FIELD ASSESSMENT OF THE ENZYME LINKED IMMUNOSORBENT ASSAY FOR FOOT-AND-MOUTH DISEASE VIRUS DIAGNOSIS AND TYPING

E. SMITSAART*, E. MARADEI**, E. FERNANDEZ**, N. FONDEVILA*, D. COMPAIRED*

* Instituto de Virología, Centro de Investigación en Ciencias Veterinarias, INTA, CC 77 (1708) Morón, Pcia. de Buenos Aires, Argentina.

**GELAB-SENASA, Av Fleming 1653 (1640) Martínez, Pcia. Buenos Aires, Argentina

Abstract

FIELD ASSESSMENT OF THE ENZYME LINKED IMMUNOSORBENT ASSAY FOR FOOT-AND-MOUTH DISEASE VIRUS DIAGNOSIS AND TYPING.

The objective of the present study was to evaluate the enzyme linked immunosorbent assay (ELISA) in comparison with the complement fixation test (CFT) for the diagnosis and typing of foot-and-mouth disease (FMD) virus (FMDV). Diagnostic material was epithelium from either suspected cases of FMD or from animals experimentally infected with FMDV. Epithelial suspensions and supernatant fluids from cell culture passage were assayed by CFT and ELISA. The superiority of the ELISA over the CFT was demonstrated:
1) the detection rate was 23% higher than that of CFT on original (epithelial) suspensions (OS) submissions of all sample (positive and negative) and 30% higher on supernatant fluids from cell culture passage, 2) the detection rate of ELISA on OS of confirmed positive samples was 28% higher than that of CFT, 3) no significant differences were observed in the detection and typing rates between the PANAFTOSA and FAO/IAEA ELISA kits ($P<0.05$) and 4) the sensitivity of the ELISA was 16 to 85 times higher than that of CFT when serial dilutions of sample homogenates were examined.

1. INTRODUCTION

Foot-and-mouth disease (FMD) is economically the most important viral disease of domestic animals. The causative agent is a Picornavirus and displays remarkable antigenic variability: seven distinct serological types and more than 60 subtypes and variants have been identified world-wide [1]. Types O, A, and C of foot-and-mouth disease virus (FMDV) are prevalent in South America.

The effectiveness of control measures for FMD relies upon rapid, sensitive and reliable diagnostic procedures. In this regard, the complement fixation test [2] has been used extensively, however, it has disadvantages mainly related to its relative insensitivity and constraints derived from the anticomplementary effects of some samples.

ELISA for antigen detection and typing has been previously described [3] and the procedure with some modifications is being applied as routine in most FMD diagnostic laboratories [4,5]. In this paper, the efficiency of the ELISA and the CFT for FMDV diagnosis has been compared using epithelial samples from animals experimentally and naturally infected with FMDV and antigens derived from their cell culture passage. Two laboratories were involved in the present study: the National Control Laboratory (SENASA) and the Virology Institute of INTA. The former conducted the CFT and cell culture passage and the latter the ELISA determinations.

2. MATERIALS AND METHODS

2.1. Samples

Field samples (N=85) from suspected cases of FMD received for diagnosis at the SENASA during 1991 and 1992 were used in the present study. Upon receipt, each sample was ground in phosphate buffered saline (PBS) using a pestle and mortar with the aid of sterile sand to yield a 20% (w/v) suspension (original suspension, OS). The suspensions were clarified by centrifugation at 1500 g for 10 min. Each OS was divided into 3 fractions: one each for CFT, ELISA and virus isolation determinations. OS which were either not typed or which gave anticomplementary results by CFT were passaged in BHK-21 roller bottles as previously described [5]. CFT and cell culture passage

were carried out at SENASA laboratory. OS and supernatants of cell culture passage were submitted to INTA laboratory for antigen detection and typing by ELISA.

2.2. ELISA

Indirect sandwich ELISA kits provided by either FAO/IAEA or PANAFTOSA for FMDV antigen detection and typing were used. The FMDV strains used to produce capture (rabbit) and detection antibody (guinea pig) for each kit are listed in Table I.

