and distributed to the five laboratories.

2.2.5. Control sera

Positive control sera: Pool of sera from bovines vaccinated and revaccinated with monovalent oil adjuvant vaccines using each one of the strains mentioned above. Positive control sera were divided in two groups: strong positive (++) and weak positive (+) and stored at -20°C.

Negative control: Pool of bovine sera from FMD and Vesicular Stomatitis Virus (VSV) free areas. All biological reagents with their respective working dilutions established, were aliquoted, labeled and distributed by PANAFTOSA to the five participating laboratories as shown in Table II and III.

2.3. ELISA chemicals, consumables, manual and software programme for data management

Chemicals (Carb. Bicarb. tablets, PBS tablets, Tween 20, Phoshp/Citr. Tablets, H_2O_2 tablets, OPD tablets, skimmed milk powder, ovalbumen grade II, V, conjugate and normal rabbit serum), consumables (tips, NUNC and polypropyelene plates, minor laboratory equipment, the ELISA software programme EDI and the protocol were supplied by the Joint FAO/IAEA Division, Vienna, Austria.

2.4. Liquid phase blocking ELISA

Values for Upper (UCL) and Lower (LCL) Control Limits for the first level of acceptance of the test were obtained based on the Optical Density (O.D.) of the antigen (1.0-1.5) as recommended by the Joint FAO/IAEA Division Manual. Virus concentrations were determined by separate titration of each of the virus strains (O, A and C).

The second level of acceptance of the controls (strong positive (++), weak positive (+), negative (-) and Antigen Control (Ca)) expressed as Percentage of Inhibition (P.I.) were obtained using four replicates of 25 tests ran over a period of several days. Replicates were done for each control and for each virus strain. A control chart developed by PANAFTOSA using physical, chemical and biological control parameters and based on O.D. values was applied. P.I. values were calculated using statistical analysis and included in the FAO/IAEA ELISA software programme EDI 2.11 as shown in Table I.

TABLE I. UPPER CONTROL LIMITS (UCL) AND LOWER CONTROL LIMITS (LCL) FOR ANTIGEN CONTROL (Ca) AND STRONG POSITIVE (C++), WEAK POSITIVE (C+) AND NEGATIVE (C-) CONTROL SERA FOR SEROTYPE A, O AND C ANTIGENS

Parameter			Value/Virus		
			O	A	C
LCL	OD	Ca	1.06	1.08	1.07
UCL	OD	Ca	1.34	1.30	1.31
LCL	PI	C++	93	89	90
UCL	PI	C++	96	95	95
LCL	PI	C+	63	59	63
UCL	PI	C+	81	75	80
LCL	PI	C-	-06	09	-01
UCL	PI	C-	38	40	36
LCL	PI	Ca	-41	-39	-40
UCL	PI	Ca	25	23	24

Ca Antigen Control
OD Optical Density
PI Percent Inhibition

TABLE II. BIOLOGICALS SUPPLIED BY PANAFTOSA FOR ARGENTINA, BRAZIL AND PARAGUAY

COORDINATED RESEARCH PROGRAMME FOR THE VALIDATION OF FMD ANTIBODIES ELISA TEST - FAO/IAEA/PAHO-PANAFTOSA - (2nd phase)

A) SERA

1) 120 bovine negative sera

2) 120 bovine sera from vaccinated cattle with oil adjuvant trivalent vaccine, bled 30 days after vaccination.

B) REAGENTS

Trapping Antibodies (Rabbit)	Amount (ml)	Final Dilution
O_1 Campos-Br.1/58	0,250	1/2.000
A_{24} Cruzeiro-Br.1/55	0,300	1/1.500
C_3 Indaial-Br.1/71	0,250	1/2.000
(Storage at - 20°C)		
Detecting Antibodies (Guinea Pig)		
O_1 Campos-Br.1/58	1,00	1/300
A_{24} Cruzeiro-Br.1/55	1,50	1/200
C_3 Indaial-Br.1/71	0,50	1/600
(Storage at - 20°C)		
Inactivated Virus/Glycerinated		
O_1 Campos-Br.1/58	40,00	1/16
A_{24} Cruzeiro-Br,1/55	20,00	1/30
C_3 Indaial-Br.1/71	10,00	1/60
(Storage at - 20°C)		
Conjugate/Peroxidase		
IgG anti Guinea Pig-Lot 83	10,00	1/80
(Storage at + 4°C)		
Positive control serum (++)		
O_1 Campos-Br.1/58	1,50	1/32
A_{24} Cruzeiro-Br.1/55	1,50	1/32
C_3 Indaial-Br.1/71	1,50	1/32
(Storage at - 20°C)		
Positive control serum (+)		
O_1 Campos-Br.1/58	1,50	1/32
A_{24} Cruzeiro-Br.1/55	1,50	1/32
C_3 Indaial-Br.1/71	1,50	1/32
(Storage at - 20°C)		
Negative control serum		
Bovine serum - Lot 02	4,50	1/32
(Storage at - 20°C)		
Blocking serum		
Bovine serum	60,00	-
(Storage at - 20°C)		

TABLE III. BIOLOGICALS SUPPLIED BY PANAFTOSA FOR COLOMBIA AND VENEZUELA

COORDINATED RESEARCH PROGRAMME FOR THE VALIDATION OF FMD ANTIBODIES ELISA TEST - FAO/IAEA/PAHO-PANAFTOSA - (2nd phase)

A) SERA

1) 120 bovine negative sera

2) 120 bovine sera from vaccinated cattle with oil adjuvant trivalent vaccine, bled 30 days after vaccination.

