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RAPID DETECTION AND TITRATION OF MAREK'S DISEASE VIRUS (MDV) VACCINE ON CER CELL LINE.

(With 2 Tables and 2 Figures)

By

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تقييم لقاح المارك على خلايا ال سى إى ر

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تم عمل تقييم لقاح المارك بأنواعه المختلفه على خلايا ال سى أى ر وعلى خلايا أجنة الكتاكيت الأولية وذلك بإجراء أختبار البلاك وتم عمل أختبار الفلورسنت المشع الغير مباشر للتأكد من نتائج المعايرة التى وصلت إلى ٣٤ر٣لو١٠ وحدة بلاك فى الجرعه الواحدة للقاح وكانت نتائج المعايرة فى خلايا ال سى أى ر وفى خلايا أجنة الكتاكيت الأوليه متطابقة ومما سبق يمكن القول أنه يمكن استخدام خلايا ال سى أى ر بدلا من خلايا أجنة الكتاكيت الاوليه فى معايرة لقاح المارك المستورد مع أستخدام أختبار الفلورسنت المشع توفيرا للوقت وتقليل النفقات حيث يمكن الأستغناء عن البيض الخالى من المسببات المرضيه عند تحضير الخلايا.

SUMMARY

A rapid assay for detection and titration of MDV vaccine using primary CEF cell culture compared with chicken Embryo Rough (CER) cell line and using plaques assay for virus titration and the titre was expressed as $\log_{10}$ plaque forming unit (PFU)/ dose using different kinds of MDV vaccine. The titer ranged from 3.14-3.34 $\log_{10}$ PFU/ dose. The obtained result was confirmed by IFA.

Key words: *Marek's Disease-Vaccine-Titration*

INTRODUCTION

The primary chicken embryo fibroblast (CEF) cells are most commonly used for the titration of cell associated and cell free (MDV) vaccine (Sharma, 1985). These CEF cells required SPF eggs which are very expensive to obtain from Europe or the USA which take long time to be obtained to Egypt. Also, primary CEF cells need long time (15 days) for preparation from embryonated eggs and give no more than 3 passages in tissue culture.

We usually, use commercial embryonated eggs for preparation of CEF cells. This type of eggs usually contaminated with Salmonella (Williams, et al. 1968), Avian Leukosis (Crittenden, 1981), Reo virus (Calnek, 1991), Mycoplasma (Yamamoto, 1991), Avian Encephalomyelitis (AE) (Monte, 1994), chicken Anaemia virus (CAV) Boer et al. 1994), which interfere with (MDV) titration.

Chicken Embryo Rough (CER) cells have been used with success for diagnosis of some animal viruses (El-Karamany et al., 1981, Saber et al., 1984 and Gihan, 1990).

Indirect immunofluorescent test have been used for early detection of viral antigen of MDV in tissue culture (Bulow, 1971; Purchase, 1973 ; and Lee &Witter, 1983). The plaque assay as described by Sharma (1985) was used for titration of MDV vaccines.

The aim of this work is to select a rapid method using CER cell line instead of primary CEF to save time and cost of titration of different kinds of MDV vaccines.

MATERIAL and METHODS

Virus and Antisera:

Marek's Disease Virus (MDV) strain-1 and MDV immune sera were kindly supplied from USDA-ARS Avian Disease and Oncology Laboratory East Lansing MI 48823.

Vaccines:

Cell free and cell associated MDV vaccine from different Sources (Intervet, TAD and Bioteke, Rhone Merieux, Mycofarm) were kindly supplied from Quality Control Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

Tissue Culture:

a. Primary Chicken Embryo Fibroblast (CEF) cells were grown and maintained as described by Witter et al. (1969).

b. Chicken Embryo Rough (CER) cell line.

The CER cell line obtained from Wister Institute in Philadelphia, USA, were grown and maintained as described by El-Karamany et al. (1981) and Saber et al. (1981) and Saber et al. (1984).

Indirect Immunofluorescent Technique (IFA):

IFA was done by using 96 well tissue culture microplate containing CEF or CER and infected with MDVV as described by Lee and Witter (1983) and Elian et al. (1996).

Plaque assay:

Plaque assay using serial dilutions of MDV vaccines and CEF or CER cells was done according to Sharma (1985) and European Pharmacopoeia (1990).

RESULTS

Table (1) showed clearly that both CEF and CER cell cultures reacted in a similar cytopathic picture to MDV vaccine. This was confirmed by IFA (Photo 1 and 2) that appeared early from 24 hours post infection (PI) till 192 hours PI.

