APPLICATION OF ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DIAGNOSIS OF FOOT-AND-MOUTH DISEASE VIRUS AND VESICULAR STOMATITIS IN COMPARISON WITH THE COMPLEMENT FIXATION

M.P.D. Gomes¹, M.S. Söndahl¹, M.A. Martins¹, R. Casas Olascoaga¹, A. Alonso F.¹

SUMMARY

The indirect sandwich enzyme-linked immunosorbent assay (ELISA) was compared with the 50% complement fixation (CF₅₀) test for the detection of O, A and C serotypes of foot-and-mouth disease virus (FMDV) and New Jersey (NJ) and Indiana (IND) serotypes of vesicular stomatitis virus (VSV), using polyvalent antisera as detector for O, A, C and IND strains and monovalent antiserum for NJ strains. The ELISA proved to be a more satisfactory procedure to identify FMDV and VSV in epithelial samples from animals affected with a vesicular disease.

INTRODUCTION

Foot-and-mouth disease (FMD) virus (FMDV) is a Picornavirus that causes a vesicular disease in cloven-hooved animals. Vesicular stomatitis (VS) virus (VSV) is a Rhabdovirus which also produces a vesicular disease in horses, cattle and swine. FMDV has seven distinct serological types and of these serotypes O, A and C, including many subtypes are prevalent in South America (2). VS is produced by the NJ and IND serotypes, which include the subtypes IND-1, IND-2 and IND-3 (8). NJ and IND-1 are found in the endemic areas of VS (southern part of the United States, Mexico, Central America, Panama,

Colombia, Ecuador, Peru and Venezuela). Subtype IND-2 is isolated sporadically in Argentina and Brazil and subtype IND-3 only in Brazil. In cattle and pigs both diseases are clinically indistinguishable (6).

The typing of FMDV and VSV in South America is performed by the 50% complement fixation (CF_{50}) test, using polyvalent and monovalent antisera (1). The enzyme-linked immunosorbent assay (ELISA) has proved to be more efficient than the conventional CF test to identify FMDV (9, 12) and VSV (10).

In the present study we compared the use of polyvalent antisera in the ELISA and $CF_{5\,0}$ test for typing the FMDV O, A and C serotypes and VSV NJ and IND serotypes which are prevalent in South America.

MATERIALS AND METHODS

Field and reference samples: A total of 291 epithelial samples from cattle, horses and swine sent to the Pan American Foot-and-Mouth Disease Center/Reference Laboratory for the Americas (PANAFTOSA) by Central and South American countries from 1952 to 1989 and conserved at -20°C in phosphate buffer saline (PBS) with 50% glycerine, were re-tested by CF₅₀ test and examined for the first time by ELISA. For the assay a 20% suspension in PBS of the epithelial samples was prepared.

Suspensions of BHK cell cultures (11) infected with FMDV of VSV from the strain collection of PANAFTOSA (2, 3), including: 29 type O; 60 type A; 21 type C; 4 type NJ; 4 subtype IND-1; 5 subtype IND-2; and 4 subtype IND-3 were also tested by ELISA and CF_{50} .

Pan American Foot-and-Mouth Disease Center (PANAFTOSA, HPV/PAHO/WHO), Caixa Postal 589, 20001 Rio de Janeiro, RJ, Brazil.

Antisera for ELISA and CF₅₀ test: Capture rabbit antisera for ELISA were prepared by the inoculation of 146S inactivated antigen of FMDV O₁ Campos-Br/58, A₂₄ Cruzeiro-Br/55 or C₃ Indaial-Br/71 propagated in BHK cells. For VSV antisera, animals were immunized with NJ Costa Rica/66, IND-1 Costa Rica/72, IND-2 Ribeirão-Br/79 or IND-3 Agulhas Negras-Br/86 grown in IBRS-2 cells (7). The immunizations were performed according to the method described (12). The capture serum for IND serotypes was a mixture of IND-1, IND-2 and IND-3 monovalent rabbit antisera, whereas the capture sera for O, A, C and NJ serotypes were the monovalent rabbit antisera.

