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

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Abstract

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

A Liquid phase blocking sandwich ELISA (LPBE) for the detection of foot-and-mouth disease (FMD) antibodies, serotypes O, A and C was validated using sera from bovines free of antibodies and vaccinated bovines. This technique proved to be sensitive and specific for the study of these antibodies. This kit has been prepared by the Pan American Foot-and-Mouth Disease Center (PAHO/ WHO) in collaboration with the Animal Production and Health Section of the Joint FAO/IAEA Division, Vienna, Austria and the Institute for Animal Health in Pirbright, United Kingdom.

1. INTRODUCTION

Foot-and-mouth disease (FMD) is the animal disease that causes the highest economic losses in the livestock industry of Argentina. The FMD eradication plan (1993-1997) is based on mass vaccination of bovines, regionalization, epidemiological surveillance and active participation of all interested sectors.

As a consequence of the implementation of this plan, no outbreaks of the disease have been observed since April 1994 and in 1997 the Office International des Epizooties (OIE) officially recognized Argentina as: "Freedom from FMD, with vaccination".

The main tasks performed in our laboratory are investigations on suspected samples for the diagnosis of vesicular disease or FMD virus, the control of all the FMD vaccines used and serological assays for epidemiological surveys and vaccine immunity studies.

An antigen detection ELISA is used to serotype virus samples and the liquid phase blocking ELISA (LPBE) is used for the detection of FMD serum antibodies [1-3]. As part of a project to extend the use of the LPBE in South America, we have evaluated an ELISA kit provided by the Joint FAO/IAEA Division and the Pan-American Foot-and-Mouth Disease Center (CPFA-OPS) for serological studies.

2. MATERIALS AND METHODS

Test serum samples and the biological reagents were supplied by CPFA-OPS. Chemical reagents, consumables, the protocol and the ELISA software were supplied by the Joint FAO/IAEA Division.

2.1. Serum Samples

2.1.1. Sera from non-infected animals

One hundred and twenty sera from 18-24 months old bovines from selected herds without neither history of FMD infection nor vaccination and tested previously by CPFA to confirm that all were FMD antibody free.

All these sera originated from Rio Grande do Sul State- Brazil, utilized by the vaccine control laboratory (MAARA-LARA/RS).

2.1.2. Sera from vaccinated animals:

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

2.2. Virus Strains

For this work the strains O1 Campos-Br.1/58, A24 Cruzeiro-Br.1/55 and C3 Indaial-Br.1/71 were used. These viruses were obtained from BHK-21, C-13 cell culture, inactivated by 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 strains and stored at -20°C after cesium chloride gradient purification.

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 immunoglobulins to guinea pig immunoglobulins) was produced by CPFA.

2.6. Control sera

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

Negative control sera: a 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 Division.

2.8. Software

The plates were read in a Multiskan spectrophotometer (MCC 340) and optical density values were interpreted by software supplied by the FAO/IAEA Joint Division (ELISA Data Interchange; EDI 2.1.1) for calculation of percentage inhibition values, control and plate acceptance.

3. RESULTS

The internal upper and lower control values are shown in Table I.

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

Parameter			Value/Virus		
			O	A	C
LCL	OD	Ca	0.957	0.967	1.013
UCL	OD	Ca	1.760	1.729	1.444
LCL	PI	C++	86	75	83
UCL	PI	C++	100	99	99
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

The group of 120 sera from non-infected cattle gave entirely negative results in the screening test (1/ 32 final dilution) for the three serotypes under study (O, A and C).

Ninety sera from the group of vaccinated animals were titrated with a five fold dilutions starting at 1/10 until 1/1250. The titers obtained for the three serotypes under study gave variable results. Figures 1, 2, and 3 show the titer for serotype C, A and O respectively. An antibody titer of ≥ 1/112 indicates that the animal is protected against infection from the homologous antigen.

According to these results the specificity was 100 % for the three serotypes, the sensitivity for virus O was 97,7 %, for virus A 92,2 % and for virus C 93,3 %.

