The Pharma Innovation Journal 2022; SP-11(4): 1108-1110

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(4): 1108-1110

www.thepharmajournal.com
Received: 07-02-2022
Accepted: 09-03-2022

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Consistency of rabies virus yield at different passages of Vero cell using traditional monolayer culture

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Abstract
Rabies virus is a neuro tropic which can be cultivated in a wide variety of host cells like primary embryo fibroblast, MRC – 5 and Vero cells using either traditional or different types of bioreactor system for bulk antigen production. Such cultivation is extremely important to obtain the knowledge about virus itself as well as for producing large quantities of virus for the production of vaccine. The production of high yield of virus depends on the factor like quality of cells, media used and multiplicity of infection (MOI). High yield of virus can be obtained when cells in suspension are infected and then allowed to form monolayer. The present study was carried out in Vero Cells from passage 141 to passage 147 using rabies virus production methods as concurrent validation for ensuring the consistency of rabies virus yield in Vero cells. Viral Harvest yielded at different passage level of Vero Cells was subjected to virus titration by fluorescent antibody technique (FAT). The analysis of result showed that the virus yield is consistent at passage level of Vero cells from 141 to 147 and all viral harvest titre were well above the set limit of $\log_{10}^5$ $CCID_{50}$/mL. Hence it is demonstrated that consistent production can be obtained with Vero cells at Passage level at the beginning (P_{141}) and end (P_{147}) of the intended span of use without having any problem of residual substrate DNA in the final vaccine formulation.

Keywords: Vero cells, passages, rabies virus, optimizing yield

Introduction
Rabies is a neuro-tropic viral disease which can affect all warm-blooded animals. In addition to a variety of wild species, dogs are important domestic reservoirs of the rabies virus and vectors of the disease, notably in developing countries where rabies is most widespread. Thus, the control of rabies is possible only by immunization in view of achieving human prophylaxis. In the twenty-first century, the majority of human rabies fatalities are due to socioeconomic factors regarding the lack of applied post exposure prophylaxis (PEP) regimens, shortages of existing rabies biologics, and inadequate canine vaccination (Wunner and Briggs, 2010) [13]. Improved approaches to safety, effectiveness, and administration of rabies biologics need to remain a primary focus in modern rabies prevention and control. Rabies vaccine development began with live rabies viruses (RABVs) in mammalian nerve tissue vaccines (MNTV), progressed to avian tissue vaccines, and thereafter resulted in primary continuous cell-culture derived vaccines. Rabies virus is cultivated in a wide variety of substrates like primary embryo culture, MRC – 5 and Vero cells for research as well as bulk production for vaccine manufacture. Today, Vero cells are considered as a more suitable substrate for the production of viral vaccines. This cell line presents several advantages over primary and diploid cell substrates (Prem Kumar *et al*., 2002) [6]. Vero cells can be used in micro carrier and suspension cultures for large scale production in bioreactors. Moreover, virus titer achieved is higher than that reached using other types of cell substrates (Duchene *et al*., 1990) [1].

Although advancement is happening with bioreactor system with serum free medium for bulk production of rabies virus using micro carriers like cytodex and fibra-cell, the traditional large scale production is still followed by biologicals manufacturers by considering the effectiveness and the residual substrate DNA level in the final vaccine. The quest for acquiring knowledge on massive production of high virus yield is demanding and also depends on qualities of substrate, media and multiplicity of infection (MOI) which is the initial ratio of virus added to the cell substrate. The prime objective of this work is to optimize to have consistent productivity of the upstream process of a rabies vaccine produced on Vero cells at different passage levels at the beginning and end of the intended span of use.

Materials and Methods

Vero cells (CCL) from passage 141 to 147 were used as substrate for propagation of rabies virus. Pasteur Virus 2061 Vero 15 Passage (PV 2061 Vero 15) strain of rabies virus was used for virus production. Minimum Essential Medium (Himedia) supplemented with 5% fetal calf serum was utilized. Ready to use grooved greiner bio cell culture bottles were used for monolayer culture. Anti nucleocapsid rabies virus FITC conjugate (Biorad, France), Phosphate Buffer Saline (PBS A) and Acetone (Qualigens) were used to carry out titration of rabies virus.

