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The specific detection of foot-and-mouth disease virus whole particle antigen (140S) by enzyme labelled immunosorbent assay

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SUMMARY

A solid-phase micro-enzyme-labelled immunosorbent assay (ELISA) using guinea pig antiserum against purified (140S) inactivated foot-and-mouth disease (FMD) virus has been used in a sandwich technique to specifically measure 140S virus in the presence of 12S material.

INTRODUCTION

The micro-enzyme-labelled immunosorbent assay (ELISA) has been successfully applied to the assay of antibodies to foot-and-mouth (FMD) virus in bovine sera (Abu Elzein & Crowther, 1978), and to the detection and quantification of purified (140S) FMD virus (Crowther & Abu Elzein, 1979). This paper shows the application of the ELISA to specific measurement of 140S FMD virus in the presence of the 12S subunit antigen. The ELISA technique offers particular advantages for the assay of FMD virus antigen for vaccines where it is important to estimate the amount of 140S antigen in unpurified tissue culture materials (see Garland, Mowat & Fletton, 1977).

MATERIALS AND METHODS

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

Viruses

FMD virus type O_1/UK/1860/1967 was grown in monolayer cultures of BHK 21 cells. A sample of infected cells was 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 virus (140S) was stored at −70 °C in siliconized glass vials.

Preparation of 12*S*

Subunit (12S) was prepared from purified O_1/UK/1860/1967. One volume of virus was acidified by the addition of 2 volumes of 0·05 M-NaH_2PO_4. After incubation for 10 min. at room temperature (RT) the pH of the reaction was adjusted to 7·4, and the sample was stored at −70 °C in a siliconized glass vial.

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Antiserum production

Antiserum against the purified 140S FMD virus type O_1/UK/1860/1967 was prepared in guinea-pigs. Virus was inactivated using acetylethyleneimine (AEI), final concentration 0·05 % (w/v), by incubation at 26 °C for 30 h. One volume of virus was emulsified with one volume of Freund's complete adjuvant (Difco Labs., Detroit, Michigan). Five guinea-pigs each received 50 μg of virus intramuscularly at two sites. Animals were exsanguinated after 28 days and the sera pooled. This is termed 140S antiserum.

Hyperimmune guinea-pig antiserum against O_1/UK/1860/1967 was obtained from the World Reference Laboratory, Animal Virus Research Institute (AVRI), Pirbright. It was prepared by the method of Brooksby (1952).

The IgG fraction of the antisera was prepared as described previously (Abu Elzein & Crowther, 1978). The IgG concentration was calculated after measuring the OD at 278nm ($E_{278} = 1{\cdot}4$). Samples were stored at -20 °C in siliconized glass vials.

Conjugation of enzyme to IgG

The enzyme was conjugated to the IgG fractions of the antisera according to the method of Avrameas (1969). The working dilution of the conjugates 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).

Sandwich ELISA

The sandwich technique as described by Voller *et al.* (1976) was used to assay the various FMD virus antigens described below. The specific IgG was diluted in carbonate buffer to a concentration of 10 μg/ml and then added to wells (200 μl). After incubation for 3 h at 37 °C the wells were washed three times. Various FMD virus antigens diluted in PBS containing a final concentration of 1 % Tween 20 (PBST) + 1 % ovalbumin were then added to the wells in two-fold dilution series (200 μl per well). Plates were incubated overnight at RT or 1 h at 37 °C. After washing three times, the enzyme-labelled homologous IgG antibody was added (200 μl per well), diluted in PBST + 3 % ovalbumin (final concentration). Plates were incubated for 1 h at 37 °C. The wells were then washed three times and substrate solution was added (200 μl). After 30 min incubation at RT, the colour reaction was stopped by the addition of 50 μl of 3 M-NaOH solution. The OD at 405 nm of the contents of each well was determined using a Uvichem spectrophotometer (Hilger and Watts).

Initial experiments involved the titration of purified 140S O_1/UK/1860/1967 and 12S material prepared from this virus after acidification, using the 140S and hyperimmune-IgG antibodies in the sandwich technique. The starting concentrations of the 140S and 12S were made equal, and dilution series were prepared as described above. The colour development using the two homologous systems to detect the antigens was as shown in Fig. 1.

