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SUBTYPING OF FOOT-AND-MOUTH DISEASE VIRUS BY THE MICRO-ENZYME-LABELLED IMMUNOSORBENT ASSAY (MICROELISA)

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Summary. — The micro-enzyme-labelled immunosorbent assay (MICROELISA) was used successfully for the subtyping of foot-and-mouth disease (FMD) virus strains recovered from field outbreaks. The rabbit anti-guinea pig globulin-peroxidase conjugate employed in the indirect MICROELISA has the advantage of being used with any of the seven types of FMD virus.

Key words: enzyme-labelled immunosorbent assay; foot-and-mouth disease virus

Though several techniques have been employed for the subtyping of foot-and-mouth disease (FMD) virus, the complement fixation (CF) and micro-neutralization tests are those routinely used for this purpose. However, there are several inherent disadvantages in these techniques. The ELISA technique has been used for FMD virus quantification and determination of antibody level in cattle (Abu Elzein and Crowther, 1978; 1979; Crowther and Abu Elzein, 1979). The present study describes its application in the subtyping of FMD virus.

Viruses. The O_5 virus which is used as the vaccine strain in this country was used as the reference strain. The field isolates of type 0 were received in the form of original tongue epithelium samples. These samples were triturated in phosphate buffer, pH 7.4 and treated with an equal volume of fluorocarbon (Mafsolvent EL 113) and centrifuged at 3000 rev/min for 30 min after which the supernatants were collected. The supernatants diluted so as to contain 1 mg/ml protein were used as virus antigens throughout. For comparison, known A_5 and A_{22} virus antigen were prepared as described above.

Antisera against O_5, A_5, A_{22} and the field isolates were obtained by hyperimmunizing guinea pigs 3 months after primary infection (Brooksby, 1952).

Micro CF test. The strains were tested as described by Rweyemamu *et al.* (1977).

MICROELISA. The rabbit anti-guinea pig globulin-peroxidase conjugation procedure and the indirect MICROELISA technique used were the same as described (Rai and Lahiri, 1981). The O_5 antiserum and O_5 virus was used as the homologous system as a reference to which all the field isolates were compared. The reagents were used in the following order: (i) O_5 virus and the field viruses diluted in coating buffer in separate wells (25 µl), (ii) O_5 antiserum in twofold dilutions (25 µl), (iii) conjugate (25µl), (iv) substrate (50 µl), and (v) 3 M NaOH (50 µl). The OD values were read at 449 nm and a curve was made for each antigen. The results were read from the linear part of the curve and the highest dilution of antiserum showing a significant difference in the OD value as compared to the control was taken as the endpoint titre. A difference of 0.075 in OD value in the reaction mixtures as compared to controls was taken to be significant. Where

Table 1. Testing of O_5 antiserum against different field FMD virus isolates

Virus	Micro-CF test		MICROELISA	
	Antiserum titre	r	Antiserum titre	r
O_5	128	1.0	2896	1.0
M 43/79	128	1.0	2048	0.7
M 44/79	45	0.35	2048	0.7
M 54/79	32	0.25	1024	0.35
M 57/79	32	0.25	1024	0.35
M 58/79	128	1.0	2048	0.7
M 59/79	64	0.5	1024	0.35
M 60/79	64	0.5	1024	0.35
M 62/79	128	1.0	1024	0.35
M 63/79	64	0.5	1024	0.35
M 64/79	45	0.35	1024	0.35
M 70/79	128	1.0	2048	0.7
M 71/79	45	0.35	1024	0.35
M 72/79	32	0.25	724	0.25
M 75/79	90	0.7	1024	0.35

the endpoint fell between two dilutions, the geometric mean of the two dilutions was calculated. The O_5 antiserum titre was determined with O_5 virus and different field isolates and r values were calculated as follows:

$$r\ (O_5\ \text{antiserum}) = \frac{O_5\ \text{antiserum titre with heterologous field virus}}{O_5\ \text{antiserum titre with}\ O_5\ \text{virus}}$$

To check whether the use of different viruses did not cause the variation, in another set of experiments r values were calculated for O_5 virus using O_5 antiserum as homologous system and sera against different field isolates as heterologous system. The order of the reagents used was (i)

Table 2. Testing of O_5 virus against antisera to different FMD virus isolates

Antiserum	Micro-CF test*		MICROELISA	
	Antiserum titre	r	Antiserum titre	r
O_5	113	1.0	2896	1.0
M 167/77	80	0.7	2048	0.7
M 170/77	113	1.0	1024	0.35
M 200/77	40	0.35	724	0.25
M 16/78	56	0.49	2048	0.7
M 20/78	80	0.7	1024	0.35
M 21/78	80	0.7	1024	0.35
M 28/78	113	1.0	2048	0.7
M 41/78	40	0.35	2048	0.7
M 42/78	56	0.49	2048	0.7
M 43/78	40	0.35	2048	0.7

* Values taken from Rai (1980) for comparison.

