PLAQUE REDUCTION NEUTRALIZATION TEST FOR THE ASSAY OF ANTIBODIES AGAINST FOOT-AND-MOUTH DISEASE

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BRIEF REPORT

The plaque reduction neutralization (PRN) test for the study of foot-and-mouth disease was described by McVicar *et al.* (4). They used secondary bovine kidney cell cultures grown for 6 days in disposable plastic plates and an overlay of 0.6% Tragacanth® gum (5). Twenty hours after the culture was inoculated with 40-50 plaque-forming-units (PFU) of the virus, the cells were fixed and stained and the results expressed as the log of the reciprocal of the dilution which neutralized 73% of the plaques.

Cultures of BHK-21 C 13 and of IB-RS-2 cells with agar overlay have been used for several years in the routine work at the Pan American Foot-and-Mouth Disease Center (PAFMDC). With the start of epidemiological studies which required testing of large numbers of samples it became necessary to introduce more practical plaque techniques which would give faster results.

For this reason PRN was adapted to our laboratory facilities. IB-RS-2 cells were cultivated in glass Petri-dishes mounted in aluminum trays and locally obtained gums for overlay made it possible to apply this test routinely to epidemiological studies under our conditions.

IB-RS-2 Clone 17 (1) cells were seeded with 1.2 x $10^{6.0}$ cells and grown in Petri-dishes of 15 x 60 mm in 6 ml of modified Eagle medium (2) containing 10% inactivated bovine serum. The Petri-dishes were mounted in aluminum trays specially constructed so that each one could hold 6 bottoms of Petri-dishes or 4 covers (Figs.1 and 2). The cultures were incubated in a humidified atmosphere of 5% CO_2 at 37° C for 48 hours.

The samples under study were previously inactivated at 56° C for 30 min (bovine serum or esophageal-pharyngeal material) and serially diluted in modified Eagle medium containing 28 mM of N-2 hydroxyehtyl-piperazine-N'2 ethane-sulfonic (HEPES). To each dilution an equal quantity of virus suspension was added, so that each mixture contained 50-60 PFU's per 0.1 ml. The virus-sample mixtures were incubated in a water-bath for 30 min at 37° C.

The medium of the cultures was removed and the cultures inoculated with the corresponding virus-sample mixture in a quantity of 0.1 ml per Petri-dish. After inoculation the cells were left for 30 min in a CO_2 incubator at 37° C with occasional redistribution of the inoculum.

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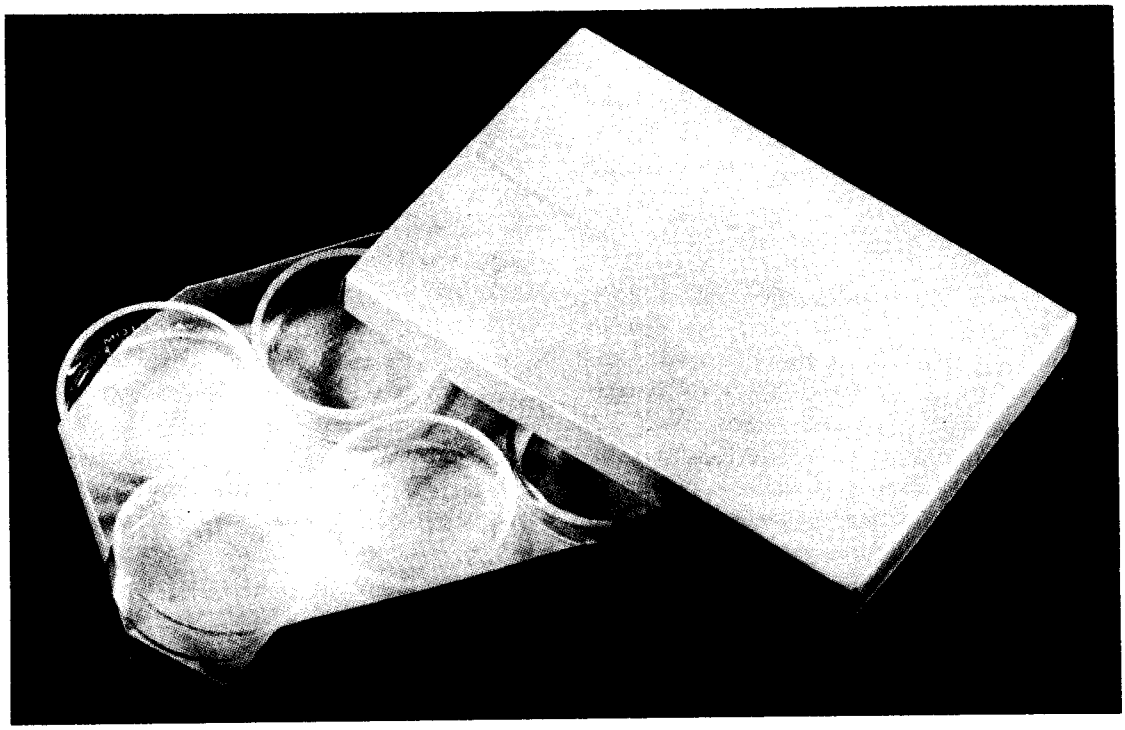

FIGURE 1. Aluminum tray designed to hold 6 Petri dish bottoms.