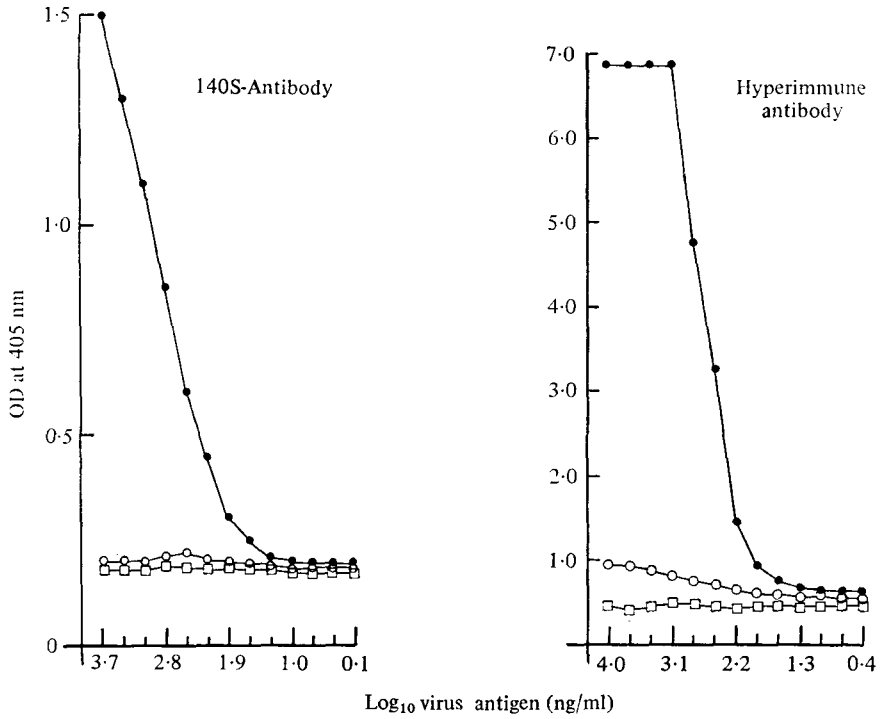

Fig. 1. Equivalent masses of purified 140S O_1/UK/1860/1967 and 12S material were diluted in twofold dilution series and assayed by sandwich ELISA using 140S-IgG and hyperimmune-IgG systems. ●—●, O_1 BFS 1860 purified 140S; ○—○, 12S; □—□, blank controls.

Various mixtures of 140S and 12S were prepared and assayed using the 140S-IgG system. The results are shown in Fig. 2.

Infected tissue culture material stored as described above was assayed with the 140S-IgG system, before and after acidification and restoration of pH to 7·4 as in the preparation of 12S material. The results are shown in Fig. 3.

The sandwich ELISA using 140S-IgG antibody was used to measure the 140S content of fractions obtained after a linear sucrose density gradient purification of O_1/UK/1860/1967 virus. The fractions were also titrated for FMD virus antigen by the tube complement fixation test used routinely in the Vaccine Research Department (VRD), Animal Virus Research Institute (AVRI), with serum from infected guinea-pigs, boosted with virus material after 3 months (Brooksby, 1952). The results are shown in Table 1. The ELISA end-points were read as the dilution of sample showing detectable colour compared with the background controls after reading by the spectrophotometer.

In order to assess the value of the ELISA tests in the measurement of 140S in samples produced during vaccine manufacture, infected tissue culture vaccine material was obtained from VRD, AVRI, Pirbright. The mass of 140S was determined using ELISA by comparing the OD developing at particular dilutions of test samples with a standard titration curve using purified 140S virus. The standard curve related the OD developing against a 'known' mass of purified virus, as

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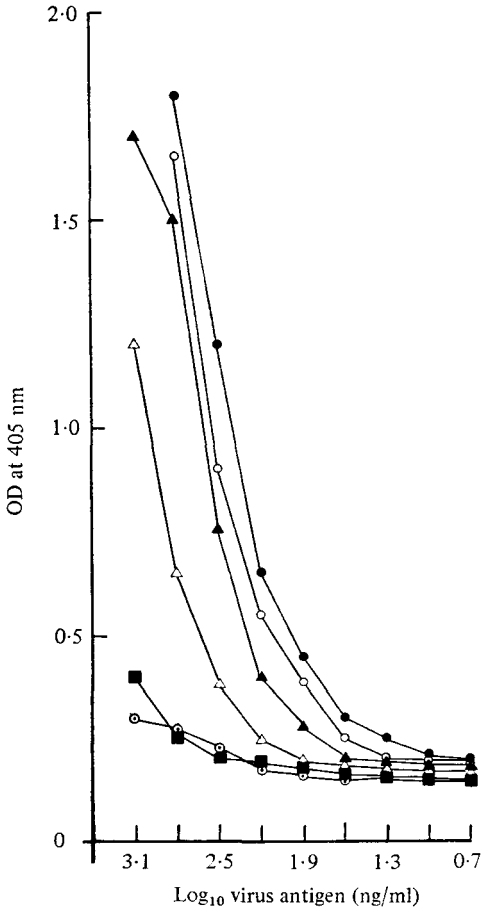