Table 3. Testing of O_5 virus against antisera to different FMD virus isolates

Antiserum	Micro-CF test		MICROELISA	
	Antiserum titre	r	Antiserum titre	tr
O_5	128	1.0	2896	1.0
M 161/78	64	0.5	1024	0.35
M 5/79	128	1.0	1024	0.35
M 12/79	90	0.7	2048	0.7
M 16/79	90	0.7	2048	0.7
M 17/79	128	1.0	1024	0.35
M 18/79	64	0.5	2048	0.7

O_5 virus (25 µl), (ii) twofold dilutions of antisera against O_5 and field isolates (25 µl), (iii) conjugate (25 µl), (iv) substrate (50 µl), and (v) 3 M NaOH (50 µl).

The r values were calculated as follows:

$$r\ (O_5\ \text{virus}) = \frac{\text{titre of heterologous antiserum with } O_5 \text{ virus}}{\text{titre of } O_5 \text{ antiserum with } O_5 \text{ virus}}$$

The results obtained by MICROELISA and the micro-CF test with different field isolates of FMD virus are shown in Table 1. In these experiments in which r values of O_5 antiserum were calculated, there was no significant difference in the antiserum titre and r values obtained by MICROELISA and the micro-CF test. The results of experiments in which values were calculated for O_5 virus in relation to antisera against different field isolates are shown in Tables 2 and 3. When A_{22} virus was tested against A_5 and A_{22} antisera, the antiserum titres were significantly different (8192 and 1024 with A_{22} and A_5 antiserum, respectively) and the r value of 0.125 with A_5 antiserum denotes that the technique could differentiate between these two known subtypes.

The indirect technique of MICROELISA standardized by us (Rai and Lahiri, 1981) may be successfully used for the subtyping of FMD virus and has advantages over the CF test. In the present experiments it gave almost similar results as the micro-CF test. The antiserum titres determined by MICROELISA were much higher than those obtained by the micro-CF test, that could be well expected since ELISA is considered to be much more sensitive and efficient than the CF test. Since r value of reference antiserum is to be determined against both homologous virus and different field strains (Pereira, 1977), it is recommended that the indirect technique be used as an additional test to the micro-CF test for subtyping work. Though the antiserum used in the present study was against the whole virus, the antibody against specific 146 S antigen would further make this test more sensitive, specific and efficient. Furthermore, unheated serum can be used and other factors like anti-complementary activity of sera and antigens encountered in the CF test are completely eliminated. The CF and microneutralization

tests which are currently employed for subtyping of FMD virus have certain inherent disadvantages. The r values obtained in the CF test may vary fourfold (Rwayemamu *et al.*, 1978) and thus the CF test may not be a reliable method. Similarly the micro-neutralization test which is quite efficient and specific may give significant variations in the neutralizing titre of sera if a constant dose of virus is used in the test and hence the two-dimensional neutralization test has been suggested to overcome this variation (Booth *et al.*, 1978; Rweyemamu *et al.*, 1978). If the present test would give comparable results on large scale testing alongwith the two tests mentioned above, it would become a test of choice because it will be free of the disadvantages inherent in the CF and neutralization tests. Another advantage of MICROELISA is that only one conjugate can be used for all the virus types. The third advantage is the small volume of reagents. If an anti-146 S serum is used, then the cell culture or tongue epithelium samples could be used as such since only 146 S antigens would participate in the reaction though VIA antigen could be present in the preparation (Abu Elzein and Crowther, 1979). This would be of great advantage in routine subtype characterization of field viruses which will not require any purification procedures.

The competition assay which is considered to be highly sensitive and efficient might prove more suitable than the indirect technique and preliminary experiments in our laboratory have given encouraging results.

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