

## RABIES VIRUS IN McCOY CELL LINE: PART II - Titration

Yeda L. NOGUEIRA  
Claudia F. C. do AMARAL

RIALA 6/722

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**ABSTRACT:** The McCoy cell line has showed high sensitivity to rabies virus. This cell line presents cytopathic effect (CPE), when infected with rabies virus. Three strains of rabies virus were tested: CVS, ERA, and PV1 and three street virus were isolated: one from a patient bitten by bat, other from a rabid dog and another from bovine. This cell line was used for virus titration purposes due to the presence of cytopathic effect, two techniques for observation of the CPE were used and were compared to the classical method of intracerebral inoculation in mice.

**DESCRIPTORS:** *McCoy cell line. Rabies Virus, (CVS, ERA, PV1 and street virus). Isolation, titration and cytopathic effect (CPE).*

### INTRODUCTION

Several authors studied a series of methods for antibody or virus titration using microtechniques for rabies virus detection. Blancou *et al*<sup>1</sup> compared four techniques to determine the levels of antibody against rabies virus (Rapid Fluorescence Focus Inhibition Test; Plaque Reduction Test; Immunoenzymatic Test) and compared all of them with Mouse Neutralization Test Habel Test as reference<sup>2</sup>.

Perrin *et alii*<sup>6</sup> used rapid enzyme immunodiagnosis (RREID) as a simple and rapid diagnosis method for detection of rabies virus in the laboratory routine, despite the necessary of a specific conjugate with peroxidase.

Webster<sup>11</sup> used the infection of tissue culture technique (a suspension of murine neuroblastom cells) using a multi-plate of 96 wells and the infection was put in evidence with immunofluorescent technique. This technique proved to be equivalent to the mouse inoculation method.

Rudd *et al*<sup>18</sup> used the murine neuroblastom cell line (C-1300; clone NA) for isolation of small quantities of street virus. Thus it was assumed by Wiktor *et al*<sup>12</sup> the slow adaptation of rabies virus into tissue

culture. In 1978, Smith *et alii*<sup>10</sup> showed the easy adaptation of rabies virus to cell line CER (murine neuroblastom) and compared it to the infection with BHK-21, clone 13, sensitized with DEAE - dextran and both cell lines showed efficiency for isolation of rabies virus *in vitro*.

Other group of authors, Mannen *et alii*<sup>4</sup>, used the microtechnique based on an enzyme immunoassay similar to the fluorescent focus inhibition assay, but they used enzyme assay method (RAMIN), that is an automatized method which permits a rapid test of many samples and the results were similar to the rapid fluorescent inhibition test (REFIT).

However, Heberling *et alii*<sup>3</sup> developed a new assay based on Dot Immunobinding Assay (DIA) for the routine detection of antibody to various viruses including rabies virus.

Following the observation from rabies virus replication and notorious cytopathic effect (CPE) caused by this virus in the McCoy cell line<sup>5</sup>, this cell line was used for titration of rabies virus. This cellular lineage was used by Nogueira<sup>5</sup> for the isolation of rabies virus from material of a suspected patient bitten by bat as well as to show the easy adaptation of vaccines (ERA, PV1 and CVS) of rabies virus.

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In this paper, the titration techniques were performed by observation of CPE *in loco* and using Karaya gum (*Sterculia tree*) as overlay for titration of rabies virus in McCoy cell line. Results obtained with both methods are shown and compared to the classical titration using intracerebral mice inoculation.

## MATERIAL AND METHODS

### Cell Culture

A cellular suspension of McCoy Cell line ( $4 \times 10^{5.0}$  cell/ml) grew in Eagle's medium supplied with 5% of foetal calf serum (FCS). Before inoculation mc virus was suspended in Eagle's medium supplied with 2% of FCS.

### Virus

Rabies virus used was the ERA strain (vaccinal origin) adapted in McCoy cell line by successive passages. The 6<sup>th</sup> passage which titre was  $10^{6.2}$  LD<sub>50</sub>/0.03 ml was inoculated intracerebrally in baby mice and the end-point was calculated by the Reed-Muench method<sup>9</sup>.

### Observing CPE *in loco*

#### TITRATION

Ten-fold dilution of the virus was prepared for titration in Eagle's medium plus 2% of FCS, 0.5 ml of each dilution were inoculated in each well of multi-well plates Limbro Flow, with 24 wells.

The plates were incubated with 5% of CO<sub>2</sub> at 33°C, during 30 minutes for virus adsorption. Then each well was completed with 1.5 ml of maintenance medium (Eagle's plus 2% FCS), and again incubated at 33°C. CPE was observed daily, and highest dilution of virus causing CPE (++) in 50% of wells was considered the titration end-point.