TABLE I. FMDV STRAINS USED TO RAISE ANTISERA FOR FAO/IAEA AND PANAFTOSA ELISA KITS

VIRUS	IAEA/FAO kit	PANAFTOSA kit [5]
FMDV-O	O1 BFS	O1Campos-Br/58, O1 Caseros-Arg/67, O2 Brescia-Italy/47, O3 Venezuela/51, O6 UK/24, O8 Bahia-Br/ 60, O Magd-Col/78, O MS-Br/ 80, O RS- Br/80
FMDV-A	A5 Allier A22 IRQ 24/64 A24 Cruz-Br/55	A5 West-Ger/48, A24 Cruz-Br/55, A32 Ven/70, A79 Arg/79, A Est-Ven/80, A 81 Arg/81, A84 S Carl-Br/84, A Col/84, A85 Col/85
FMDV-C	C1 Noville-Swit/65	C1 Noville-Swit/65, C2 Pando-Ur/44, C3 Res-Br/55, C3 Indaial-Br/71, C3 Arg/85, C4 T.Fuego-Arg/66, C5 Arg/69
VSV-New Jersey	New Jersey	New Jersey Costa Rica/66
VSV-Indiana	Indiana C	Indiana 1-2-3

Briefly, ELISA plates (Nunc- Maxisorp, Denmark) were coated overnight at 4°C with O, A, and C FMDV rabbit antiserum at the appropriate dilution in 0.05 M carbonate-bicarbonate buffer, pH 9.6. Samples and control antigens were added and plates incubated at 37°C for 1 hour on a rotary shaker. Detection of captured virus was performed by adding specific guinea pig antisera to O, A and C FMDV followed by the addition of peroxidase conjugate (rabbit anti guinea pig IgG, Dako Corporation). Guinea pig antisera and conjugate were diluted in PBS containing 0.05% Tween 20 and 5% skimmed milk. Plates were washed with PBS after each incubation step. O-phenylenediamine and hydrogen peroxide composed the chromogen/ substrate mixture and plates were incubated for 15 min at room temperature (RT). The reaction was stopped with 1.25M sulphuric acid and read on a Dynatech Multiskan reader at 492 nm. Positive samples to a specific serotype showed a corrected absorbance value of above 0.1.

The ELISA using the PANAFTOSA kit was operated as described above but with some modifications: 1) after coating with rabbit antisera, plates were blocked for 1 hour at RT with PBS containing 1% ovalbumin and 2) control antigens, detection antibody and conjugate were diluted in PBS containing 0.05% Tween 20, 1% ovalbumin, 2% non-immune bovine serum and 2% non-immune rabbit serum.

2.3. Complement fixation test

The CF_{50} tube test [6] as used for vesicular stomatitis virus and FMDV typing by diagnostic laboratories in South America and at the PANAFTOSA was employed and carried out at SENASA laboratory.

2.4. Virus isolation

Field samples giving negative results by CFT were inoculated onto BHK-21 roller bottles at SENASA laboratory as previously described [5].

Cultures which either showed no evidence of cytopathic effect CPE up to 48 hours post inoculation or which were negative by CFT were further passaged in cell culture a maximum of two times. Aliquots from cell culture passage were stored at -20°C for 4-8 weeks before submission to INTA laboratory for ELISA determinations. It should be noted that not all the material passaged in cell culture was available by the time the ELISA was performed.

2.5. Relative sensitivity between the ELISA and CFT

Serial dilutions of several OS were prepared in PBS containing 0.05% Tween 20 for ELISA determinations (double dilution range from 1/2 up to 1/512) and in PBS for CFT determinations (dilutions 1/1.5, 1/2, 1/3 and 1/4) to compare the relative sensitivity between the two assays.

3. RESULTS

3.1. Comparison of FAO/IAEA ELISA with CFT

The numbers of OS and cell culture harvests which were found to be positive by use of the CFT and the FAO/IAEA ELISA are recorded in Table II.

