

A MICRO-ENZYME-LABELLED IMMUNOSORBENT ASSAY (MICROELISA) FOR THE DETECTION OF FOOT-AND-MOUTH DISEASE VIRUS ANTIGEN AND ANTIBODY

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Summary. — The indirect technique of a micro-enzyme-labelled immunosorbent assay (MICROELISA) was standardized and found efficient in detecting the foot-and-mouth disease virus antigen in cell culture fluids, mice carcasses and cattle tongue epithelium as well as the antibody titre of sera.

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

Introduction

The application of enzyme-labelled immunosorbent assay (ELISA) for the detection of foot-and-mouth disease (FMD) virus (genus Aphthovirus) antigen has been described by Abu Elzein and Crowther (1979) and Crowther and Abu Elzein (1979). But the volume of the reagents used by them was rather high. The present study describes a microplate technique (MICRO-ELISA) with wide applications and several advantages over the method described above.

Materials and Methods

Virus. FMD virus type 0 (subtype 0₅) was used in the form of cattle tongue epithelium, mice carcasses and cell culture fluid (BHK-21 cell culture-adapted). The virus suspensions were treated with fluorocarbon and the supernatants collected for further use.

Hyperimmune serum was raised in guinea pigs against 0₅ virus as described by Brooksby (1952).

The complement fixation (CF) test was done by the micromethod described by Rweyemanu *et al.* (1977).

Protein estimation. The virus concentration in the purified suspensions was determined by estimating protein as described by Lowry *et al.* (1951).

Rabbit anti-guinea pig globulin. The globulin from healthy guinea pig serum separated by ammonium sulphate precipitation was mixed with an equal volume of Freund's complete adjuvant and injected into rabbits, 0.2 ml intramuscularly into each of the four feet. After 21 days a similar injection but with incomplete Freund's adjuvant was given; it was repeated 7 days later. Ten days after the last injection, the rabbits were bled and the sera collected. The globulin was separated by ammonium sulphate precipitation.

Conjugation with horseradish peroxidase. The rabbit anti-guinea pig globulin was conjugated with horseradish peroxidase by the glutaraldehyde two-step procedure (Avrameas and Ternynck, 1971). The conjugate was tested in 1 : 10, 1 : 100, 1 : 200, and 1 : 400 dilutions; the latter gave a satisfactory reaction and was used throughout.

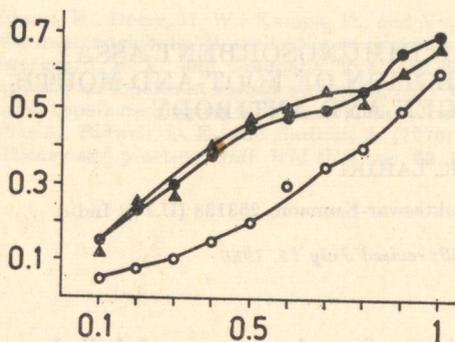


Fig. 1.

Results of MICROELISA with different concentrations of FMD virus antigen and a 1 : 128 dilution of antiserum. Antigens: cattle tongue (●), cell culture (○), mouse (▲). Abscissa: virus concentration (mg/ml); ordinate: OD₄₄₉ values.

MICROELISA. The indirect technique with polystyrene 96-well flat-bottom microtitre plates (Laxbro) was used. Various dilutions of antigen were prepared in 0.015 M sodium carbonate buffer (pH 9.6) and 25 μ l of each dilution were delivered into the wells and kept at 4 °C overnight. Thereafter the plates were incubated at 37 °C for 1 hr and the wells washed with PBS-Tween buffer (pH 7.4); then 25 μ l of the antiserum were added. The plates were again incubated and washed as above, after which 25 μ l of the conjugate were added and allowed to react for 1 hr at 37 °C. After washing the wells, the substrate (50 μ l) was added and allowed to react at 37 °C for 30 min after which the reaction was stopped by adding 50 μ l of 3 M NaOH. The substrate used was Nadi reagent (α -naphthol — 15 mg; p-phenylenediamine — 22 mg; dissolved in 20 ml of PBS-Tween buffer, pH 7.4, with few drops of H₂O₂; after thorough mixing, it was centrifuged at 3000 rev/min for 15 min and the supernatant was used). The reaction mixture was collected in tubes and the volume was made up to 2 ml by addition of distilled water. The OD values were read in a spectrophotometer at 449 nm.