Foci of CPE appeared after 72 hours post infection with MDV. Plaques are counted from 144 hours to 192 hours PI and the number of plaques unit (PFU) per dose of MDV calculated on both CEF and CER cells. The titer of MDV reached 3.27 $\log_{10}$ PFU/dose in CEF and 3.25 $\log_{10}$ PFU/ dose in CER cells after 192 hours PI. (Photo 3).

The result of titration of different kind of MDV vaccine on CEF and CER cells appeared almost the same as shown in Table (2a and 2b).

DISCUSSION

From the obtained result, the early detection of MDV in primary CEF cell and CER cell lines within 24 hours would reduce the long time in the diagnosis of MDV by 2 days to 2 weeks depending on the virus and kind of cell culture used. These findings agreed with those obtained by Bulow (1971); Lee and Witter (1983) and Sharma (1985) where they used primary CEF cell culture and IFA for detection and titration of MDV.

The Obtained titre of MDVV ranged from 3.14-3.34 $\log_{10}$ PFU/dose in both primary CER cells (no big difference between the mean value of several batches of MDVV titrations) agreed with those reported by Witter et al. (1969); Bulow (1971); Sharma (1985) and European Pharmacopoeia (1990) when they used the PFU for MDVV titration.

From the above mentioned data, we can say that CER cell line and IFA could be used with success for rapid diagnosis and early detection of both kind of cell free and cell associated MDV vaccines and for minimizing the cost of evaluation of imported MDV vaccines instead of using CEF primary cell cultures.

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Table (1) : Susceptibility of CEF and CER cell culture to MDV.

Type of cell culture	Hours post inoculation														Log10 PFU per ml	
	24		48		72		96		120		144		168		192	
	CPE	IFA	CPE	IFA	CPE	IFA	CPE	IFA	CPE	IFA	CPE	IFA	CPE	IFA	CPE	IFA
CEF	-	+	-	+	++	+	++	+	++	+	+++	+	+++	+	+++	+	6.27
CER	-	+	-	+	++	+	++	+	++	+	+++	+	+++	+	+++	+	6.25

CPE : Cytopathic effect.
IFA : Indirect fluorescent antibody assay.
CEF : Chicken Embryo Fibroblast cells.
CER : Chicken Embryo Rough cells.
+ : IFA positive.
++ : Foci appeared.
+++ : Plaques appeared.
- : No CPE.

Tables (2) : Titration of different kind of MDV virus on CEF and CER cells.

A. Cell free MDVV

Vaccine Name	Company Name	Inspected titre * (expressed in log10 PFU per dose)	
		Cell free MDV vaccine	
		CEF	CER
Delvax MTHV **	Mycofarm	3.32	3.25
TAD Marek vaccine	TAD	3.23	3.15
Marek vaccine Nobilis	Intervet	3.34	3.27

B. Cell associated MDVV

Vaccine Name	Company Name	Inspected titre * (expressed in log10 PFU per dose)	
		Cell free MDV vaccine	
		CEF	CER
Cryomarex	Rhone Merieux	3.20	3.23
Bio Marek bivalent	Bioteke	3.25	3.27
Risma vac.	Intervet	3.14	3.15

** MTHV : Marek Turkey Herpes Virus.

CEF : Chicken Embryo Fibroblast cells.

CER : Chicken Embryo Rough cells.

* : Each value represents the mean value of several batches titrations.

PFU : Plaque Forming Unit.

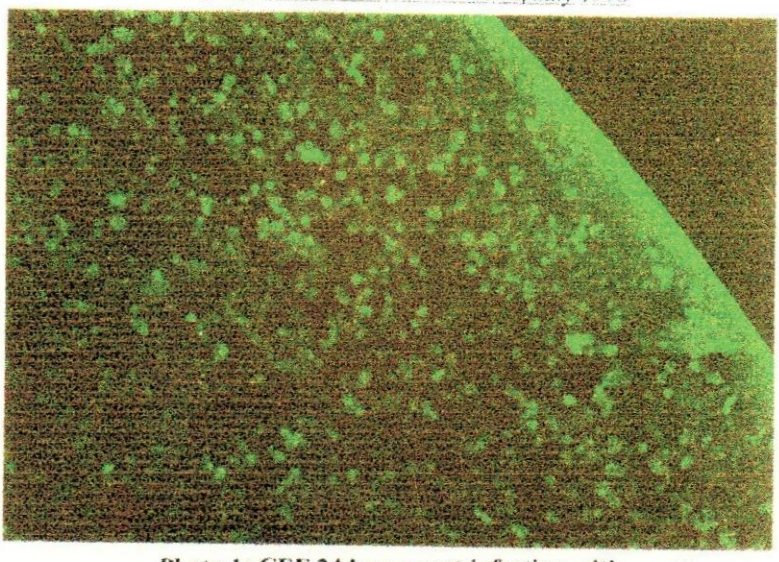

Photo 1: CEF 24 hours post infection with MDV vaccine (dilution) as done by IFA (X 40).

