

VALIDATION OF FAO/IAEA/PANAFTOSA ELISA KIT FOR THE DETECTION OF ANTIBODIES AGAINST FOOT-AND-MOUTH DISEASE VIRUS IN VENEZUELA



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Abstract

VALIDATION OF FAO/IAEA/PANAFTOSA ELISA KIT FOR THE DETECTION OF ANTIBODIES AGAINST FOOT-AND-MOUTH DISEASE VIRUS IN VENEZUELA.

A liquid phase blocking ELISA (LPBE) supplied by FAO/IAEA/PANAFTOSA has been evaluated for the qualitative and quantitative detection of specific antibodies to "O" and "A" serotypes of foot-and-mouth disease (FMD). A total of 240 bovine sera were analyzed. 120 sera from non-infected and non-vaccinated cattle were tested in a screening test showing a specificity of 99,2%. 120 from vaccinated cattle were tested in a titration assay giving a sensitivity of 99,2%. For serotype "O" the titration test showed a protection of 80% and for serotype "A" 75,6%. Antibody titers fluctuated between >112 and >1250 which indicates protection.

1. INTRODUCTION

The liquid phase blocking ELISA [1,2] developed to identify specific antibodies to foot-and-mouth disease (FMD) [3] in serum of cattle in the field is a very useful method. It is able to determine the status of immunity in cattle and has been used to monitor the success of vaccination against FMD.

The Joint FAO/IAEA Division and the Panamerican Center for Foot-and-Mouth Disease (PANAFTOSA) have agreed on a protocol [4] to validate a LPBE kit. Five countries (Argentina, Brazil, Colombia, Paraguay and Venezuela) were selected to standardize a technique that supports the vaccination campaign through seroepidemiological studies and which can be used to control the quality of FMD vaccine.

The national livestock population in Venezuela represents approximately 18.000.000 bovine, 5.000.000 swine (126.512 sows) and a less important number of ovine and caprine species. Efforts to eradicate FMD in Latin America have been agreed through the establishment of a hemispheric eradication plan. In June 1996 in Brasilia countries have committed themselves to eradicate FMD from the continent by the year 2009. Following this plan, Venezuela implemented a control and eradication programme, which is based on three pillars: 1) mass vaccination of bovine 2) epidemiological surveillance and 3) active participation of all sectors involved. Encouraging results have been reported from the south of Venezuela, Bolivar state, where no outbreaks have been observed since June 1994. The reason for this development is the very strict application of a vaccination plan, covering approximately 90% of the cattle and it is estimated that this area will be declared free of FMD in 1998.

In the view of the existing eradication plan, it is necessary to replace the present diagnostic technique, which uses inoculation of live virus in suckling mice with a LPBE, which uses inactivated virus as the assay antigen.

2. MATERIALS AND METHODS

Test serum samples and the biological reagents were supplied by PANAFTOSA. Chemicals, consumables and the ELISA software were supplied by the Joint FAO/IAEA Division.

2.1. Serum Samples

2.1.1. Sera from non-infected, non-vaccinated animals

One hundred and twenty sera from 18-24 months old cattle from selected herds without neither history of FMD infection nor vaccination. Sera were tested previously at PANAFTOSA to confirm freedom of FMD antibody. Sera were supplied from the vaccine control laboratory, MAARA-LARA/RS, Rio Grande do Sul State, Brazil.

2.1.2. Sera from vaccinated animals

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

2.2. Virus strains

For this work the strains O₁ Campos-Br.1/58 and A₂₄ Cruzeiro-Br.1/55 were used. Viruses were obtained from BHK-21, C-13 cell cultures, inactivated with binary ethylenimine (BEI), treated with sterile glycerol (50% v/v) and stored at -20°C.

2.3. Trapping antibodies

Hyperimmune sera to each of the serotypes were obtained by inoculation of rabbits with one of the previously mentioned virus stains, after cesium chloride gradient purification, and stored at -20°C.

2.4. Detecting antibodies

Hyperimmune antisera were produced in guinea pigs against the strains previously mentioned using live virus adapted to this species and stored at -20°C.

2.5. Conjugate

The conjugate (Peroxidase labeled goat anti guinea pig immunoglobulin) was produced at PANAFTOSA.

2.6. Control Sera

2.6.1. Positive control sera

Pools of sera from vaccinated and revaccinated cattle with monovalent oil adjuvanted vaccines manufactured to each of the virus strains previously described and divided in two groups: strong positive (C++) and weak Positive (C+), and stored at -20°C.

2.6.2. Negative control sera

Pool of bovine sera from FMD and Vesicular Stomatitis Virus (VSV)-free areas.

