COMPARISON OF COMPLEMENT FIXATION AND ELISA FOR DIAGNOSIS OF FOOT-AND-MOUTH DISEASE

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Abstract

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Foot-and-mouth disease (FMD) virus is characterised by its rapid transmission and its great antigenic variability which require a rapid and accurate diagnosis in the laboratory, in order to initiate an immediate response for control. From these studies it is clear that Enzyme linked immunosorbent assay (ELISA) has the advantage over the Complement fixation test (CFT) of being a test of high sensitivity and specificity. Therefore, this technique is now used in our laboratory for diagnosis to detect FMD virus (O-A-C) in epithelia from animals affected by the disease.

1. INTRODUCTION

Foot-and-mouth disease has a great capacity for spread due to the movement of infected animals, contaminated animal products and escape of virus from the laboratory. FMD is caused by a virus, classified as an Enterovirus, belonging to the family Picornavirus and genus Aphtovirus. There are 7 serotypes of virus and within each serotype there is a spectrum of antigenic variants.

2. MATERIALS AND METHODS

2.1. Field samples

Epithelium from the mouth or feet of affected cattle (38 samples) and from BHK cell culture (4 samples) [1,2]. The epithelia samples had previously been submitted to SENACSA, kept at - 20°C. in glycerine and phosphate buffer. The samples were examined by the ELISA [3] and compared with the Complement fixation test [4,5] for diagnosis and typing.

2.2. ELISA procedure

For the diagnosis and typing of FMD virus by ELISA the following materials were used:

- Rabbit Capture Antisera (O-A-C-NJ-I-and negative sera)
- The samples: Epithelium samples or BHK tissue culture suspension.
- Antigen reference: 0-A-C-NJ-I-and negative
- Plates
- Coating buffer
- PBS
- Tween 20
- Conjugate
- Substrate
- Multichannel and single channel pipettes
- Sulphuric Acid.
- Assay tubes
- Shaker
- Spectrophotometer Multiskan plus (filter: 492 mm.)

TABLE I. RESULTS ON FMD ELISA AT SENACSA

No	Date	Plate	Test sample	ELISA	CFT
01.	10.01.94.	1	Epit. Mouth	0	0
02.	10.01.94.		Epit. Mouth	0	0
03.	10.01.94.		Epit. Foot	Neg.	Neg.
04.	14.01.94.	2	Epit. Foot	0	0
05.			Epit. Mouth	Neg.	Neg.
06.			Epit. Mouth	Neg.	Neg.
07.	31.01.94.	3	Epit. Mouth	0	0
08.			Epit. Foot	0	0
09.			Epit. Foot	0	0
10.	22.02.94.	4	Epit. Mouth	0	0
11.			Epit. Mouth	0	0
12.			Epit. Mouth	0	0
13.	22.02.94.	5	Epit. Foot	0	0
14.			Epit. Foot	0	0
15.			Epit. Foot	Neg.	Neg.
16.	22.02.94.	6	Epit. Mouth	0	0
17.			Epit. Mouth	0	0
18.			Epit. Mouth	0	0
19.	22.02.94.	7	Epit. Mouth	0	0
20.			Epit. Mouth	0	0
21.			Epit. Mouth	0	0
22.			Epit. Mouth	0	0
23.			Epit. Mouth	0	0
24.	23.02.94.		Epit. Mouth	0	0
25.			Epit. Mouth	0	0
26.			Epit. Mouth	0	0
27.	23.02.94.	9	Epit. Mouth	0	0
28.			(BHK3)	C	C
29.			Epit. Mouth	0	0
30.	23.02.94.	10	Epit. Foot	0	0
31.			BHK2	A	A
32.			BHK3	C	C
33.	23.02.94.	11	Epit. Mouth	0	0
34.	23.02.94.		Epit. Mouth	0	0
35.			Epit. Mouth	0	0
36.			Epit. Mouth	0	0
37.			BHK3	A	A
38.	23.02.94.		Epit. Mouth	0	0
39.			Epit. Mouth	0	0
40.			Epit. Mouth	0	0
41.			Epit. Mouth	0	0
42.			Epit. Mouth	0	0

The assay procedure was as follows:

1- Solid Phase: Antisera Rabbit (O,A,C-NJ-I-and negative sera) dilution 1/100, 18 h, 4°C, Wash 5 times
2- Control Antigen: Dilution used O,A,C, 1/40 - 1/160 with PBS, 0.01 M, pH 7,4, NJ-I 1/50 - 1/200
Test samples: Suspension of epithelium
1 h 37°C on shaker washing 5 times
3- Detector guinea pig antisera: Dilution 1/100 (O,A,C-NJ-I-and negative)
4- Conjugate anti guinea pig: Dilution 1/300
5- Substrate OPD + $H_2 0_2$ 15 minutes
6- $H_2 SO_4$ (0.25 M)
7- Read at 492 nm

3. RESULTS

Virus types O, A and C were identified from the samples prepared from epithelia and suspensions of BHK cell cultures. Results were calculated using the background control adding the optical density (OD) value of the columns five and six divided by two; this result is the mean background OD. The mean OD of the antigen controls of the serotypes O-A-C-NJ-I-Neg were calculated and subtracted from the OD value of the background. This was repeated for every sample. All positive samples by complement fixation were also positive by ELISA (Table I).

4. DISCUSSION

Rapid and specific laboratory diagnosis is required for FMD, in order to differentiate this disease from others caused by vesicular viruses, and to identify the serotype of FMD virus. In the results reported here no difference was found between the ELISA and complement fixation test, however, other workers have reported increased sensitivity of ELISA, and the reagents used in ELISA can more easily be standardised and stored.

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