Fig. 2

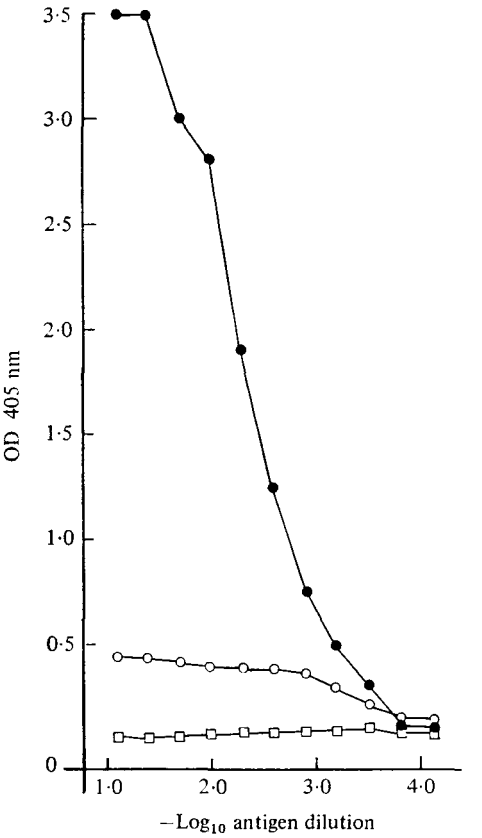

Fig. 3

Fig. 2. Purified 140S O_1/UK/1860/1967, 12S and mixtures of 140S and 12S were added to wells and titrated using ELISA sandwich technique using 140S-IgG. ●—●, 140S only; ■—■, 12S only. ○—○, 75% 140S, 25% 12S; ▲—▲, 50% 140S, 50% 12S; △—△, 25% 140S; 75% 12S; ⊙—⊙, blank controls.

Fig. 3. Infected tissue culture material was assayed using the sandwich ELISA with 140S-IgG (●—●). The same material was assayed after acidification and restoration of pH to 7·4 (○—○). □—□, Blank control.

determined after sucrose density gradient centrifugation and determination of OD (Bachrach, Trautman & Breese, 1964). The standard antigen titration curve was produced each time test samples were examined. Fig. 4 shows a typical standard curve for FMD virus type O_1/UK/1860/1967, relating the $\log_{10}$ OD of the colour developing in ELISA with the virus concentrations in μg/ml. The figure also includes the titration curve of an infected tissue culture sample, diluted in a 2-fold series as was the standard antigen, in order to assess the similarity of antigen/antibody reactions in the test and standard system. The samples were also assessed in the VRD for 140S antigen using a routine method as described by Capstick *et al.* (1967).

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Table 1. *Examination of linear sucrose gradient fractions of O_1/UK/1860/1967 FMD virus by sandwich ELISA and CF tests*

Sucrose gradient fraction	OD 259 nm	Reciprocal of end-point ELISA	Complement fixing units/ml
1 (bottom)	0·23	<2	<19
2	0·21	<2	<19
3	0·20	16	<19
4	0·20	32	19
5	0·36	4096	433
6	0·53	32768	850
7	0·24	512	155
8	0·16	64	35
9	0·16	32	23
10	0·17	32	20
11	0·22	16	<19
12	0·45	4	42
13	0·84	4	>293
14	1·20	<2	139
15 (top)	1·80	<2	84

RESULTS

The titration curves for 140S and 12S in Fig. 1 indicate that both the 140S-IgG and hyperimmune-IgG systems react predominantly with 140S-specific determinants of the FMD virus. The OD measurement for the hyperimmune-IgG system was extended to approximately 7 OD units (obtained after dilution of well contents), to examine the 12S reaction at high concentrations, since the titre for 12S was increasing where 0–2 OD units were measured. However, the extent of reaction with 12S was low compared with purified 140S. The 140S-IgG system showed no cross-reactivity for 12S.

Results shown in Fig. 2 indicate that 12S had no effect on the titration curve of 140S. Increasing the proportion of 12S had the effect of diluting the 140S, so that the titration curves were moved to the left.

Infected tissue culture material, presumably containing 140S and 12S virus material, gave the titration curve shown in Fig. 3. After acidification and conversion of 140S to 12S subunit material, a plateau was obtained above background at approximately 0·4 OD, indicating that a low maximum of 12S reacts in the system. The effect was reduced after dilution of 1/1000 of the tissue culture material.

The assessment of FMD virus antigen from sucrose density gradients by ELISA showed a high peak in the expected 140S region (fractions 5 and 6). This peak was also shown in the CF test and OD readings. However, no antigen was detected by ELISA at the top of the gradient, where CF tests indicate FMD virus antigen with increase in protein, presumably due to the 12S content at this region.

