

Semi-Micro XC Cell Assay Technique for Murine Leukemia Virus

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The XC cell assay employed in *in vitro* titration of murine leukemia viruses was modified for use as a semi-micro procedure.

The murine leukemia viruses (Mulv) which are noncytopathic in cell culture have eluded quantitation by plaque assay methods. Consequently, serological and bioassay methods have been used to determine their growth in cell culture. The XC cell plaque assay developed by Rowe et al. (5) provides an excellent *in vitro* method for assay of Mulv that is as sensitive as the COMUL or immunofluorescence assays. This report concerns the modification of the XC cell assay for use in a semi-micro procedure to facilitate the rapid assay of multiple virus samples.

National Institutes of Health (NIH) Swiss mouse embryo fibroblast (MEF) cells and the XC cell line, provided by Wallace P. Rowe, NIH, Bethesda, Md., were grown in Eagle's minimal essential medium with Earle's salts, 1% L-glutamine (200 mM), penicillin (100 units/ml), streptomycin (100 μ g/ml), and 10% fetal calf serum.

The procedure for the semi-micro XC assay for Mulv is similar to the XC assay procedure described by Rowe et al. (5). Briefly, wells in Microtest II tissue culture plates were seeded with MEF cells and incubated at 37 C for 24 hr in 5% CO₂ in air. The cell cultures were infected with 0.1 ml of virus and incubated for 24 hr. A 0.1-ml amount of growth media was then added to each well, and the cultures were incubated for an additional 5 days. After 6 days of total incubation, the cultures were treated with ultraviolet (UV) light, and a 0.2-ml volume of XC cell suspension was added. Four days after the addition of XC cells, the cultures were fixed and stained as described by Rowe et al. (5). The cultures were scored microscopically for the presence or absence of multinucleated giant cells. The 50% infectious dose (ID₅₀) of Mulv was estimated by the method of Reed and Muench (4).

Factors considered in adapting the XC assay to microplates were (i) the seeding densities of MEF and XC cells and (ii) the intensity of UV exposure of virus-infected MEF cells. Infection of semiconfluent monolayers of MEF cells with Mulv has been shown to result in higher titers than Mulv titrated on confluent monolayers of MEF cells (1, 3). In addition, Rowe et al. showed that the density of XC cells and the dose of UV used to treat Mulv-infected cultures were critical factors in achieving the highest titer for Mulv by the XC cell plaque assay (5). Therefore, the optimum cell densities as well as UV dosage were determined to ensure expression of maximum titer of Mulv in the semi-micro XC assay.

Table 1 shows that titers of Moloney leukemia virus (MLV) obtained in microplates did not appear to differ when the cell densities employed varied from 1.6×10^3 to 6.3×10^3 MEF cells per microwell and from 4.5×10^3 to 9.0×10^4 XC cells per microwell. However, 3.15×10^4 MEF cells per well yielded lower titers of MLV. Although MLV titer appeared to be independent of MEF and XC cell densities over a broad range, the XC cell response was most clearly demonstrated with the lowest cell densities tested. Therefore, 1.6×10^3 MEF cells and 4.5×10^3 XC cells were used in the microwells for subsequent experiments.

The UV dose resulting in the highest observed MLV titer in microplates was considered the optimum UV exposure and was 60 ergs per mm² per sec for 30 sec (Table 2). From four determinations at 60 ergs per mm² per sec, the 95% confidence interval for the MLV titer was $5.87 \leq \mu \leq 6.18$. With UV intensities of 45, 75, or 90 ergs per mm² per sec for 30 sec, MLV titers all fell below this range.

Pools of MLV, Rauscher leukemia virus (RLV), Friend leukemia virus (FLV), and

TABLE 1. Effect of MEF and XC cell density on the titer of MLV in microplates employing the XC assay

MEF cell density/well ^a	Titer of MLV (log ₁₀ ID ₅₀ /ml) ^b			
	4.5 × 10 ³ ^c	9.0 × 10 ³	1.8 × 10 ⁴	9.0 × 10 ⁴
1.6 × 10 ³	6.1	6.0	6.0	5.8
3.15 × 10 ³	6.1	5.8	5.6	5.9
6.3 × 10 ³	5.8	5.7	5.8	6.0
3.15 × 10 ⁴	5.3	4.8	4.8	5.1

^a Various cell densities of MEF cells was dispersed in 0.2-ml volumes. Microplate wells of MEF cells infected with MLV according to the procedure described in the text of this communication were treated with UV (60 ergs per sec per mm² for 30 sec) 6 days after infection.

^b Three wells were infected with each dilution of virus. The absence or presence of XC syncytium was recorded, and ID₅₀ titer was determined.

^c XC cell density per well. XC cells were delivered to each well in 0.2-ml volume.

TABLE 2. Effect of UV dose on the titer of MLV in MEF cells as determined by the XC micro-titer assay

Intensity of UV (ergs per mm ² per sec) ^a	Titer of MLV (log ₁₀ ID ₅₀ /ml) ^b
45.0	5.5
60.0	6.0 ^c
75.0	5.7
90.0	5.4

^a Applied for 30 sec to MLV-infected MEF cells. Wells of the microplates were seeded with 1.6 × 10³ MEF cells and 4.5 × 10³ XC cells after UV treatment.

^b See footnote b, Table 1.

^c Average titer, standard error (±0.05), and a 95% confidence interval of 5.87 ≤ μ ≤ 6.18 were based on four determinations. The confidence interval was determined by a procedure previously described (6).

Gross leukemia virus (GLV) were titered by the semi-micro XC assay technique (Table 3). Variance of the titers measured by standard error was greatest for RLV and GLV, although from the sampling size the standard error was not statistically significant. Titers of all four Mulv were observed to be 0.7 to 1.6 logs lower than those exhibited by the same viruses in the macro-XC plaque assay (see footnote a, Table 3). This reduced sensitivity of the semi-

TABLE 3. Titers of murine leukemia viruses determined by the semi-micro XC cell assay technique

Virus ^a	Virus titer (log ₁₀ ID ₅₀ /ml) in microplates	
	Multiple assays	Average titer ± SE ^b
MLV	5.9, 6.0, 6.1, 6.1	6.02 ± 0.05
RLV	5.0, 5.3, 4.8, 4.9	5.00 ± 0.11
FLV	4.9, 4.9	4.9
GLV	4.3, 4.0	4.15 ± 0.15

^a MLV, RLV, FLV, and GLV show a titer of 10^{6.7}, 10^{6.4}, 10^{6.0}, and 10^{5.8} plaque-forming units/ml when titrated on MEF cultures in 60-mm plastic culture plates by the XC plaque assay.

^b Standard error (SE) was determined by the method previously described (6).

micro XC assay for Mulv could be due to (i) a reduced efficiency of Mulv absorption in microwell cultures, (ii) the greater quantitative sensitivity of plaque assay procedures as compared to ID₅₀ procedures for enumeration of infectious virus, or to both. Preliminary results indicated that diethylaminoethyl dextran treatment of MEF cells before Mulv infection had no significant effect on titer of the virus in the semi-micro XC assay. The present semi-micro XC assay nevertheless provides a rapid and reproducible in vitro method for titration of multiple samples of Mulv and their neutralizing antibodies.

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