Results

The results obtained with different types of virus antigen, viz. tongue epithelium, cell culture and mouse antigen, using different concentrations of the antigen and a constant dilution of antiserum (1 : 128) are shown in Fig. 1. With increasing concentration of the virus antigens, the OD values in-

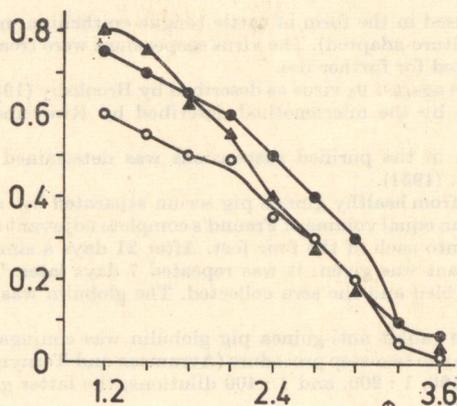


Fig. 2.

Results of MICROELISA with twofold dilutions of antiserum and a constant concentration of virus antigen (1 mg/ml protein). Antigens: as in Fig. 1. Abscissa: antiserum dilution reciprocals (log₁₀); ordinate: OD₄₄₉ values.

creased. The results obtained with twofold dilutions of antiserum with a constant antigen concentration (1 mg/ml) are shown in Fig. 2. The OD value decreased with increasing dilutions of the antiserum. With all types of virus antigen, the antiserum titre obtained by MICROELISA was 2896 while by micro-CF test it was 128. By MICROELISA it was possible to detect antigen in a very low concentration whereas the micro-CF test detected no virus antigen when it was below 1 mg/ml of protein.

Discussion

We standardized the indirect technique of MICROELISA for use in FMD virus research. Almost a linear curve was obtained with increasing concentration of the virus antigen at a particular antiserum dilution. Since the technique was able to detect a very low concentration of the virus antigen, it could be advantageously used for quantification of FMD virus antigen in various preparations, particularly the vaccines manufactured. At a constant concentration of virus antigen, the OD value obtained with different dilutions of antisera varied and gave a trend of a linear curve from which the endpoint titre of antisera could be easily determined. The titre of O₅ antiserum by this technique was 2896 while it was 128 by the micro-CF test. Thus MICROELISA could be a suitable method for determining titres of sera against FMD viruses and may have wide applications. The technique can be applied for subtyping of FMD virus (Rai and Lahiri, 1981).

Though other workers used ELISA for quantification of FMD virus antigen and for measuring the antibody level in cattle sera (Abu Elzein and Crowther, 1978, 1979), they used a larger volume of reagents which may not be advantageous when working with numerous samples. The present technique uses a very low volume of reagents which is both economic and convenient in handling. Moreover, the above workers used type-specific guinea pig globulin-peroxidase conjugate which would require that a conjugate for each type-specific antigen be prepared while the present technique uses a single rabbit anti-guinea pig globulin-peroxidase conjugate which can be used with any of the type-specific antisera and thus makes the test simplified. Though alkaline phosphatase as well as horseradish peroxidase can be used for ELISA, we preferred the latter because it can be used for detecting virus antigens in cell cultures and tissue sections by light microscopy as well as in electron microscopic studies in addition to the ELISA. Such other studies using horseradish peroxidase conjugate are being carried out in this laboratory successfully (Rai and Prasad, 1980) and thus a single conjugate makes possible to carry out a wide spectrum of studies.

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References

- Abu Elzein, E. M. E., and Crowther, J. R. (1978): Enzyme-labelled immunosorbent assay technique in foot-and-mouth disease virus research. *J. Hyg. (Camb.)* **80**, 391—399.
- Abu Elzein, E. M. E., and Crowther, J. R. (1979): The specific detection of foot-and-mouth disease virus whole particle antigen (140 S) — by enzyme labelled immunosorbent assay. *J. Hyg. (Camb.)* **83**, 127—134.
- Avrameas, S. and Ternynck, T. (1971): Peroxidase labelled antibody and Fab conjugates with enhanced intracellular penetration. *Immunochem.* **8**, 1175—1179.
- Brooksby, J. B. (1952): The technique of complement fixation in foot and mouth disease research. *A.R.C. Rep. Ser.* No. 12, H. M. Stationery Office, London.
- Crowther, J. R., and Abu Elzein, E. M. E. (1979): Detection and quantification of foot and mouth disease virus by enzyme labelled immunosorbent assay techniques. *J. gen. Virol.* **42**, 597—602.
- Lowry, C. H., Rosebrough, N. J., Far, A. L., and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265—275.
- Rai, A. and Lahiri, D. K. (1981): Subtyping of foot-and-mouth disease virus by the micro-enzyme-labelled immunosorbent assay (MICROELISA). *Acta virol.* **25**, 53—56.
- Rai, A., and Prasad, I. J. (1980): The immunoperoxidase technique in rapid detection of foot and mouth disease virus. *Ind. J. anim. Sci.* **50**, in press.
- Rweyemanu, M. M., Booth, J. C., Parry, N., and Pay, T. W. F. (1977): Neutralization kinetics studies with type SAT 2 foot and mouth disease virus strains. II. Antigenic differentiation of vaccine strains *J. Hyg. (Camb.)* **78**, 429—438.