Detection antibodies for O, A, C and IND serotypes to be used in ELISA and CF₅₀ test were polyvalent antisera which were produced in guinea pigs with infectious virus adapted to this species. The immunizing schedule was the same as the one previously described (4).

For the preparation of polyvalent antisera the following strains were used: O₁ Campos-Br/58. O₁ Caseros-Br/67, O₂ Brescia-Italy/47, O₃ Venezuela/51, O₆ UK/24, O₈ Bahia-Br/60, O Magdalena-Col/78, O MS-Br/80 and O RS-Br/80 for type O; A₅ Westerwald-Ger/48, A₂₄ Cruzeiro-Br/55, A₃₂ Venezuela/70, A-79 Argentina/79, A Esteller-Ven/80, A-81 Arg/81, A-84 SC-Br/84, A Col/84 and A-85 Col/85 for type A; C₁ Noville-Smith/65, C₂ Pando-Uru/44, C₃ Resende-Br/55, C₃ Indaial-Br/71, C₃ Arg/85, C₄ T.del F.-Arg/66 and C₅ Arg/69 for type C; and IND-1 Costa Rica/72, IND-2 Ribeirão-Br/79 and IND-3 Agulhas Negras-Br/86 for type IND.

The detector antibody for the NJ type was monovalent antiserum produced in guinea pig with the NJ Costa Rica/66 strain using the same methodology as described above.

All sera were inactivated at 56°C for 30 minutes in 2 ml volumes and stored at -20°C.

CF₅₀ test: The CF₅₀ tube test used by PANAFTOSA for FMDV and VSV typing was employed. Briefly: guinea pig polyvalent O, A, C and IND antisera and NJ monovalent antiserum were used in the dilution containing 2.5 fifty per cent complement fixation units against homologous O₁ Campos-Br/58, A₂₄ Cruzeiro-Br/55,

C₃ Indaial-Br/71, NJ Costa Rica/66 and IND-1 Costa Rica/72 viruses, respectively. The fifty per cent complement hemolytic units for each antigen were determined previously by titrating the complement against the antigens.

The antigens were used in their original suspension. Serum, antigen and complement were used in 200 μ l volumes and after incubation at 37°C for 30 min., 400 μ l of hemolytic system with an optical density (OD, 545 nm) of 0.66 was added. The mixture was allowed to react for 30 min. at 37°C. Afterwards, the tubes were centrifuged and the degree of the reactions was determined by OD measurement. Samples with OD of 20% or lower for one antiserum, in comparison with the other antisera and control serum, were considered positive for the corresponding type.

ELISA procedure: We used the indirect sandwich ELISA based on the method described for FMDV diagnosis (12) as follows:

- (a) ELISA plates were coated overnight at 4° C with 100 μ l of the optimal dilution, in carbonate/bicarbonate buffer, pH 9.6, of rabbit antisera and normal rabbit serum. The plates were subsequently blocked for 1 h at 24-25°C with 1% ovoalbumine in PBS.
- (b) The antigens were tested in 50 μ l volumes of the original suspension and left to react for 1 h at 37°C on a plate shaker.
- (c) Guinea pig sera and conjugate were added successively in 50 μ l volumes of the optimal dilution and incubated at 37°C for 30 min. on a plate shaker.
- (d) OPD substrate was added at 50 μ l volume. The reaction was allowed to proceed for 15 min. at 24-25°C and stopped by acidification.
- (e) ODs were read with a Flow Multiskan photometer at 492 nm. Antigens, guinea pig sera and conjugate were diluted in PBS containing 0.05% Tween 20, 1% ovoalbumine, 2% normal rabbit serum and 2% normal bovine serum. The plates were washed four times between each step with PBS containing 0.05% Tween 20. Samples with OD of 20% or greater for one antiserum, in comparison with the other antisera and control serum, were considered positive for the corresponding type.