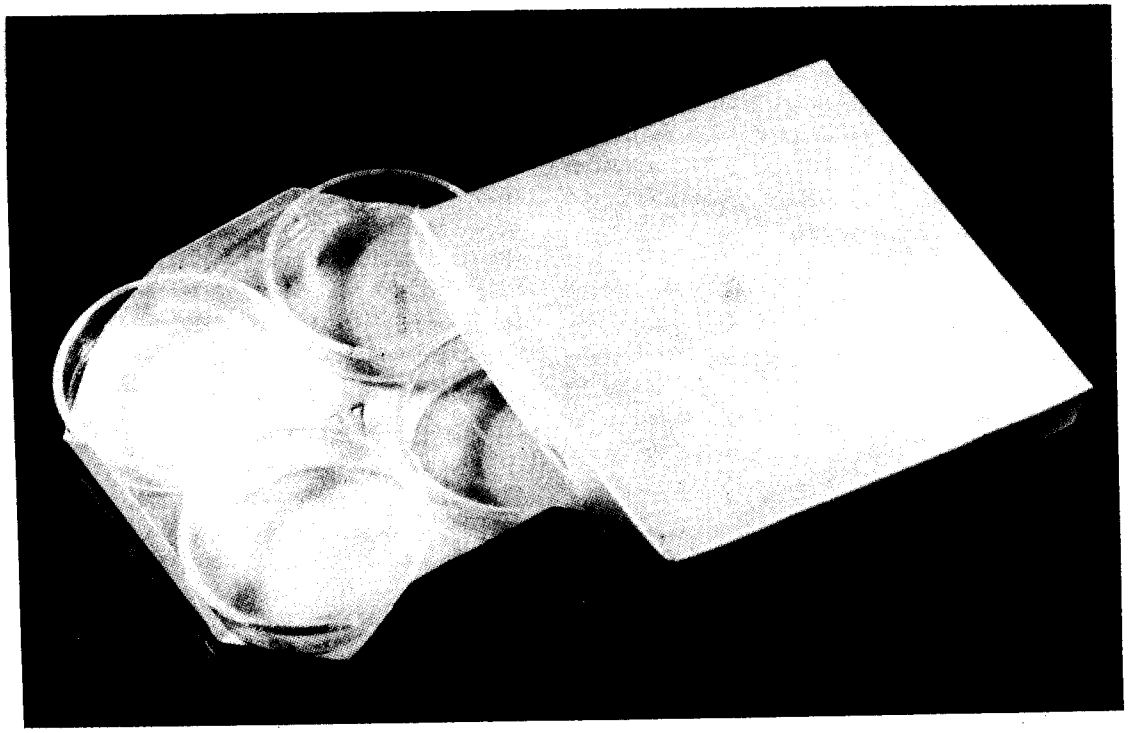

FIGURE 2. Aluminum tray designed to hold 4 Petri dish lids.

Two types of gum were found on the local market: Adraganth* and Karaya** (3). These gums, to be used as overlay, were prepared as follows:

Adraganth gum

Adraganth gum was prepared in a 2.5% (w/v) concentration, dissolving the powder under strong agitation in demineralized water previously heated to 50° C. Once a homogeneous colloidal suspension was formed, 2.5 ml of fenol red at 1% (w/v) per liter of solution were added. The gum was autoclaved at 120° C for 20 min and then stored at 4° C. At the moment of use the gum was pre-heated to 37° C, after adding an equal amount of double concentrated modified Earle's medium with 500 I.U. penicillin, 1 mg of streptomycin and 5.0 mg of fungizon per ml.

Karaya gum

Karaya gum was prepared at 2% (w/v) in demineralized water in the same form as described for the adraganth gum. Just before use, an equal quantity of double concentrated modified Earle's medium with antibiotics added and the pH adjusted to 7.4-7.6 by means of a 7.5% solution of $NaHCO_3$. The addition of approximately 10% of $NaHCO_3$ was required to reach the desirable pH.