The comparison of standard and test antigen titration curves (Fig. 4) shows the lines to be parallel over a range equivalent to 320–30 ng/ml for the standard antigen preparation, with an OD range of 1·14–0·28 units. This region of the standard curve was used to titrate test samples. OD readings obtained for test samples within the range 0·28–1·14 were taken as showing the same reaction as standard

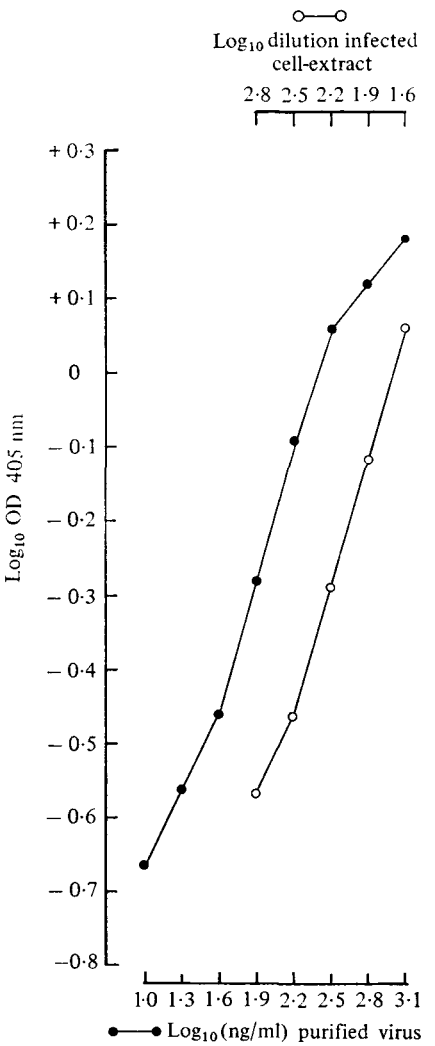

Fig. 4. A twofold dilution series of a known mass of purified type O_1/UK/1860/1967 and tissue culture material infected with same virus was assayed using sandwich ELISA. ●—●, Purified 140S; ○—○, infected tissue culture.

purified virus. The curves for all test samples were parallel to standard curves. The concentration of virus in test samples was thus easily calculated by reading the mass of standard virus at the OD obtained and multiplying by the dilution factor used. Table 2 compares the masses of 140S virus obtained from the same samples as determined by ELISA and CF tests, and indicates similar values.

DISCUSSION

Two antisera were examined in this study: a hyperimmune guinea-pig antiserum produced with live FMD virus, and a guinea-pig antiserum raised against purified inactivated FMD virus. These sera react with both 12S and 140S FMD

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Table 2. *Measurement of 140S virus antigen using ELISA and CF tests*

Sample	Virus concentration (μg/ml)	
	ELISA	CFT
(1) Sucrose gradient material	40	34
(2) Sucrose gradient material	10	(5–7)
(3) Infected cell material	0·03	0
(4) Infected cell material	9	(5–7)
(5) Infected cell material	5	(3–4)

virus antigens in tests such as immunodiffusion, complement fixation and double antibody radioimmunoassay. However, the sera did not show reactivity with 12S FMD antigen under the conditions used in the sandwich-ELISA test. Studies using similarly prepared guinea-pig antisera against FMD virus types A and SAT 1 have shown essentially the same results, with specific detection of 140S FMD virus antigen in the presence of 12S.

Since the preparation of antiserum against inactivated virus is simpler in respect of safe handling it is recommended that this kind of antiserum is used for the specific measurement of 140S particles. Further studies using the sandwich ELISA test with 5- to 7-day post-infection bovine or guinea-pig antisera to FMD virus, which react specifically with 140S determinants, have also proved successful for the specific detection of 140S virus particles. These results will be discussed in connexion with the use of the ELISA test to compare FMD virus strains.

This study indicates a useful application of the ELISA test to measure specifically 140S in unfractionated tissue culture samples obtained during the manufacture of vaccines. The parallel lines obtained for the ELISA titration of virus with tissue culture samples and purified virus indicate that at the relevant concentrations a similar antigen/antibody reaction is being observed. Table 2 confirms that similar values for the amount of 140S in samples were obtained compared with those obtained in the routine CF test employed. The ELISA technique offers two major advantages over previous techniques. Antigen is measured directly from samples without the need to separate 140S from 12S and VIA virus antigens, and owing to the high sensitivity of the test, samples containing only small amounts (down to approximately 25 ng/ml) of 140S can be assessed without need for concentration.

The use of the sandwich ELISA to measure the 140S during the manufacture of vaccines is now being compared more extensively with methods used routinely in the Vaccine Research Department, AVRI, Pirbright. The method is rapid, sensitive and specific and can be used to assay a large number of samples. The application of the test to control quality through the various stages of vaccine manufacture is also of interest.

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