The arrangement of the reagents on the plate for FMDV and VSV typing is shown in Figure 1.

RESULTS

Reference samples: BHK cells suspensions of 110 FMDV and 17 VSV from PANAFTOSA strain collection were examined by ELISA and CF₅₀ test to compare the ability to identify the most important VSV and FMDV strains isolated in Central and South America from 1944 to 1989 by both tests. Table 1 shows that the ELISA gave positive results in all 127 samples examined, while de CF₅₀ test did not detect virus in the virus C Leticia-Col/70.

Field samples: A total of 291 suspensions of epithelial samples from cattle, horses or swine affected with a vesicular disease were examined by ELISA and CF_{50} test. The ELISA gave positive results in 67.4% (196/291), whereas the CF_{50} test only typed virus in 54.0% (157/291) of the same samples. All specimens positive by CF_{50} test were also positive by ELISA.

There was complete agreement between the typing results obtained by both tests.

DISCUSSION

Since FMD and VS in cattle or swine cannot be distinguished on clinical signs alone, laboratory identification of the agent involved is essential for diagnosis. The improvement of diagnostic

TABLE 1. Results of typing achieved by ELISA and CF₅₀ test on suspension of BHK cells infected with FMD and VSV of the PANAFTOSA strain collection

	No box of	Positive results			
Virus type	Number of strains	ELISA	CF ₅₀		
0	29	29	29		
Α	60	60	60		
С	21	21	20		
NJ	4	4	4		
IND	13	13	13		
Total	127	127	126		

FIGURE 1. Arrangement of reagents on plate for FMDV and VSV typing by the indirect sandwich ELISA

Rabbit/guinea pig serum											
6	A	С	NJ	IND	N	0	A	С	NJ	IND	N
1	2	3	4	5	6	7	8	9	10	11	12
A B		TS		1				TS		5	
C D		TS	;	2				τs		6	
E F		TS		3		0	A	С	NJ	IND I	No Aç
G H		TS	·	4			1	No A	ntig	en	1, 1,
				5.		1					

N = Normal serum.

TS= Test sample.

Ag= Antigen.

techniques thus is of great importance in order to help the prevention, control and eradication programs of vesicular diseases.

The higher sensitivity of the ELISA was clear when compared with the conventional CF test to identify FMDV (9, 12) and VSV (10) using monovalent antisera. Polyvalent antisera were more efficient than the monovalent antisera to detect FMDV by CF_{50} test (4). This aspect was investigated by ELISA testing of 418 reference and field samples of FMDV and VSV. Both tests showed similar effectiveness to identify samples passed in BHK cells (Table 1: reference samples). However, when epithelial samples from the field were examined (Table 2) the ELISA provided consistently more positive results with both FMDV and VSV samples.

The high percentage of positive results obtained on BHK suspensions by both tests relates to the greater antigen concentration in BHK suspensions than in epithelial samples, together with the broader spectrum of polyvalent antisera to identify heterologous virus when compared with monovalent antisera (4).

Samples								
Origin	Number	Test	0	A	С	NJ	DIND	Neg.
FMDV	201	ELISA	37	61	17	0	0	86
FMDV	201	CF ₅₀	30	42	13	0	0	116
VSV	90	ELISA	0	0	0	72	9	9
VSV	90	CFen	0	0	0	63	9	18

TABLE 2. Results of typing achieved by ELISA and CF₅₀ test on origina; suspensions of epithelial samples from animals affected by foot-and-mouth disease (FMD) or vesicular stomatitis (VS)

The broad spectrum of the polyvalent antisera as antibody detector (4) along with the high sensitivity of the ELISA (9, 10, 12) increases the possibility of identifying, directly from epithelial samples, markedly different strains that sporadically appear in the field in the O, A and C FMDV serotypes (2) and VSV IND serotype (3, 8).

