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An Improved Method for Enumeration of X-C Cell Assay for Murine Leukemia Virus

GERARD J. SPAHN,[1] ROBERT M. NIMS, ROBERT L. PETERS, AND KATHRYN KENYON
Microbiological Associates, Inc., Walkersville, Maryland 21793

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A modification for the enumeration of X-C syncytia is described wherein the projected image of the entire infected culture is observed. This method is rapid, reliable, and reproducible.

The X-C test has proven a rapid and accurate means for the in vitro quantitation of infectious C-type ribonucleic acid murine leukemia oncornavirus (MuLV) (2, 3). Recently, the semimicro test for enumeration of X-C-induced plaques was described (1). These recent modifications have increased the use of the X-C test as a diagnostic tool for quantitation or neutralization, or both, of infectious MuLV, and chemotherapeutic enhancement or inhibition of MuLV infection. This report describes a modification which permits rapid, accurate, and efficient enumeration of syncytia by means of an enlarged, projected image.

The X-C tests are prepared on 60-mm grid dishes (Lux Scientific Corp., Thousand Oaks, Calif.) by infecting secondary mouse embryo cultures with MuLV, treating with ultraviolet irradiation, overlaying with X-C cells, and then fixing and staining as previously described (3). Quantitation consists of counting all syncytia on the plate. Use of a microscope (×10 objective) requires multiple linear scans to observe and count all grids on the plate.

Use of the X-C test in this laboratory requires the preparation and reading of 300 to 400 plates each week. Trained technicians using a microscope can count about 10 plates per hr with good reproducibility. Accurate counting, however, is a laborious process with eye fatigue and boredom leading to decreasing accuracy after about 2 hr of continuous counting.

The volume of testing in this laboratory and the resultant amount of technician time spent in counting the plates led to a search for an improved means of quantitation.

The image of the stained plates can be visualized using a 3.25- by 4-inch (ca. 8.26 by 10.2 cm) lantern slide projector (Charles Beseler Co., East Orange, N.J.) (Fig. 1). The projected image, at a radius of about 28 inches (ca. 71.1 cm), has a quality of resolution comparable to a lantern slide image of the same magnification. A piece of ⅛ inch (ca. 0.3 cm) plywood was cut 3.25 by 4 inches, with a centered circular hole in which the 60-mm grid plate fit snugly. In the initial application, the side of the grid plate was removed with a small circular saw to allow the grid plate to pass through the slide carrier. The altered grid plate was placed in the plywood blank and then inserted into the slide carrier for projection. After the concept of projection proved feasible, the width of the slide carrier and its aperature in the projector was increased 3/16 inch (ca. 0.48 cm) to allow an unaltered grid plate to be inserted for projection (Fig. 2).

Advantages of the slide projector over a microscope are several. (i) Since the entire plate can be viewed, linear scanning is not required for counting. This results in reduced counting time and subjects the technician to less eye strain. More than 60 plates per hr can be counted, compared to 10 per hr by microscope. (ii) A series of five plates with varying numbers of syncytia were counted by five different technicians. Although variation was greater as syncytia per plate increased, both systems proved comparably accurate (Table 1).

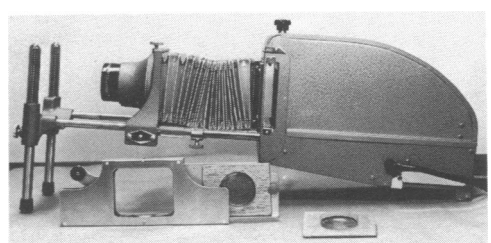

FIG. 1. *Lantern slide projector for enumeration of X-C syncytia.*

[1] Present address: Frederick Cancer Research Center, Ft. Detrick, Frederick, Md. 21701.

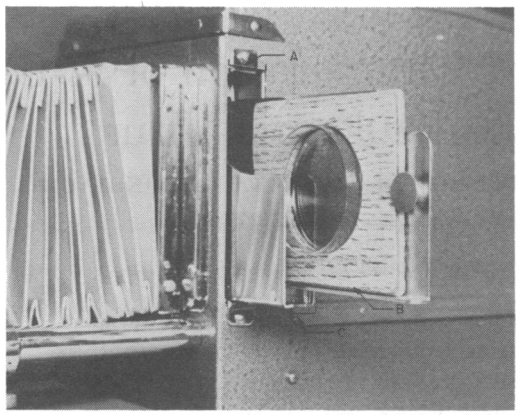

FIG. 2. *Modified lantern slide projector; A, enlarged slide carrier aperature; B, plywood grid plate holder with centered hold; C, enlarged slide carrier in place.*

TABLE 1. Comparison of microscopic to projected observation of X-C-treated plates

Plate no.	No. of syncytia observed			
	Microscopic		Projection	
	Mean	Range	Mean	Range
1	113	102–118	110	99–123
2	58	53–63	58	55–63
3	61	53–69	63	57–66
4	>250	>250	>250	>250
5	146	132–154	142	131–149

In our test system, attempts to infect secondary mouse embryo cells with 100 syncytia-forming units is standard procedure (3). (iii) The ability to view the entire plate simplifies counting a single plaque which covers many grids, a difficult and error-prone task by microscope. (iv) The technique has proven equally effective for counting of foci using murine sarcoma virus-infected 3T3 cells (4). The infected cultures, when fixed and stained, have distinct foci which are easily visualized. With the projected image, it is possible to identify satellite foci directly and avoid their erroneous incorporation as part of the total count.

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LITERATURE CITED

1. Bass, L. R., and W. Turner. 1972. Semi-micro X-C cell assay technique for murine leukemia virus. Appl. Microbiol. **23**:200–201.
2. Klement, V., W. P. Rowe, J. W. Hartley, and W. E. Pugh. 1969. Mixed culture cytopathogenicity: a new test for growth of murine leukemia viruses in tissue culture. Proc. Nat. Acad. Sci. U.S.A. **63**:753–758.
3. Rowe, W. P., W. E. Pugh, and J. W. Hartley. 1970. Plaque assay techniques for murine leukemia viruses. Virology **42**:1136–1139.
4. Todaro, G. J., and S. A. Aaronson. 1969. Properties of clonal lines of murine sarcoma virus transformed BALB/3T3 cells. Virology **38**:174–179.