Foot-and-Mouth Disease Virus Antibodies: Comparison of a Tissue Culture Microneutralization Test with the Assay in Suckling Mice

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A microneutralization test for foot-and-mouth disease virus antibodies is described and compared to the standard mouse test.

The neutralization of foot-and-mouth disease virus (FMDV) strains in tissue cultures (2, 6) and in mice (1) has been reported. The introduction of microtitration techniques (14) has greatly facilitated similar assays in several other virus systems (3, 7-11, 13, 15). This report describes a microneutralization (MN) assay for titration of FMDV antibodies and compares the results with those obtained in suckling mice.

The FMDV used in this investigation were: FMDV, type A, subtype 12, strain 119 (A-119); FMDV, type A, strain CANEFA-1 (A-1); FMDV, type O, subtype 1, strain CANEFA-2 (O-2); and FMDV, type C, subtype 3, strain Rezende (C-3). All viruses were isolated from infectious bovine tongue epithelium and passed seven to eight times in primary calf kidney cell cultures. Samples of infectious tissue culture fluids were frozen and stored at -70°C.

Antiseras, obtained from 30 Hereford steers used in an FMDV A-1, O-2, and C-3 monovalent and trivalent vaccine trial, were titered by the mouse neutralization assay, and the results were reported by Graves (5). The present study, which used the same set of antisera, provides a direct correlation between the mouse test and the MN test described.

The medium used for diluting serum, virus, and cells for cell growth consisted of equal volumes of Eagle's minimum essential medium and Hanks balanced salt solution, the latter containing 0.5% lactalbumin hydrolysate. The medium contained 4% heat-inactivated bovine serum, 100 units of penicillin per ml, and 200 μg of streptomycin per ml.

Appropriate virus dilutions for infectivity assays and serum-virus mixtures for MN assays were prepared in tubes (typically 2 ml) and incubated as necessary. Two drops from a 1-ml pipette of a cold, uniform suspension of cells containing three to five million cells/ml were added to each tube and to a diluent control. The contents of each tube were then mixed by pipetting, and 0.2-ml samples were distributed into four wells in one row of a microplate (model IS-FB-96-TC, Linbro Chemical Co. Inc., New Haven, Conn.). The plates were incubated at 37°C in a humidified atmosphere containing 5% CO₂. Virus cytopathic effect was determined microscopically, and results were recorded after 48 hr of incubation. Infectivity titers as TCID₅₀/ml and neutralization titers as MN₅₀/ml were calculated by the method of Kärber as described by Finney (4). Additional virus infectivity and serum-neutralizing activity titrations were performed in suckling mice (1), as necessary for comparative purposes.

All of the cell types used regularly in this laboratory proved applicable to the procedure. Plating of 20,000 to 30,000 cells per well with primary bovine thyroid, secondary bovine, or porcine kidney cells and 30,000 to 50,000 cells per well with three cell lines (PK-15, IBRS-2, and BHK-21) resulted in monolayers within 24 to 48 hr of incubation.

However, end points with the primary and secondary cell cultures were often difficult to determine because of clumps of cells rather than clear monolayers in the "uninfected" wells. Also, 72 to 96 hr of incubation was often required to establish an end point. With the cell line cultures, however, end points could be determined at 48 hr. The BHK-21 cell cultures appeared the most suitable.

1This work was conducted during G. G. Wagner’s tenure as a Postdoctoral Resident Research Associate of the Agricultural Research Service and the National Academy of Sciences, National Research Council.
Table 1. Comparison of virus infectivity titers obtained by micro-tissue culture (MTC) and mouse tests

| Tests    | A-1 | O-2 | C-3 | A-119 |
|----------|-----|-----|-----|-------|
| MTC, ID₅₀ | 6.28| 5.68| 6.73| 7.11  |
| Mouse, LD₅₀ | 6.95| 5.83| 6.41| 7.05  |

* Coefficient of correlation equals 0.7435.

Table 2. Median serum microneutralization values of cattle vaccinated against foot-and-mouth disease at time of exposure to virulent virus

| Vaccine  | Virus type | A-1 | O-2 | C-3 |
|----------|------------|-----|-----|-----|
| Monovalent |            | 2.7 | 1.65| 2.24|
| Trivalent |            | 2.0 | 1.65| 2.0 |
| Mean     |            | 1.97| 1.70| 2.31|

* Analysis of variance was determined as follows. When the virus types were the source of variation, the degrees of freedom were 2, the sum of the squares was 1.8290, and the mean square was 0.9145. When the monovalent versus the trivalent vaccine trial was the source of variation, the degree of freedom was 1, the sum of the squares was 0.4539, and the mean square was 0.4539. When the interaction was the source of variation, the degrees of freedom were 2, the sum of the squares was 1.2055, and the mean square was 0.6027. For the residual variation, the degrees of freedom were 24, the sum of the squares was 4.0003, and the mean square was 0.1666. The total degrees of freedom were 29, and the sum of the squares was 7.4887.

The infectivity titer obtained with all viruses tested in BHK-21 cells was invariably similar to the titer obtained by mouse titration. Such comparisons are shown in Table 1. Analysis of the data (12) yielded no significant difference in the titers, with a coefficient of correlation equal to 0.7435.

Serum neutralizing MN₅₀ values and analysis of variation are shown in Table 2. The residual mean square value, a test of the variation in the test itself (12), is 0.1666. This value agrees well with the value of 0.1785 found by Graves (5) in similar analysis of the PD₅₀ values for the identical sera. Thus, there are no significant differences in the 50% end points estimated by the two tests.

The correlation of the end-point titers obtained by both tests is shown in Fig. 1. The MN₅₀ values (Table 1) paired with the PD₅₀ values from Graves (5) are shown plotted, with regression lines for each test. The high degree of correlation between the two tests is noted since the regression lines are almost superimposed (12). The coefficient of correlation is 0.76.

Designed with four well replicates, the MN procedure described is equal in sensitivity, precision, and reproducibility to the “standard” mouse test with eight suckling mice per litter. The titers obtained with three sera, each tested on five separate occasions, yielded a standard deviation (12) of ±0.106 log units. Results are obtained in 48 hr instead of 96 hr. In our laboratory, the economy is on the order of 30 times less expensive. Above all, the use of one cell line minimizes the undefinable “biological variation” in results obtained from animal tests.

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