TABLE II. COMPARISON OF ANTIGEN DETECTION AND TYPING BY FAO/IAEA ELISA AND CFT ON ORIGINAL (EPITHELIAL) SUSPENSIONS AND ON SUPERNATANT FLUID DERIVED FROM THEIR CELL CULTURE PASSAGE

Test comparison	Sample	No. samples	No. +ve FAO/IAEA ELISA	No. +ve CFT
(1)	OS	85	65 (76)*	45 (53)
	Cell culture	106	76 (72)	45 (42)
	Total	191	141 (74)	90 (47)
(2)	OS	71	57 (80)	37 (52)

Test comparison of ELISA and CFT performed on: (1) test samples from all submissions and (2) OS from virus-positive submissions

OS, original suspension* : Percentage of total

The results demonstrate that a higher typing rate was achieved by use of the ELISA. The typing rate obtained by ELISA was similar in tests on OS and cell culture antigens from all sample submissions. This was also true for the CFT but the detection rate(s) was/were lower. In test comparisons on confirmed FMDV positive submissions, the ELISA on both OS and cell culture harvests again gave a higher detection rate.

Six out of 192 samples gave cross-reactivity between serotypes, 5 of which typed as O and A (cell culture passage), and the other (OS) typed as O and C.

3.2. Comparison of PANAFTOSA ELISA kit with FAO/IAEA ELISA and with CFT

The results achieved by use of the ELISA kits received from PANFTOSA and FAO/IAEA and CFT are summarised in Table III and again illustrate that a higher typing rate was obtained by the ELISA.

TABLE III. COMPARISON OF ANTIGEN DETECTION AND TYPING BY BOTH FAO/IAEA AND PANAFTOSA ELISA AND CFT ON ORIGINAL (EPITHELIAL) SUSPENSIONS AND ON SUPERNATANT FLUID DERIVED FROM THEIR CELL CULTURE PASSAGE

No. samples	No. +ve	
	PANAFTOSA ELISA	CFT
64	47 (73)*	35 (55)
	No. +ve	
	PANAFTOSA ELISA	FAO/IAEA ELISA
73	53 (73)	58 (79)

*Percentage of total

No significant differences were found between the detection rate of the two ELISA kits ($P<0.05$). One sample was scored positive by the PANAFTOSA ELISA but not by the FAO/IAEA ELISA, but a positive diagnosis was achieved by both assays after sample cell culture passage and retesting by ELISA. Conversely, 6 samples were scored positive by the FAO/IAEA ELISA but not by the PANAFTOSA ELISA. Cell culture passage of these samples led to positive results by ELISA in 5 out of the 6 cases.

One out of 73 samples derived from cell culture passage gave a cross-reaction between types C and O by both ELISA kits.

3.3. Relative sensitivity between the FAO/IAEA ELISA and CFT

ELISA was found to be 16 to 85 times more sensitive than CFT in detecting FMDV (Table IV).

TABLE IV. RELATIVE SENSITIVITY BETWEEN THE FAO/IAEA ELISA AND CFT FOR DETECTION OF FMDV ON SERIALLY DILUTED ORIGINAL (EPITHELIAL) SUSPENSIONS (OS)

Sample	CFT	ELISA
R636	1/2*	1/32
L20	1/3	1/32
227	1/1.5	1/32
228	1/3	1/64
6466	1/1.5	1/16
OC	1/3	1/256

*Final dilution of OS which was found to be positive

4. DISCUSSION

The superior sensitivity of the ELISA over the CFT has been extensively documented [3,4,7]. The ELISA kits provided by FAO/IAEA and PANAFTOSA have been applied as routine diagnostic procedures and typing in many FMD laboratories. The results obtained with the ELISA under our laboratory conditions have confirmed the advantages of this method over the CFT [3,4,5,7].

We detected FMDV antigen by ELISA in 73% of OS samples that gave positive results in OS or cell culture passage by either ELISA or CFT, and in 52% by CFT. This superiority was lower to that reported by Westbury et al. (1988) [7] who found 58% more positives by ELISA than by micro-CFT in confirmed positive samples. The lower figure achieved in this study could be due to a higher sensitivity of the CFT in tubes applied here [5] and the quality of epithelium samples.