The ELISA results show some cross-reactions, specially between O and C FMDV sertoypes (results not shown) which we related to the common epitopes of the 146 particles of FMDV strains. However, these cross reactions did not produce false-positive results.

The ELISA technique performed with polyvalent antisera will probably prove to be a more satisfactory test in support of the prevention, control and eradication programs for the FMD and VS.

ACKNOWLEDGEMENTS

The authors are extremely grateful to Carlos Alberto Senna and Jorge Sebastião dos Santos for their excellent technical assistance. We also wish to thank Miss Carla Prete Gonçalves for typing the paper.

REFERENCES

- ALONSO FERNANDEZ, A. Manual de diagnóstico de laboratorio de las enfermedades vesiculares. Rio de Janeiro, PANAFTOSA, 1986. (Ser. Man. Didáct., 15).
- ALONSO FERNANDEZ, A., CASAS OLASCOAGA, R., ASTUDILLO, V.M., SÖNDAHL, M.S., GOMES, I., VIANNA FILHO, Y.L. Actualización de cepas del virus de la fiebre aftosa de importancia epidemiológica en América del Sur. Updating of foot-and-mouth disease virus strains of epidemiological importance in South America. Bol. Centr. Panam. Fiebre Aftosa, 53:3-10, 11-18, 1987.
- ALONSO FERNANDEZ, A., SÖNDAHL, M.S. Caracterización antigénica e inmunogénica de varias cepas del serotipo Indiana de estomatitis vesicular aisladas en Brasil. Antigenic and immunogenic characterization of various strains of the Indiana serotype of vesicular stomatitis isolated in Brazil. Bol. Centr. Panem. Fiebre Aftosa, 51:23-26, 27-30, 1985.
- 4. ALONSO FERNANDEZ, A., SÖNDAHL, M.S., FERREIRA, M.E.V. Preparación de un suero polivalente para el diagnóstico del virus de la fiebre aftosa por fijación dei complemento. Preparation of a polyvalent antiserum for diagnosis of foot-and-mouth disease virus by complement fixation. Bol. Centr. Panam. Fiebre Aftosa, 47-49:3-6, 7-10, 1983.
- ASTUDILLO, V.M., ESTUPIÑAN, J., ROSENBERG, F., SILVA, A.J.M., DORA, J.F.P. Vesicular stomatitis in South America: description of data taken from the continental vesicular disease surveillance system. In: Proceedings of an International Conference on Vesicular Stomatitis. Mexico City, Mexico, 1984, p.23-83.
- COTTRAL, G.E. Diagnosis of bovine vesicular disease, J. Am. Vet. Med. Assoc., 161:1293-1298, 1972.
- De CASTRO, M.P. Behaviour of the foot-and-mouth disease virus in cell cultures: susceptibility of the IBRS-2 cell line. Arch. Inst. Biol., S.Paulo, 31:63-78, 1964.

- FEDERER, K.E., BURROWS, R., BROOKSBY, J.
 Vesicular stomatitis virus: the relationship between some strains of the Indiana serotype. Res. Vet. Sci., 8:103-117, 1967.
- FERRIS, N.P., DAWSON, M. Routine application of enzyme-linked immunosorbent assay in comparison with complement fixation for the diagnosis of footand-mouth disease and swine vesicular disease. Vet. Microbiol., 16:201-209, 1988.
- FERRIS, M.P., DONALDSON, A.I. Enzyme-linked immunosorbent assay for the diagnosis for the vesicular stomatitis virus antigen. Vet. Microbiol., 18:243-258, 1988.
- McPHERSON, I.A., STOKER, M.G.P. Polymer transformation of hamster cell clons. An investigation of genetic factors affecting cell competence. *Virology*, 16:147-151, 1962.
- ROEDER, P.L., Le BLANC SMITH, P.M. Detection and typing of foot-and-mouth disease virus by enzyme-linked immunosorbent assay: a sensitive, rapid and reliable technique for primary diagnosis. *Res. Vet. Sci.*, 43:225-232, 1987.