J. Hyg., Camb. (1979), **83**, 513
Printed in Great Britain

Application of the enzyme linked immunosorbent assay to the detection and identification of foot-and-mouth disease viruses

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(*Received* 11 *April* 1979)

SUMMARY

An indirect enzyme linked immunosorbent assay (ELISA) was applied to the detection and identification of foot-and-mouth disease (FMD) virus types. The test proved successful for the specific detection of virus from infected tissue culture, and from epithelial tissues from bovines suspected of having FMD. The ELISA compared favourably with the complement fixation (CF) test, being more sensitive and unaffected by anticomplementary factors.

INTRODUCTION

Enzyme linked immunosorbent assays (ELISA) have been successfully applied to the detection of bovine antibodies against foot-and-mouth disease (FMD) virus (Abu Elzein & Crowther, 1978), the detection and quantification of FMD virus (Crowther & Abu Elzein, 1979) and the specific detection of whole particle (140S) FMD virus in the presence of subunit and tissue culture material (Abu Elzein & Crowther, 1979). This present work investigated the use of the ELISA in detection and identification of FMD virus types in epithelial tissue from cattle suspected of having FMD. Results are compared with the current complement fixation test used for this purpose.

MATERIALS AND METHODS

The solid phase microtitre plates, buffers, enzyme, substrate and washing procedures were as described by Abu Elzein & Crowther (1978).

Viruses

FMD virus types O_6/UK/1924 (OV1), A_5/Germany/Westerwald/1933 (A_5-West), C/Germany/1933 (CGC), SAT 1/Botswana/1/68 (Bot 1/68), SAT 2/South West Africa/1/1969 (Swa 1/69), SAT 3/Bechuanaland/1/1965 (Bec 1/65), and Asia 1/Pakistan/1/1954 (Pak 1/54), were grown in monolayer cultures of BHK-21 cells. These viruses are used to raise typing antisera in guinea-pigs in the World Reference Laboratory (WRL), Animal Virus Research Institute (AVRI), Pirbright. A sample of infected cells in growth medium was taken, half of which was acidified as described by Abu Elzein & Crowther (1979), and both infected cell

0022-1724/79/0031-1979 $01.00

samples were stored at −70 °C. Virus was purified from the rest of the cells by sucrose density gradient centrifugation as described by Brown & Cartwright (1963), using 1% SDS instead of deoxycholate. Purified (140S) viruses were stored at −70 °C in siliconized glass vials.

Epithelial samples from cattle sent for typing of FMD virus infection were obtained from the WRL, AVRI. These had been stored in PBS/glycerol (50:50 pH 7·6) since being sent to the WRL. Samples were processed by being ground with sterile sand and phosphate buffer (0·1 M, pH 7·6), as in the routine treatment, before typing by complement fixation (CF) carried out in the WRL, as described by Buckley, Osborne & Pereira (1975). The reference numbers of all the strains are those given by the WRL, AVRI.

Antisera

Guinea-pig antisera against the current virus strains used for typing FMD viruses by means of the CF test were obtained from the WRL, AVRI, and were prepared by the method described by Brooksby (1952).

Convalescent cattle antisera against FMD virus types O_1/UK/1860/1968 (O_1 BFS), A_5/W. Germany/Eystrup/1952 (A_5 Eys), C/Switzerland/Noville/1958 (C Nov), SAT 1/Turkey/323/1973 (SAT 1 Tur 323/73), and Asia 1/Israel/3/1963 (Asia 1 Isr 3/63), were obtained from the Vaccine Research Department at the AVRI, and were prepared according to the method of Garland (1974).

Goat anti-guinea-pig antiserum was obtained from Miles Laboratories Ltd., U.K.

The IgG fractions of antisera were prepared as described by Abu Elzein & Crowther (1978). The IgG concentrations were calculated after measuring the OD at 278 nm (E 278 = 1·4). Samples were stored at −20 °C in siliconized glass vials.