B) REAGENTS

Trapping Antibodies (Rabbit)	Amount (ml)	Final Dilution
O_1 Campos-Br.1/58	0,250	1/2.000
A_{24} Cruzeiro-Br.1/55	0,300	1/1.500
(Storage at - 20°C)		
Detecting Antibodies (Guinea Pig)		
O_1 Campos-Br.1/58	1,00	1/300
A_{24} Cruzeiro-Br.1/55	1,50	1/200
(Storage at - 20°C)		
Inactivated Virus/Glycerinated		
O_1 Campos-Br.1/58	40,00	1/16
A_{24} Cruzeiro-Br,1/55	20,00	1/30
(Storage at - 20°C)		
Conjugate/Peroxidase		
IgG anti Guinea Pig-Lot 83	10,00	1/80
(Storage at + 4°C)		
Positive control serum (++)		
O_1 Campos-Br.1/58	1,50	1/32
A_{24} Cruzeiro-Br.1/55	1,50	1/32
(Storage at - 20°C)		
Positive control serum (+)		
O_1 Campos-Br.1/58	1,50	1/32
A_{24} Cruzeiro-Br.1/55	1,50	1/32
(Storage at - 20°C)		
Negative control serum		
Bovine serum - Lot 02	4,50	1/32
(Storage at - 20°C)		
Blocking serum		
Bovine serum	60,00	-
(Storage at - 20°C)		

The assay was developed following strictly the previously established protocol, published and distributed by the Joint FAO/IAEA Division, Vienna, Austria to assure a maximum of standardization between participating laboratories.

3. RESULTS

A total of 46 ELISA plates were used and the following results obtained:

3.1. Spot test

Performed with 120 non-infected bovine sera diluted 1:32. Out of 9 ELISA plates 4 plates were "outside limits" (2 because of Ca values , 1 because of the C++ P.I. values and 1 because of Ca and P.I. values). Five serum samples were positive for virus A only. Specificities for the different antigens of the spot test are given in Table IV.

TABLE IV. SPECIFICITY OF SCREENING ASSAY (Spot test 1/32) FOR SEROTYPES O, A AND C

Virus	C	O	A
Total neg. sera	120	120	120
Test pos.	0	4	0
Test neg.	120	116	120
Specificity (%)	100	97.5	100

3.2. Antibody titration

Performed with 120 sera from cattle vaccinated with FMD trivalent oil adjuvant vaccine using O_1 Campos-Br.1/58, A_{24} Cruzeiro-Br.1/55 and C_3 Indaial-Br.1/71 virus. Out of 36 ELISA plates 15 were "outside limits" (11 because of OD values for the Ca, 2 because of C+ PI values and 2 because of C++ PI values). Four dilutions were used for this titration: 1:10, 1:50, 1:250 and 1:1250. Results are shown in Tables IV-VII.

3.3. Antibody titration of positive sera in the spot test

Five sera, which were positive in the spot test were further titrated 1:32, 1:64, 1:128 and 1:256 against virus A. Three sera were positive at dilution of 1/32 dilution. The two remaining resulted negative.
Sensitivity of the titration assay according to the dilution is shown in Tables V-VIII.

TABLE V. SENSITIVITY OF TITRATION ASSAY (CUT-OFF > 1:10)

Virus	C	O	A
Total of pos. sera	120	120	120
Test pos.	120	120	120
Test neg.	0	0	0
Sensitivity (%)	100	100	100

TABLE VI. SENSITIVITY OF TITRATION ASSAY (CUT-OFF > 1:50)

Virus	C	O	A
Total of pos. sera	120	120	120
Test pos.	119	93	111
Test neg.	1	24	8
RT	-	3	1
Sensitivity (%)	99.16	77.5	92.5

RT = retest

TABLE VII. SENSITIVITY OF TITRATION ASSAY (CUT-OFF > 1:250)

Virus	C	O	A
Total of pos. sera	120	120	120
Test pos.	48	14	44
Test neg.	60	104	71
RT	12	2	5
Sensitivity (%)	40	11.6	36.6

RT = retest

TABLE VIII. SENSITIVITY OF TITRATION ASSAY (CUT-OFF > 1:1250)

Virus	C	O	A
Total of pos. sera	120	120	120
Test pos.	3	1	3
Test neg.	113	119	114
RT	4	0	3
Sensitivity (%)	2.5	0.83	2.5

RT = retest

4. DISCUSSION

The validation of a liquid phase blocking ELISA for detection of FMD antibodies proposed by FAO/IAEA, together with PAHO-PANAFTOSA and 5 laboratories (Argentina, Colombia, Brazil, Paraguay, and Venezuela) proved to be a valuable exercise.

To detect FMD positive animals in the field, the spot test established with a cut-off of 1:32 demonstrated in this work a good specificity with the non-vaccinated group, where 3 animals out of 120 were considered positive.

O.D. values for the antigen control established by PANAFTOSA fell within the predetermined range (1.0-1.5) and were included in the FAO/IAEA ELISA software EDI. A high degree of precision in the results was observed. It is recommended that P.I. values which are established as border values for the acceptance of the plate should be accepted e.g. a test value $\geq$ the Lower Control Limit (LCL) or a test value $\leq$ the Upper Control Limit (UCL) should be taken as accepted by the software programme EDI.

The antibody titration test is an excellent tool to determine the level of antibodies in cattle populations. The protocol indicates that positive sera from the spot test should be tested in the titration assay in a starting dilution of 1:32. We suggest to use a lower starting dilution (1:16) in order to start below the discriminative of positive spot test sera 1:32 for the titration assay procedures.

Looking on the results there was no difference using the PANAFTOSA software or FAO/IAEA software (EDI). The main difference between the two programmes is that PANAFTOSA software expresses results in logarithmical functions and EDI uses values expressed as percentage of inhibition P.I. as recommended by OIE (Office International des Epizooties).

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