### Observing CPE Using Karaya Gum as Overlay

#### CELL CULTURES

The same cellular suspension of  $4.0 \times 10^{5.0}$  cells/ml was used in each well, on the multi-well plates (24 wells) with Eagle's medium plus 5% of FCS for cellular growth.

#### VIRUS

It was used 0.2 ml of inocula of the same dilution of the 6th passage of ERA strain in McCoy cell line already referred to.

Overlay Karaya gum used was from Sigma Labs. It was prepared to 2% (p/v) in deionnized water, at the moment of use equal volume of Earle's solution twice concentrated and also antibiotic/penicilin/streptomycin (500 U.L./mg) were added. The pH was adjusted to 7.4-7.6 with NaHO<sub>3</sub> 7.5%.

#### PROCEDURE

After the incubation period of 48 hours, the cells were fixed during 30 minutes with solution of formalin 20% in alcoholic solution 20%, the plates were washed in distilled water and stained with

alcoholic solution 20% and violet crystal 0.5%, again the plates were washed and dried.

A unique large plaque was evidenced with different kinds of stain, the cells showed CPE and there was a great difference in the morphology of the cells in different stains.

Forty-eight hours were necessary for the occurrence of CPE and the end-point considered was the highest dilution were 50% of the area was affected in 50% of the wells, figures not shown.

### Titration in Baby Mice

Five-to-nine-day-old Swiss-Webster baby-mice were used. The virus titration followed the same dilutions of the 6th passage of ERA strain already referred to. The animals were inoculated intracerebrally with 0.03 ml of each dilution ten-fold performed.

Control animals with the same age were inoculated intracerebrally with 0.03 ml of sterile saline solution, and observed during twenty-one days.

## RESULTS

Preliminary studies showed the CPE caused in McCoy cell line by rabies virus (Nogueira<sup>5</sup>), this cellular lineage is now use for titration purposes of rabies virus.

Figure 1 shows the observation of CPE directly on the bottle of tissue culture. The end point was obtained with 50% (++) of infectivity at dilution  $10^{-8}$  (in 50% of wells) at the 12<sup>th</sup> day. This figure shows the curve of the increase in infectivity during the days of observation.

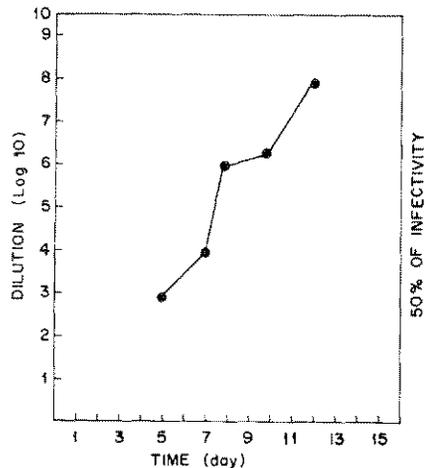


FIGURE 1  
Percentage of Infection Observed With The Presence of Cytopathic Effect During the Observation Days.

For each dilution only one point was plotted (50% of infection).

The percentage of infectivity was obtained from the percentage of CPE observed in the microscope field (+ = 25%, ++ = 50%, +++ = 75%, ++++ = 100%) in four wells for each dilution according to the Reed-Muench method<sup>9</sup>.

When Karaya gum was used as overlay the rapid action of virus in the cell monolayer was observed and 48 hours later the presence of CPE was also observed. Figure 2 shows the difference of cell mor-

phology caused by virus action. In this case the percentage of infectivity was evident, and the criteria used was the same.