Conjugation of Enzyme to IgG

The enzyme alkaline phosphatase was conjugated to the IgG fraction of described antisera according to the method of Avrameas (1969). The working dilution of each conjugate was determined as described by Voller, Bidwell & Bartlett (1976). Stock conjugates were stored at 4 °C after the addition of 5% ovalbumin and 0·02% sodium azide (final concentrations).

Typing experiments

In order to ensure best conditions for attachment of test antigen, a double sandwich test was developed in which plates were coated with type-specific bovine sera before addition of antigen, as shown in Fig. 1. Each test plate received a single row of each of the 7 type-specific bovine IgGs diluted in carbonate buffer to 10 μg/ml (200 μl per well). Plates were covered and incubated at 37 °C for 3 h. After washing off non-attached IgG, a dilution series of the virus sample to be typed (as described in Results) was added to each row diluted in PBS containing 0·05% (v/v) Tween 20 (PBST) and a final concentration of 1% (w/v) ovalbumin. Virus samples and typing-IgGs were allowed to react for 1 h at 37 °C, after which

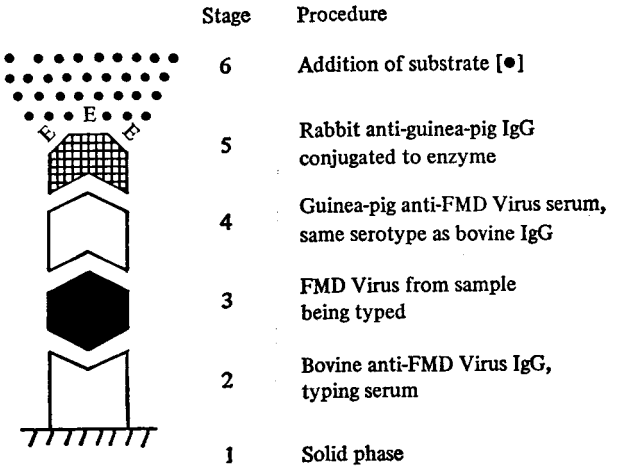

Fig. 1. Principle of double sandwich ELISA for identification of FMD virus types.

wells were washed. Each well then received 200 μl of whole guinea-pig typing serum, of the same serotype as the bovine IgG originally attached to the row, diluted 1/300 in PBST containing 1% ovalbumin (w/v) final concentration. Plates were incubated for 1 h at 37 °C and washed. Enzyme-labelled anti-guinea-pig IgG was then added (200 μl per well) at a suitable pretitrated dilution in PBST + 3% ovalbumin (final concentration). Plates were incubated for 1 h at 37 °C and washed, after which substrate addition and incubation procedures were as described by Abu Elzein & Crowther (1978). The colour developing in wells was read by eye and then by spectrophotometer at 405 nm.

Preliminary experiments were made where plates were sensitized with a single type-specific bovine IgG. Purified viruses (140S) of all seven types were then added to each plate and the test followed as described above.

RESULTS

The results showing the reactions of two 140S virus preparations in the double antibody-ELISA are shown in Fig. 2(*a*) and (*b*). They illustrate results observed with all 140S preparations that were typed on plates sensitized with a single bovine type IgG. Fig. 2(*a*) shows that only type A FMD virus is measured on plates sensitized with bovine type A IgG. Fig. 2(*b*) shows that only type O is measured on plates sensitized with bovine type O IgG. The same background colour (approximately 0·25 OD) was obtained for all other heterotypic reactions. The results indicate the limits for virus detection by this method as judged by the highest dilutions showing specific colour measured above background levels, as indicated by the arrow on the figures.