The trypsinized Vero cells were infected with rabies virus seed with different passage level of Vero cells to know the consistency of rabies virus yield. The virus seed and Vero cell substrate ratio (MOI) was maintained at the rate of 1:750 (0.0013) for virus production at each passage of Vero cells until a complete monolayer was formed. The infection was carried out using MEM with 5% FBS and incubated between 35 and 37^{0}C for 72 hours. After 72 hours, the infective media was decanted in a discarding bowl containing 0.2% citric acid solution and then washed with fresh MEM without serum. After washing, 300 ml of fresh virus maintenance medium (VMM) was added to grooved bottles and incubated for further 72 hours between 35 and 37^{0}C. Henceforth rabies virus harvest was collected at every 72 hours by adding virus maintenance medium until four harvests. All four harvest collected from a single lot were subjected to sterility and virus titration by fluorescent antibody test to quantify the rabies virus yield. After fourth harvest, grooved bottles were discarded in the discarding bowl containing 0.2% citric acid solution and then decontaminated by autoclaving.

Results and Discussion

The consistency of virus yield from series of harvests from a lot is very important in view of optimizing the production of vaccine in any in house validated manufacturing facility. The mode and method of harvest depends on the product being grown in substrates and the virus can be intracellular or lysed into the cell culture medium in case of lytic viruses or otherwise the cell culture supernatant is collected at different time points during production, sometimes over a few days.

Special design of culture media (In House) is also required for maintaining high concentration of Virus yield and indeed the medium should supply all the nutrients needed without impairing cell growth. Important factors like glucose concentration and additional requirement of glutamine in virus maintenance medium also play major role and particular consideration should be given to osmolarity since this parameter is considered as the most important physical property of cell culture media and could have a detrimental effect on cell growth and adherence and/or integrity (Freshney, 1994) [2]. The virus titres of harvests produced at each passage level (P141 to P147) were found to be almost in a consistent manner with its trend analysis being represented (Fig.1).

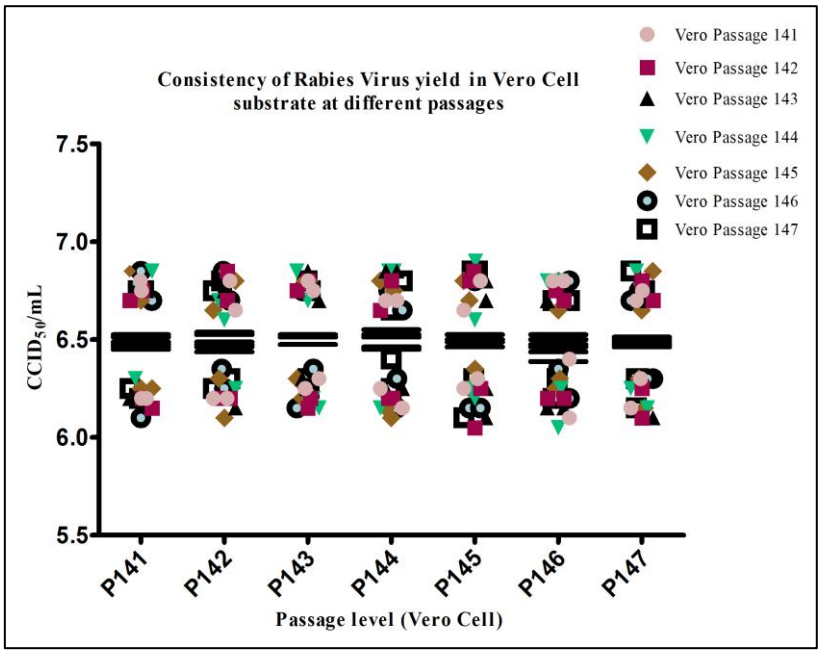

Fig 1: Consistency of Rabies Virus yield in Vero cell substrate at different passages

Based on the virus yield and the trend analysis of the same at different Vero Cell Passage level (P_{141} to P_{147}), the virus yield seems to be consistently above $\log_{10}^6$ $CCID_{50}$/mL well above the acceptance limit of $\log_{10}^5$ $CCID_{50}$/mL with statistically no significant difference ($p<0.01$) to have better quality vaccine production through high glycoprotein content which is considered as important protein for inducing neutralizing antibodies against rabies virus in immunized individuals. These results were in agreement with those reported by Mendonca *et al.* (2002) and Khaled Trabelsi *et al.*, (2006) [9] who showed that regular feeding of Vero cell culture with virus maintenance medium containing l-glutamine and glucose had prevented apoptosis and resulted in a high cell density level. Hence it is demonstrated that consistent production could be obtained with Vero cells at Passage level at the beginning (P_{141}) and end (P_{147}) of the intended span of use as it is one of the important elements of vaccine manufacturing.

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