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Plaque Assay for Polyoma Virus on Primary Mouse Kidney Cell Cultures

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A plaque assay for polyoma virus using primary baby mouse kidney cells is reported.

Methods for the assay of polyoma virus have been reported (1-5) in which plaques have been observed 7 to 10 days after infection in mouse embryo cells. However, a plaque assay for polyoma virus using primary baby mouse kidney cells has not been previously reported.

The procedure described in this communication reveals that reproducible plaque titers are obtained after only 7 days of incubation. Plaque development in the polyoma-mouse kidney system was found to be dependent on the following: (i) the number of mouse kidney cells seeded, (ii) age of the monolayer prior to infection, (iii) species of serum used in overlay, and (iv) volume of overlay.

Mouse kidney cells were prepared from 10-day-old Swiss albino CR1 mice. Excised kidneys were repeatedly trypsinized in 0.2% trypsin (Difco, Trypsin 1-250) in isotonic buffer (NaCl, 0.136 M; KH_2PO_4, 10^{-4} M; Na_2HPO_4, 5×10^{-4} M; KCl, 4×10^{-3} M; 0.015% phenol red; 500 U of penicillin G/ml, and 100 μg of streptomycin sulfate per ml, at pH 7.5). The mouse kidney cells were suspended in modified Dulbecco medium (6) containing the following: sodium pyruvate, 0.018 M; glucose, 2×10^{-3} M; and $MgSO_4 \cdot 7H_2O$, 8×10^{-4} M, thereafter referred to as culture medium, plus 10% calf serum (Microbiological Associates).

The trypsinized mouse kidney cells were enumerated by counting nuclei (nuclei stain—a filtered solution of 0.05% crystal violet and 0.25 M citric acid) in a hemocytometer. The cells were diluted with culture medium to a concentration of 1.7×10^6 cells/ml. Plastic petri dishes (Falcon, 60 by 15 mm) were each seeded with 5 ml of culture medium containing the diluted cells (8.5×10^6 cells per dish) and incubated at 37 C in a humidified air-CO_2 atmosphere. Mouse kidney cultures were found most suitable for plaquing 72 h after initial seeding. The cultures were washed once with isotonic buffer (omitting phenol red and containing 0.025 M tris(hydroxymethyl)aminomethane to remove extraneous cellular debris. Then, 0.2 ml of a suspension of wild-type polyoma virus was added and the virus was allowed to adsorb for 60 min at 37 C. All dilutions of virus were made with serum-free culture medium. The cultures were then overlaid with 10 ml of a solution containing culture medium with 0.9% agar (Difco, purified), 0.025% fresh glutamine, and 10% horse serum (Microbiological Associates).

After incubation for 6 days at 37 C, 3 ml of the agar-culture medium containing neutral red (0.01%) was added to each dish. The plaques were counted after overnight incubation at 37 C; they appeared as clear areas, 1 to 3.5 mm in diameter. It is of interest to note that this plaque procedure will produce visible plaques as early as 4 to 5 days after infection. However, when cultures are stained this early the plaques produced by the small-plaque polyoma mutants can not yet be observed. Thus, we routinely added the overlay stain on day 6 after infection to quantitate both the large and small polyoma plaque types.

Under the above conditions, a polyoma stock virus preparation yielded the same titer, $2.03 \pm 0.94 \times 10^8$ plaque-forming units/ml, over a 7-month period. A representative plaque assay is shown in Fig. 1.

There are several advantages in using mouse kidney cell cultures for plaquing polyoma virus. It allows the investigator currently using the polyoma-mouse kidney system for biochemical and genetic studies to utilize the same host cell type to quantitate polyoma virus. Furthermore, this method provides a rapid and dependable technique for plaquing polyoma virus.

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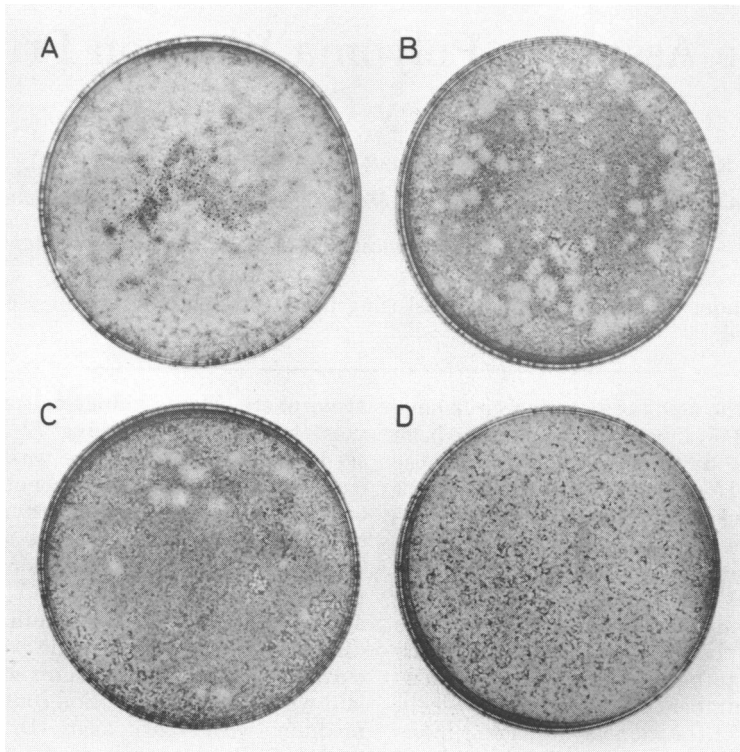

FIG. 1. *Representative plaque assay of wild-type polyoma virus on primary mouse kidney cells. Medium-stain overlay was added 6 days after infection. A, Polyoma virus stock diluted to 10^{-5}, plaques TNTC; B, polyoma virus stock diluted to 10^{-6}, 93 plaques expressed; C, polyoma virus stock diluted to 10^{-7}, 13 plaques expressed; D, uninfected mouse kidney cells.*

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