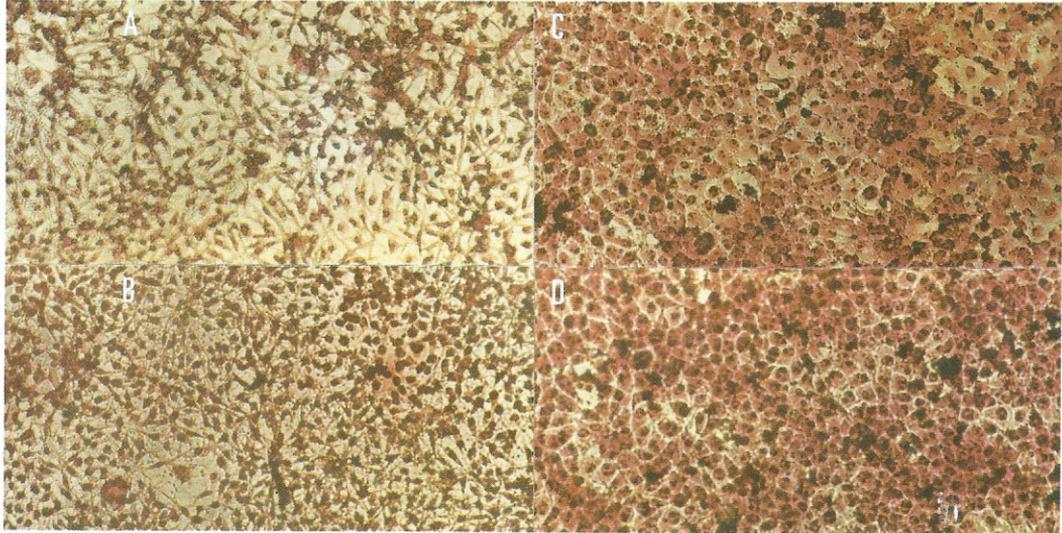


FIGURE 2

Differences of Morphology with Inoculation of Rabies Virus, (ERA strain), on McCoy Cell Line Using Karaya Gum (SIGMA LABS).

After 48 hours: A - Dilution  $10^{-4}$ ; B - Dilution  $10^{-5}$ ; C - Dilution  $10^{-6}$  and D - Control cells. This observation was performed with planar objective 16/0.35 with IM - Zeiss microscope.

Figure 3 shows the observation of CPE at a different magnitude in the microscope observed with planar objective 6.3/0.16 in IM-Zeiss microscope

where morphological alterations caused by rabies virus, as well as stains differences, can be seen.

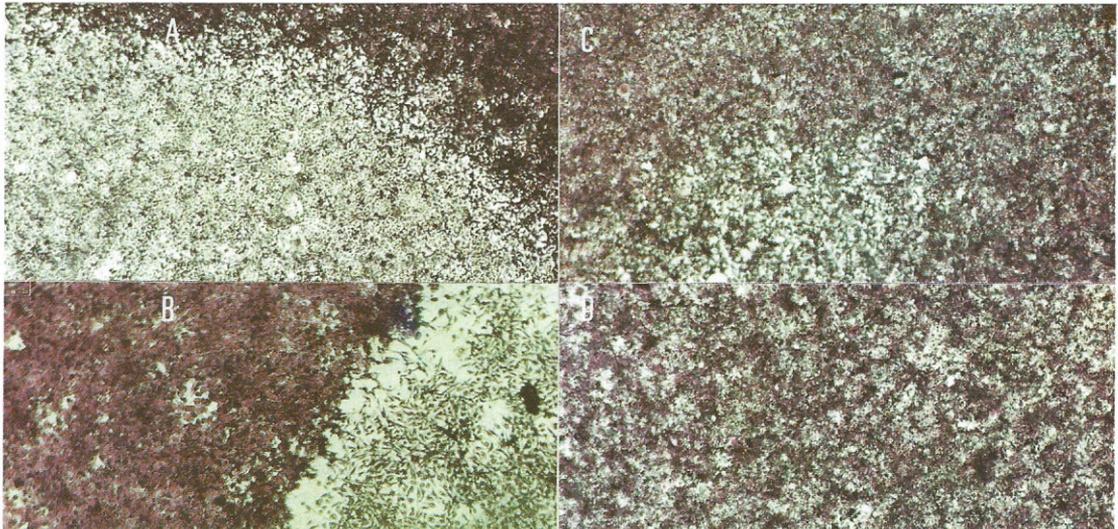


FIGURE 3

Differences of Morphology with Inoculation of Rabies Virus (ERA strain) Where the Action of the Virus on McCoy Cell Line is Observed.

The dilution observed is the same as figure 2, but observed with other magnitude. A - Dilution  $10^{-4}$ ; B - Dilution  $10^{-5}$ ; C - Dilution  $10^{-6}$  and D - Control cells. This observation was performed with planar objective 6.3/0.16 with IM - Zeiss microscope.

CPE Compared to Classic Mice Inoculation.

The titration of the three systems mentioned above, with different volumes of inocula used is

shown in figure 4. Their results were equivalent when the values were adjusted to the same volume, as it can be seen in table I.

