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An Improved Method for Titration of Mouse Hepatitis Virus Type 3 in a Mouse Cell Culture

Brief Report

By

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With 1 Figure

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Summary

Plaque assay in DBT cells with DEAE-dextran and trypsin presents a titration system for MHV 3 as sensitive as the LD_{50} assay in mice.

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Plaque assay of mouse hepatitis virus type 3 (MHV 3), a member of the genus coronavirus (7), was carried out in mouse embryo cells (3) as well as microfocus assay in mouse macrophages (5). However these methods were either not so sensitive as the LD_{50} assay in mice or else not convenient for routine use. In this paper we describe an improved method for the titration of MHV 3 in the same continuous cell-line that makes the growth of MHV 2 possible (4).

The cell-line SR-CDF 1-DBT (DBT), originating from a mouse brain tumor, was kindly supplied by Dr. Hirano (Institute of Medical Sciences, University of Tokyo, Japan). DBT cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10 per cent calf serum (CS) and 10 per cent tryptose phosphate broth (TPB).

When DBT-monolayers were infected with MHV 3 (American Type Collection Strain previously submitted to three cycles of plaque purification in our laboratory), the formation of syncytia became visible at 6 hours postinfection (p.i.) and increased in both size and number parallel to the virus titer. Plaque assays were performed on DBT-monolayers in 3.5 cm-diameter petri dishes, following the method described by Hirano *et al.* (4). In DBT cells overlaid with MEM containing 1 per cent Noble agar, 5 per cent CS, 10 per cent TPB, and 1:10,000 neutral red, MHV 3-plaques of 0.6—1.4 mm (mean value = 0.9 mm) in diameter could be counted at 40 hours p.i. The plaques were clearly-defined and sometimes contained a deeply-stained area at their center. Comparative titrations showed that

the plaque assay gave titers lower by about half a log than did the LD_{50} method in mice (Table 1).

Table 1. *Comparative assay of MHV 3 by plaque formation on DBT-monolayers and LD_{50} in mice*

Virus	PFU/ml	LD_{50}/ml	PFU/LD_{50}
Sample 1	$10^{4.4}$	$10^{5.2}$	$10^{-0.8}$
Sample 2	$10^{4.5}$	$10^{3.7}$	$10^{0.8}$
Sample 3	$10^{4.5}$	$10^{4.7}$	$10^{-0.2}$
Sample 4	$10^{3.9}$	$10^{5.3}$	$10^{-1.4}$
Sample 5	$10^{5.0}$ ($10^{5.6}$)[a]	$10^{5.4}$	$10^{-0.4}$

[a] PFU obtained when a 50 μg/ml dose of DEAE-dextran was given to DBT-monolayers with virus suspension

To improve the sensitivity of the plaque assay we examined the effect of diethylaminoethyl-dextran (DEAE-D) on the plaque formation and plaque diameter of MHV3, as BRADBURNE and TYRRELL had reported that DEAE-D added to overlay agar increased the plaque number of human coronavirus (2). When DEAE-D was added to the overlay medium, no significant increase in the mean plaque number was produced compared with that of cultures overlaid with a DEAE-D-free medium. When DEAE-D was given along with the virus suspension to DBT cells, a clear-cut increase in the plaque number was found (Fig. 1). The most remarkable increase was observed in those cells which received a 50 μg/ml dose of DEAE-D. With a 25 μg/ml dose of DEAE-D the mean plaque number was still 4 times greater than that of the control. With regard to the plaque diameter,

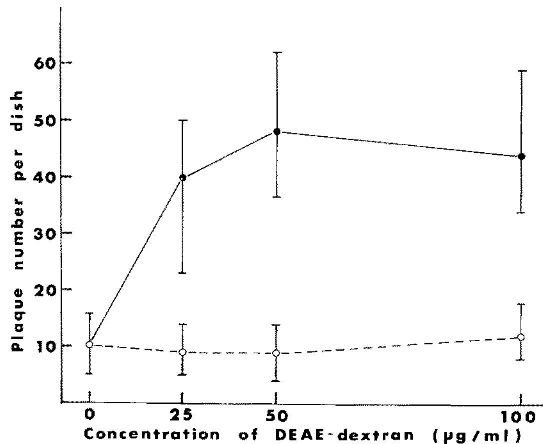

Fig. 1. Effect of DEAE-dextran on the plaque formation of MHV 3
DEAE-dextran was added to DBT-monolayers at the same time as the virus inoculum; after adsorption the cells received the agar medium without DEAE-dextran (•). DBT-monolayers were infected with MHV 3 in the absence of DEAE-dextran; DEAE-dextran was added to the agar overlay (○). Open and filled circles show the mean plaque numbers and vertical lines illustrate the range of plaque numbers

no significant difference was found between the control group and any of the treated groups.

The effect of trypsin on MHV3-plaque formation in DBT-monolayers was also investigated. After infection, DBT cells were overlaid with MEM containing 1 per cent Noble agar and trypsin at a concentration of 5, 10 or 25 μg/ml. The DBT-dishes were incubated at 37° C for 24 hours and plaques were stained with a second overlay medium consisting of MEM with 1 per cent Noble agar, 5 per cent CS, 10 per cent TPB and 1:10,000 neutral red at 37° C for 16—18 hours. Whereas an enhancing effect on the plaque formation of myxoviruses has been reported (1, 6), no significant increase in the mean plaque number of MHV3 was observed. However, the plaques were larger (mean value = 1.4 mm in diameter; range = 0.6—2.3 mm) and clearer than those in the control group.

The sensitivity of the plaque assay in DBT-monolayers was equal to that of the LD_{50} assay in mice when 50 μg/ml of DEAE-D were added to the virus dilutions and the plaques could be counted without difficulty when the overlay medium contained 25 μg/ml of trypsin (Table 1). As DBT is an established cell-line, it is far less troublesome in handling than mouse macrophages and, through the plaque assay on DBT cells, virus titers are determined in a shorter period of time than through the LD_{50} assay in mice which is widely used as a titration method of MHV3 today. Consequently, the assay method of MHV3 described above can advantageously replace not only plaque assay on mouse embryo cells or mouse macrophages but also the LD_{50} assay in mice.

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