2.7. Liquid Phase Blocking ELISA

The assay was followed strictly in accordance to the established protocol distributed by the Joint FAO/IAEA.

2.8. Software

The plates were read in a Multiskan spectrophotometer (MCC 340) and optical density values were interpreted by software supplied by the Joint FAO/IAEA (ELISA Data Interchange, EDI 2.1.1).

3. RESULTS

The antigen control (Ca) and Percentage of Inhibition (PI) values for the upper and lower control limits for serotype "O" and "A" Antigen were redefined during the final Research Coordination Meeting in Vienna in April, 1997. These values are 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 O AND A ANTIGENS

Parameter			Value/Virus	
			O	A
LCL	OD	Ca	0.96	0.97
UCL	OD	Ca	1.76	1.73
LCL	PI	C++	86	75
UCL	PI	C++	100	99
LCL	PI	C+	63	59
UCL	PI	C+	81	75
LCL	PI	C-	-06	09
UCL	PI	C-	38	40
LCL	PI	Ca	-41	-39
UCL	PI	Ca	25	23

Ca Antigen Control
 OD Optical Density
 PI Percent Inhibition

The analysis of results obtained from 38 ELISA-plates that were processed in relation to the acceptance levels is shown in Table II.

TABLE II. PERCENTAGE OF ACCEPTED ANTIGEN CONTROL (CA) AND PI CONTROL VALUES (SEROPTYPE O AND A)

Antigen control (Ca) (%)		Accepted PI control values (%)		
Serotype	Controls	O	A	
O	68	C++	89	89
A	37	C+	68	58
		C-	84	74
		Ca	100	100

The screening test of 120 sera from non-vaccinated an non-infected bovine gave a specificity of 99,2%. The same percentage was obtained for the positive sera to "O" and "A" serotypes of FMD virus, coming from vaccinated bovines.

One hundred and twenty positive sera from vaccinated cattle were titrated to both "O" and "A" serotypes using five fold dilution series (1/10-1/1250). 95 (79%) out of 120 cattle have antibody titers $\geq 1/112$ against serotype O and 91 (76%) out of 120 cattle have antibody titers $\geq 1/112$ against serotype A. It is concluded that 79% of the cattle are protected against an infection with serotype O and 76% are protected against serotype A (Table III).

TABLE III. ANTIBODY TITER OF 120 VACCINATED CATTLE IN THE TITRATION ASSAY

	1/112	1/250	1/560	1/1250	>1/1250
Serotype O	59	8	26	1	1
Serotype A	66	8	14	1	2

4. CONCLUSIONS AND DISCUSSION

Results shown in Table II demonstrate that the first level of acceptance based on the average of the control value of the antigen of both serotypes needs to be adjusted in order to reach a significant percentage of "accepted" plates. Since the antigen is dissolved in glycerin the pipetting techniques may also play a significant role for the consistency of the Ca values. Our study revealed that 68% of the plates were accepted for serotype O and 37% for serotype A at the first level of acceptance (antigen control).

Concerning the second level of acceptance e.g. values for upper and lower control sera for C++, C+, C- Ca, Percentage of Inhibition, PI our results showed a high percentage of "within limits" status (> 80 %). Only the values for the weak positive control (C+) need adjustment. In this category only 68% were "within limits" for serotype O and 58% for serotype A respectively.

A certain variation between serotypes O and A was observed. Another source of variation was observed when different microplates were used. The EDI program rejects a plate when the value of a control serum is a border value. EDI should be modified in the sense that border values still are regarded as "within limits". Another solution is to take this particularity into account when control values are established.

In the group of non-vaccinated and non-infected animals one animal out of 120 was classified as positive. We therefore conclude that the screening assay at a standard dilution of 1/32 (cut-off value) gave a specificity of 99,2%.

The titration assay is an excellent tool to determine serotype specific antibody levels in cattle and to assess their immune status. Out of 120 vaccinated cattle, 95 were classified as positive (titer \geq 1/112) giving a sensitivity of 79% for serotype O. Out of the same group 91 were classified positive (\geq 1/112 for serotype A giving a sensitivity of 76%.

Taking into consideration the adjustments and recommendations made in this paper we conclude that the liquid phase blocking ELISA showed good specificity and sensitivity. Performance, standardization and interpretation of results are easy and the fact that only inactivated virus material is involved makes the test suitable to support an FMD eradication campaign e.g. in FMD free areas. In these aspects the test clearly exceeds other more cumbersome serological tests e.g. the serum protection test or virus neutralization test which at present are still in use. Nevertheless, a new ELISA, which detects antibodies to non-structural FMD virus protein and which differentiates FMD-infected from vaccinated cattle is needed to round up the diagnostic spectrum to effectively support an FMD eradication campaign.

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