Table 1 shows the results of typing FMD virus-infected tissue culture of all seven serotypes by ELISA. Only three dilutions of each sample were necessary to obtain specific typing. No colour was obtained for any sample after acidification,

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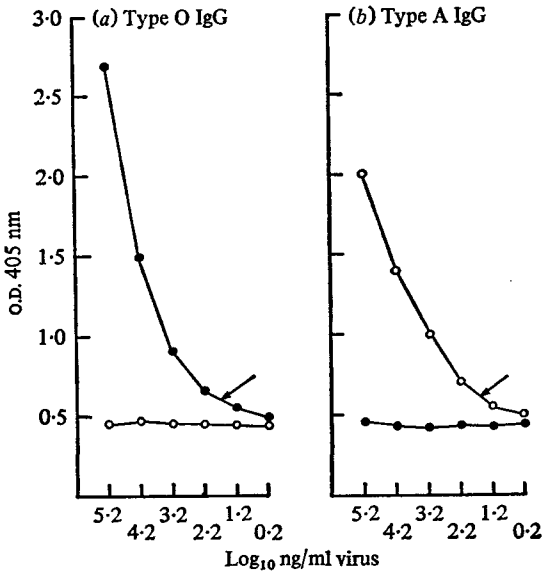

Fig. 2. Typing of FMD virus using ELISA. Addition of purified FMD virus type O (●—●) and type A (○—○) to wells with bovine IgG against (*a*) type O and (*b*) type A. End point shown by arrows.

indicating that 12S antigens were not measured in this test. The background colour obtained for heterologous types was low, indicating that any cross-reactivity due to 12S and VIA antigens present in untreated samples was reduced to low levels. When the typing results were measured 'by eye', the specificity of the homologous reactions was clear in all cases. The eye did not detect colour in any well reading less than 0·1 OD after the background of approximately 0·3 OD units had been subtracted, i.e. in wells reading 0·4 OD or less.

Table 2 compares the results of typing virus from 23 epithelium samples using the ELISA and the routine CF test. Positive typing by ELISA was made on the initially prepared dilutions of the epithelium in 19 cases, with four negative results, when reading by eye. Eight samples were found positive by CF using the *same* material and five samples proved to be anti-complementary. A further 11 samples were then typed by CF test after passage in tissue culture. The same serotypes were found in positive samples using both tests, and the same four samples were found negative.

DISCUSSION

The double sandwich ELISA test was developed after examination of a more straightforward indirect-ELISA where antigen to be typed was allowed to adsorb directly to plates, typing serum was added and the anti-species antibody conjugated to enzyme was used as the indicator system. This technique was successful when results were read by spectrophotometer but gave consistently higher backgrounds than the double sandwich technique, making reading by eye difficult.

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TABLE 1. *Typing of FMD viruses from infected tissue culture*

	FMD virus type added to plates																							
	A			O			C			SAT 1			SAT 2			SAT 3			ASIA 1					
DILN^{-1} sample	2	4	8	2	4	8	2	4	8	2	4	8	2	4	8	2	4	8	2	4	8			
Typing IgG																								
A	0·40	0·20	0·10	0·10	0	0	0	0	0	0·13	0	0	0	0	0	0·08	0	0	0·11	0·02	0			
O	0	0	0	0·40	0·23	0·16	0	0	0	0·10	0	0	0	0	0	0·10	0·02	0	0	0	0			
C	0	0	0	0	0	0	0·90	0·54	0·26	0	0	0	0	0	0	0·08	0	0	0·17	0·06	0			
1	0	0	0	0	0	0	0	0	0	0·42	0·19	0·07	0	0	0	0·14	0·02	0	0	0	0			
2	0	0	0	0·10	0·02	0	0	0	0	0·06	0	0	0·54	0·39	0·17	0·05	0	0	0·09	0	0			
3	0	0	0	0·09	0	0	0	0	0	0·19	0·05	0	0	0	0	0·30	0·15	0·10	0·09	0	0			
Asia 1	0	0	0	0	0	0	0	0	0	0·05	0	0	0	0	0	0·09	0	0	0·61	0·40	0·30			

Figures represent OD at 405 nm minus control OD value.
Control = non-immune guinea-pig serum attached to wells.