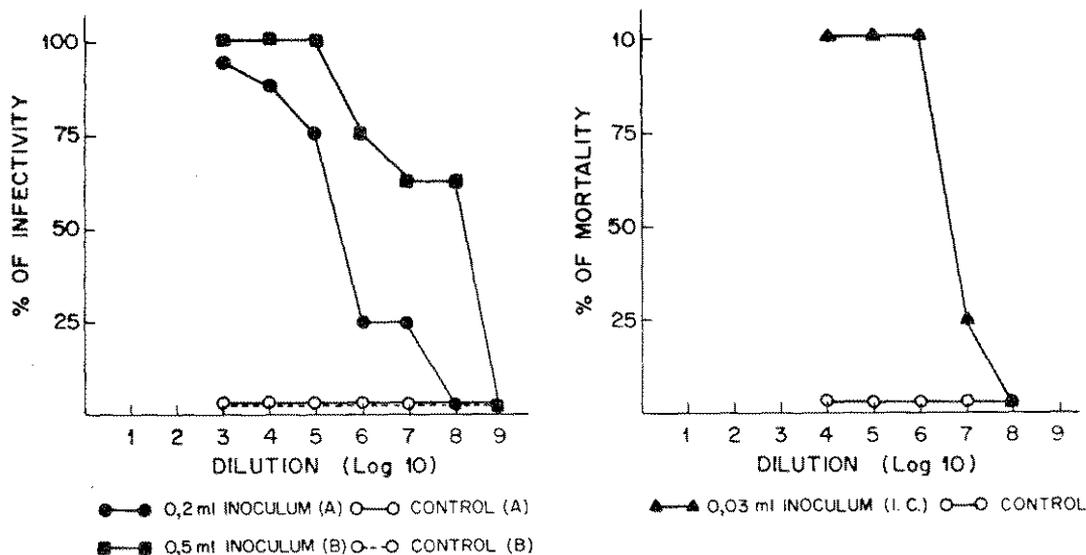


FIGURE 4

Titration of Rabies Virus (ERA strain) Observed with Two Techniques (A AND B) Compared to the Classic Intracerebral Inoculation in Baby Mice (C).

TABLE I  
Titres Observed with the 6<sup>th</sup> Passage of Rabies Virus (ERA strain), on the McCoy Cell Line.

TITRE VOL. INOCULATED	I.D <sub>50</sub> MICE	IDTC 50%	
		IN LOCO	WITH OVERLAY
0.03 ml/mice	10 <sup>6.2</sup>	-	-
0.2 ml/well	-	-	10 <sup>6.0</sup>
0.5 ml/well	-	10 <sup>8.0</sup>	-
1.0 ml*	10 <sup>8.18</sup>	10 <sup>8.3</sup>	10 <sup>8.23</sup>

\* The values of the titres were adjusted to the same inoculum volume, evidencing the similarity of data.

Three different methods of observation were used. The titers were obtained by the end point technique according to Reed-Muench's method.

DISCUSSION

Data obtained in these preliminary studies show the possibilities of the use of McCoy cell line for titration of rabies virus. The use of Karaya gum as overlay speeded the action of the virus, and the results using 0.2 ml of inoculum per 2 ml of total volume was comparable to the results obtained with intracerebral inoculation of baby mice (0.03 ml).

On the other hand, the direct observation of CPE is efficient to obtain an increase of the titre during viral replication. When the logarithm of each dilution is plotted against the days of CPE observation (with more than 50% of infectivity) a sigmoid curve

is obtained and its inflection is equivalent to the result obtained with mice inoculation on the eight day of CPE observation and with a dilution of about 10<sup>6.0</sup> (see Nogueira<sup>5</sup>). It was observed that the obtainment of CPE in McCoy cell line after inoculation with rabies virus can detect small amounts of antigen and is as sensitive and efficient as another techniques such as RIFT (Rapid Inhibition Focus Test) on murine neuroblastoma cells.<sup>10</sup> Besides, it has the advantage that the intermediary reaction of IFA or enzyme linked reaction or UV microscope are unnecessary. The costs decreased due to the absence of laboratory mice as well as another kinds of reagents.

In spite of the relatively small number of samples the isolation of street virus (patient bitten by bat;

bovine and rabid dog) was efficient and confirmed by mice inoculation and by IFA technique.

RIALA6/722

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RESUMO: A linhagem celular McCoy tem demonstrado elevada sensibilidade ao vírus da raiva. Essa linhagem apresenta efeito citopático (ECP) quando infectada com o vírus rábico. Foram testadas três cepas de vírus da raiva: CVS, ERA e PVI e foram isolados três vírus de rua: um proveniente de paciente mordido por morcego, outro de origem canina e outro de origem bovina. Utilizamos essa linhagem celular também para fins de titulação de vírus. Dada a presença do efeito citopático, foram empregadas duas técnicas de observação do ECP e ambas foram comparadas ao método clássico de inoculação em cérebros de camundongos.

DESCRITORES: Linhagem celular McCoy. Vírus da raiva (CVS, ERA, PVI e vírus de rua). Isolamento, titulação, e efeito citopático (ECP).

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