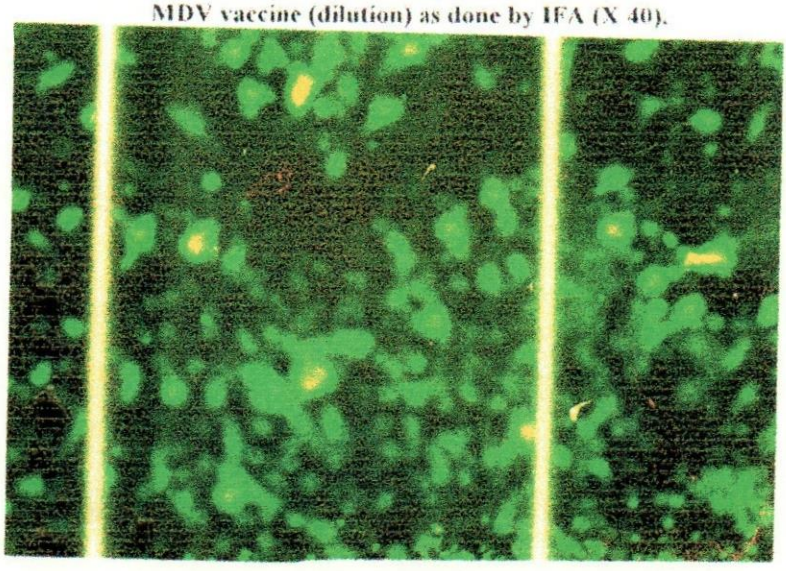

Photo 2: CER 24 hours post infection with MDV vaccine (dilution) as done by IFA (X 160).

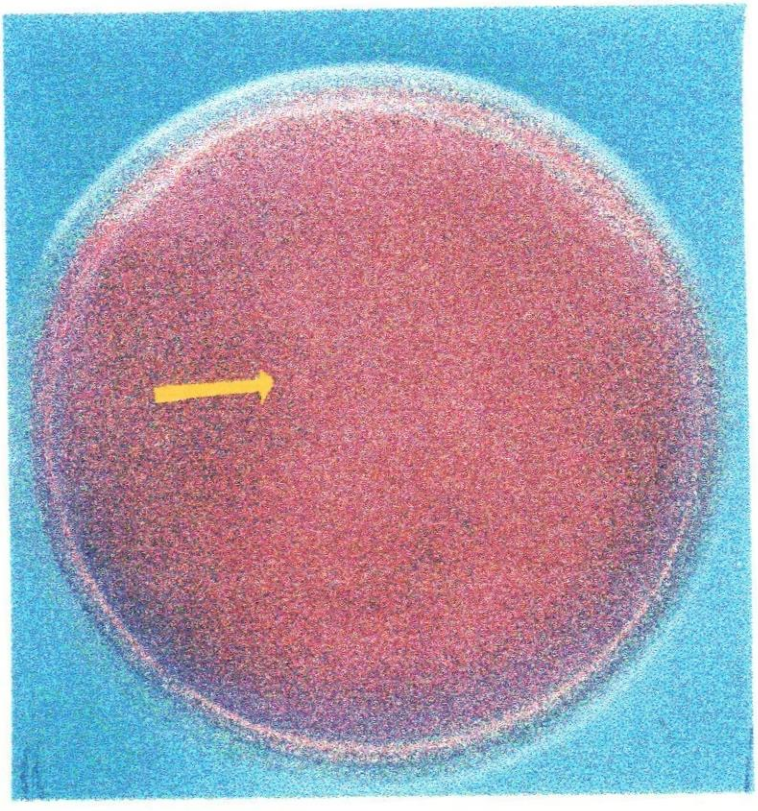

Photo 3: Shows plaques at 10^{-6} dilution of MDV on CER cells stained with neutral red 1% (arrows) (X 1).