ELISA was more efficient than CFT in detecting antigen either in OS or in cell culture passage. Detecting rates were similar in both categories of test material. Previous studies have reported a higher detection rate on cell culture supernatants than on OS [4,5]. The lower detection rate obtained by us on cell culture samples could be due to antigen degradation after storage at -20°C for 4-8 weeks before running the ELISA. Additionally, there were probably supernatant fluids tested which were derived from cell cultures either showing no or low amounts of CPE.

Similar detection and typing rates were achieved by both ELISAs (FAO/IAEA and PANAFTOSA kits) in spite of differences in strain components, suggesting an adequate coverage of the different antiserum reagents to FMDV strains prevalent in Argentina in 1991 and 1992.

The cross-reactivity shown with samples originated from cell culture passage could be due to reactivity of capture and detector antibody to BHK-21 components of cell culture supernatants. Antisera included in the FAO/IAEA kit were prepared against purified, inactivated 146S antigens derived from FMDV propagated in BKK-21 cells. Besides having an anti-FMDV activity and antisera contain antibodies to BHK-21 cells and bovine gamma globulin. Antibodies to the latter component have been "blocked" but not those to BHK cells. The phenomen was also shown by PANAFTOSA kit in 1/64 samples tested. The cross-reactivity between O and C found in this OS sample could be explained by the presence of two serotypes in the one specimen [8] as a consequence of either a natural mixed infection or by contamination of the sample by another serotype. However, virus neutralization analysis using antisera against O and C revealed the presence of one type (C FMDV type) (data not shown).

The sensitivity of the ELISA was 16 to 85 times higher than that of the CFT when serial dilutions of sample homogenates were examined. This estimation is consistent with previous sensitivity assessment: minimum detection levels of 8 ng/ml of 140S can be detected by ELISA [3], whereas a minimum of 500 ng/ml of 146S is needed to achieve a positive result by CFT [4].

A rapid confirmation of the presence and type of FMDV achieved by use of the ELISA has significant impact in initiating rapid emergency activities to restrict an outbreak.

In conclusion, the higher detection rate of the ELISA over the CFT and its easy performance, strongly recommends its use for swift and reliable diagnosis of FMDV.

ACKNOWLEDGEMENTS

The authors wish to thank Nigel P. Ferris for his contributions in the preparation of this paper. This work was supported by the Joint FAO/IAEA Division (RC No 6516/SD).

REFERENCES

[1] PEREIRA, H.G., Subtyping of foot-and-mouth disease virus, In: "Developments in Biological Standardization" (C. Mackowiak and R.H. Regamey, eds.) Vol.35 (1977) pp.167-174 Karger, Basel.

[2] BUCKLEY, L.S., OSBORNE, R.W. & Pereira, H.G., Laboratory diagnosis of foot-and-mouth disease and swine vesicular disease, Bull. Off. Int. Epizoot. **83** (1975) 123-129.

[3] 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** (1987) 225-232.

[4] FERRIS, N.P. & DAWSON, M., Routine application of enzyme-linked immunosorbent assay in comparison with complement fixation for the diagnosis of foot-and-mouth and swine vesicular diseases, Vet. Microbiol. **16** (1988) 201-209.

[5] ALONSO, A., MARTINS, M., GOMES, M.P.D. et al., Foot-and-mouth disease virus typing by complement fixation and enzyme-linked immunosorbent assay using monovalent and polyvalent antisera, J. Vet. Diagn. Invest. **4** (1992) 249-253.

[6] ALONSO, A. MARTINS, M., GOMES, M. P.D., et al. (1991). Development and evaluation of an enzyme-linked immunosorbent assay for detection, typing, and subtyping of vesicular stomatitis virus, J.Vet. Diagn. Invest. **3** (1992) 287-292.

[7] WESTBURY, H.A., DOUGHLY, W.J., FORMAN, A.J. et al., A comparison of enzyme linked immunosorbent assay, complement fixation and virus isolation for foot-and-mouth disease diagnosis, Vet. Microbiol.**17** (1988) 21-28.

[8] FERRIS, N.P., OXTOBY, J.M. & HUGHES, J.F., Multiple-infected diagnostic specimens from foot-and-mouth disease endemic regions, Rev.sci.tech.Off.int.Epiz. **14** (1995) 557-565.