

Miniculture Plaquing Technique for Mouse Cytomegalovirus

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A method for plaquing mouse cytomegalovirus under agar in miniature cultures is described. Maximal virus adsorption was found to take approximately 5 hr.

Two methods for plaquing mouse cytomegalovirus (MCMV) have been described, one employing starch (2) and the other agar (1) as the overlay. Because MCMV produces only microscopic plaques in each system, however, a convenient miniature culture system seemed desirable to reduce the overlay area and thus the plaque counting time. This report describes an agar-plaquing method employing reusable Pyrex rings on slides and a study of MCMV cell adsorption time.

Primary mouse embryo tissue culture (METC; reference 2) was prepared in 75-cm² plastic tissue culture flasks. Cells from confluent primary cultures were dispersed with 0.25% trypsin, washed and resuspended in growth medium [medium 199, NaHCO₃ to achieve pH 7.2, 10% inactivated calf serum (CS), and antibiotics] at a concentration of 500,000 cells per ml. To produce miniature cultures, sterile Pyrex rings (6 by 11 mm inside diameter) were heated, touched to sterile petroleum jelly, and placed on washed sterile slides in sterile 100-mm petri dishes. Three or four ring cultures were placed on each slide (Fig. 1). The resulting chambers were completely filled with approximately 0.5 ml of METC cell suspension. Cultures were incubated in a 37 C humidified CO₂ incubator for 24 hr when monolayers covered the slide surface.

The Smith strain of MCMV which had been passaged approximately 80 times in mice was obtained from Donald Medearis of Pittsburgh, Pa. Virus stock was a 10% clarified suspension of infected mouse salivary gland in Hanks solution with 25% sorbital. It contained 10^{5.2} tissue culture doses 50% effective (TCID₅₀) per 0.1 ml. Virus dilutions were made with cold Hanks solution containing 5% CS.

To produce MCMV plaques, media were aspirated and each culture was infected with approximately 100 TCID₅₀ of virus in 0.1-ml

volumes. After 2 hr of incubation at 37 C, maintenance medium (growth medium but with 2% CS) was added and incubation was continued for an additional 22 hr. The medium was aspirated and approximately 0.3 ml of agar overlay was added. Incubation was continued for 72 hr when cultures were again overlaid with 0.2 ml of agar overlay containing 10% neutral red (1:10,000).

The agar overlay was made by combining 25 ml of melted agar solution (3% agar in sterile water), 22 ml of distilled water, 2 ml of 5% NaHCO₃, 1 ml of antibiotic solution, and 50 ml

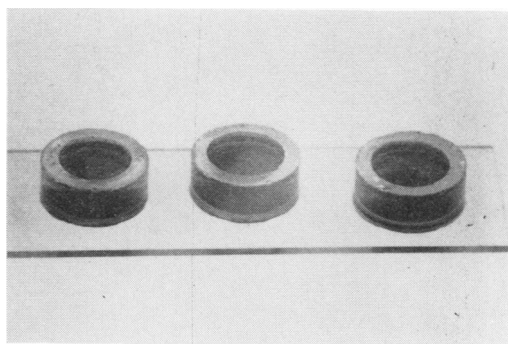


FIG. 1. Standard microscopic slide (25 by 75 mm) with three ring cultures.

of overlay medium. The overlay media contained 1% lactalbumin hydrolysate, 0.2% yeast extract, 0.2% bovine albumin fraction V, 20% Earles balanced salt solution (10X), 1% phenol red (1:1,000), and 77.5% deionized water. Inactivated fetal calf serum (2%) was added to complete the overlay.

Plaques were counted with a dissecting microscope 8 or 24 hr after the second agar overlay. Cultures were placed over a transparent lined grid of 1-mm squares which allowed accurate,

TABLE 1. *Plaque counts after timed mouse cytomegalovirus adsorption*

Time (hr)	Plaque counts for culture						Avg
	1	2	3	4	5	6	
1	20	11	0	10	6	0	7.9
3	30	30	20	10	6	16	18.6
5	30	40	40	30	60	30	38.3
7	120	70	60	48	90	70	76.3
24	110	60	70	64	98	112	85.6

rapid counts of over 200 plaques per culture. To avoid the toxic effects of agar to unadsorbed MCMV (2, 3), the first overlay was delayed until virus adsorption was complete.

To determine maximal virus adsorption time, cultures were infected, incubated for 1 hr, and then supplied with media. At 1, 3, 5, 7, and 24 hr after inoculation, groups of six cultures were washed three times with Hanks solution. Twenty-four hours after inoculation, the first agar overlay was applied followed 72 hr later by the second. Table 1 shows that maximal virus adsorption occurred after 5 hr but before 7 hr, with little change in plaque counts in the 7- and 24-hr cultures.

The described method may be employed for virus titrations as well as plaque-reduction neutralization antibody assays. By placing three or four cultures on a slide, a virus or serum dilution can be assayed on a single slide. Plaques can be counted rapidly by scanning across the cultures on a slide. The components are economical and reusable. An alternative miniaturized plaquing technique has been described by Webb et al. which uses disposable plastic trays (4).

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