TABLE 2. *Typing of FMD virus from bovine epithelium samples*

Epithelium sample	ELISA† Original material	CF Original material	CF After passage
Gha 23/73	A	—	A
Bur 6/77	0	*	0
Phi 20/75	—	—	—
Nig 5/75	A	—	A
Tan 1/77	1	1	1
Ivy 1/76	—	*	—
Tai 3/74	As 1	As 1	As 1
Sud 3/77	A	—	A
Srl 2/76	C	—	C
Hkn 12/77	—	—	—
Tur 12/73	A	A	A
Uga 43/73	1	—	1
Alg 1/77	A	A	A
Mtn 28/75	0	0	0
Nig 1/75	2	2	2
Uga 15/75	0	*	0
Tai 2/74	A	—	A
Bel 1/76	0	0	0
Nig 3/77	—	*	—
Fra 1/74	C	C	C
Sar 23/74	1	—	1
Ger 2/73	A	—	A
Bel 5/74	0	*	0

† Results obtained using neat, $\frac{1}{2}$ and $\frac{1}{4}$ of original sample.
A = type A FMD virus; O = type O; C = type C; 1 = type SAT 1; 2 = type SAT 2; As 1 = type Asia 1; — = no typing obtained.
* = anti-complementary.

The results in this paper indicate that the double sandwich technique can be used to type FMD viruses, using the initial preparations from bovine epithelial tissue. Only three dilutions of sample were necessary to give specific typing of plates read by 'eye'. The higher sensitivity of the ELISA test over the CF test, as shown by Crowther & Abu Elzein (1979), was demonstrated by the fact that all the 19 positive samples in this study were typed by ELISA from original material, whereas the CF test typed only eight samples and further tissue culture passage was necessary before CF typing was obtained. The ELISA method was not affected by anti-complementary factors as was seen for five samples using the CF test before passage. Clear typing results were also read by eye using the ELISA for all three dilutions when examining infected tissue culture material. Some difficulty is observed with cross-reactions with samples using the CF test, particularly between types O and C, and SAT 1 and SAT 3 viruses. This probably reflects the cross-reactivity of the guinea-pig antisera, containing antibodies to 12S and VIA antigens, with these components in crude infected tissue culture fluids. This reaction was greatly reduced in the ELISA tests and confirms earlier results by Abu Elzein & Crowther (1978), who showed that sandwich techniques

gave results specifically detecting whole particle (140S) antigen in the presence of tissue culture and 12S material.

In practical terms the test is simple to perform. Bovine antisera to all seven serotypes are readily available and, since only small amounts of specific IgG are needed to coat typing plates, a single isolation procedure of gamma globulin provides enough material for several years' work. Plates, once sensitized, may be stored at 4 °C and remain usable for long periods (up to 1 year so far in this laboratory), and thus are readily available for typing. Hyperimmune guinea-pig typing serum is already prepared for typing by complement fixation tests. A single enzyme conjugate of commercially available anti-guinea-pig IgG is used to detect antibody and this reagent is easily standardized, a single batch of IgG remaining stable in small samples at − 20 °C before conjugation and the conjugated reagent being stable when stored as described for up to 1 year (so far). Revision of the method used to prepare typing antisera, e.g. the use of antiserum prepared against purified inactivated FMD virus strains, may eliminate all cross-reactivity in the ELISA test, thus making the detection of 'mixed' populations of types of FMD virus feasible.

The high sensitivity and specificity achieved by the ELISA, making it possible to type FMD virus from 'original' material, may be of most benefit to laboratories where tissue culture techniques are not available.

The authors wish to thank Dr J. B. Brooksby and Dr H. G. Pereira for reading the manuscript, and for their useful suggestions.

The skilful technical assistance of Mrs B. Newman is highly appreciated.

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