The gum (adraganth or karaya) was added in 3 ml quantities to each Petri-dish and then incubated for 24 hours at 37° C in a humidified atmosphere with 5% CO_2. During that period it was necessary not to move the Petri-dishes in order to prevent the formation of "comet" plaques, as seen in Fig. 4, upper right plate.

After incubation, the cell cultures were fixed for 30 min in a solution of 20% formol and rinsed in tap water and stained for 10 min with a violet crystal 0.5% solution in 20% ethyl alcohol in distilled water. The stained solution was recovered and cells washed in tap water.

Plaques were counted and the results expressed as the percentage of neutralization with titers calculated as described by McVicar *et al.* (4): 1) calculation of the percentage of plaque reduction in each dilution of the sample; 2) transformation of the percentages into probits; 3) calculation of the regression lines of the probits; and 4) calculation of the titers of the sample on the basis of 73% plaque reduction. For our test 70% reduction was used.

The results obtained with adraganth and karaya gums in concentrations of 1.25% and 1%, respectively, were similar. There were no signs of toxicity with the IB-RS-2 cells, which always had a healthy appearance and survived longer when compared with those under a standard agar overlay. With IB-RS-2 cells under gum overlay well-defined plaques were formed by FMD virus in only 24 hours (Figs. 3 and 4); this did not occur with the BHK-21 C 13 cells. The virus titers obtained, when compared to agar, were always similar or higher and for that reason we adopted the gum overlay for routine virus titrations.

The use of aluminum trays to mount the Petri-dishes doubled the number of available dishes, simplified the work and permitted better use of the CO_2 incubator's capacity.

With the introduced modifications it was possible to adapt the plaque reduction neutralization test for routine use and for practical and economical titrations of FMD virus plaques readings in 24 hours.

* Adraganth is the same as Tragacanth - gum derived from several species of the plant *Astragalus*.

** Karaya - gum derived from several species of the plant *Sterculia*.

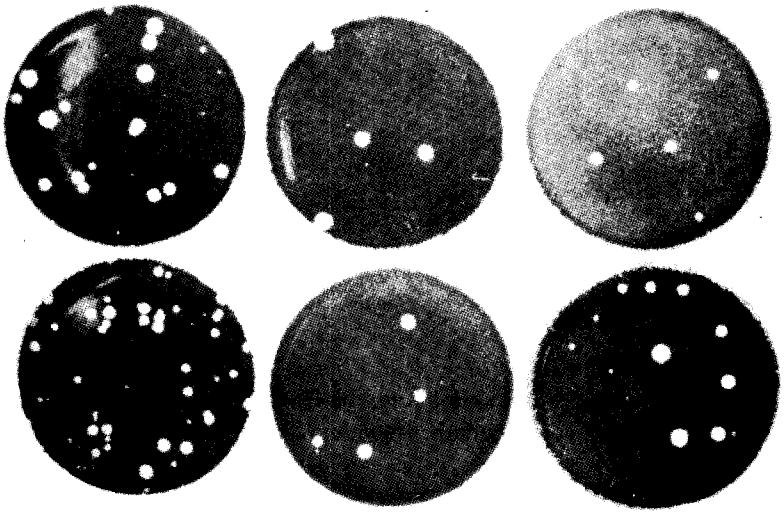

FIGURE 3. Plaques produced by foot-and-mouth disease with type O virus in IB-RS-2 cells with gum overlay after 24 hours incubation.

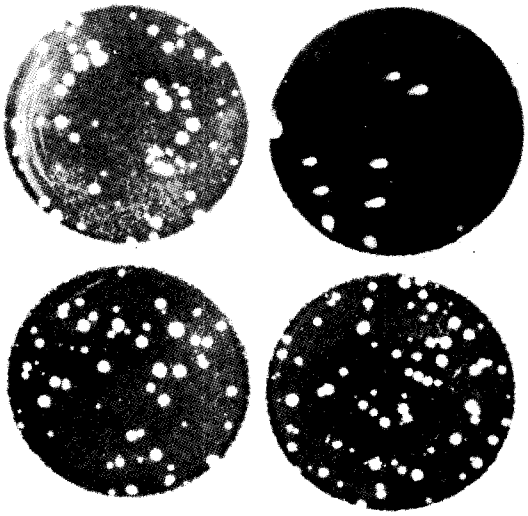

FIGURE 4. Plaques produced by foot-and-mouth disease with type O virus in IB-RS-2 cells with gum overlay after 24 hours incubation; upper right plate shows development of "comets".

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Paul Sutmoller who suggested this technique, and Mr. Pedro J. Vieira for his technical assistance.

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