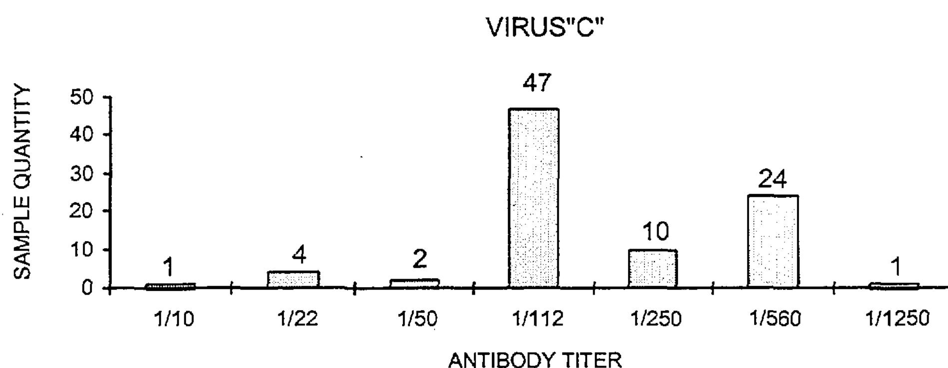

FIG. 1. Antibody titer of 120 sera from cattle vaccinated against serotype "C" (titers of ≥ 1/112 are considered as protective).

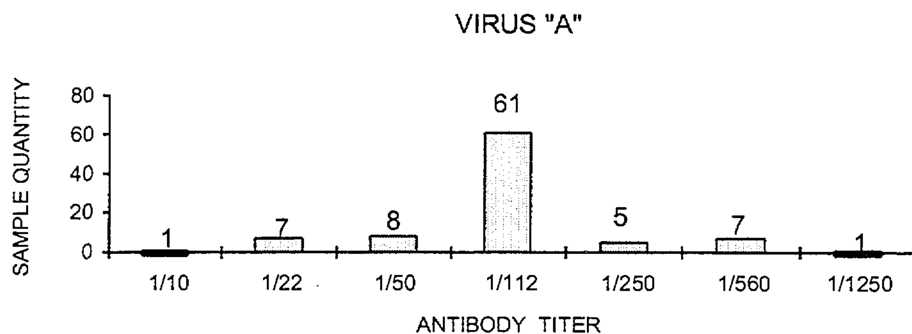

FIG. 2. Antibody titer of 120 sera from cattle vaccinated against serotype "A" (titers of ≥ 1/112 are considered as protective).

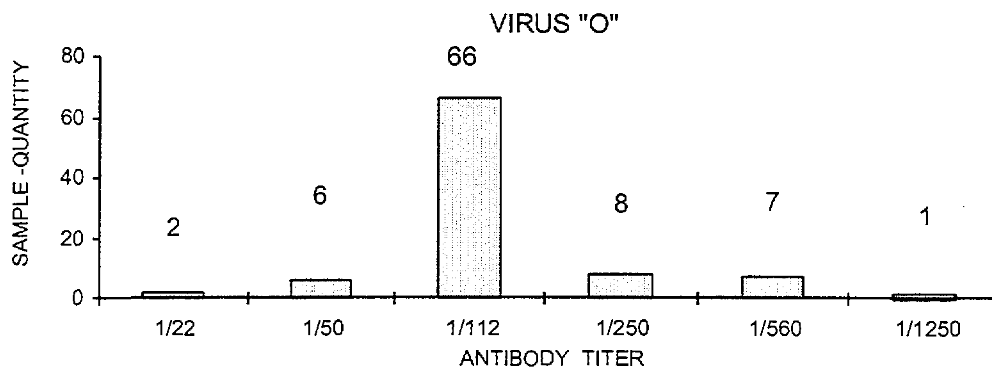

FIG. 3. Antibody titer of 120 sera from cattle vaccinated against serotype "O" (titers of ≥ 1/112 are considered as protective).

4. DISCUSSION

The LPBE proved to be specific and sensitive to be used for FMD antibody detection. In FMD free areas without vaccination it can be applied for import/export testing of animals in order to determine the absence or presence of the disease. In areas where vaccination against FMD is carried out the LPBE is a useful tool to determine the protection level of the vaccinated animals, but since the technique does not discriminate antibodies due to vaccination from antibodies due to infection it can not be used to determine the presence or absence of the disease. During the first run the internal control values were too narrow and a high percentage of plates were rejected due to outside limits status. The values for the upper and lower internal control limits were analyzed and reestablished taking into consideration all data produced by the 5 participating laboratories and by PANAFTOSA. The new values, which are shown in Table I proved to be more suitable.

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