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Review Article

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Study on Preparation of New Purified Vero Cell Rabies Vaccine

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Abstract

Rabies is a 100% vaccine-preventable disease. A cheap, rabies vaccine for humans that could be used in mass vaccination campaigns would be a valuable weapon against rabies. Despite the existence of safe and effective vaccines, rabies disease still causes an estimated more human deaths a year in the endemic areas in Asia and Africa. Rabies virus is single stranded negative sense RNA from genus lyssavirus and _family rhabdoviridae.

Abbreviations

SRID: Single Radial Immunodiffusion Assay
TFF: Tangential Flow Filtration
HCP: Host Cellular Protein
BSA: Bovine Serum Albumin
BPL: Heta-propidiactone
PRA: Purified rabies antigen

Introduction

In manufacturing process, each step in production of rabies vaccine is important but the inactivation and purification steps are very crucial. BPL is used as inactivation agent and column chromatography with cellufine sulfate as matrix is used for purification. By using the column chromatography optimal recovery of rabies glycoproteins as well as effective removal of impurities such as residual cellular DNA, BSA and host cell protein were found. In this study, vero cells ATCC CCL-81 are used as substrate for the growth of rabies virus (PM 3218). The rabies antigen is purified by using column chromatography (cellufine sulfate resins) and subsequent tangential flow filtration. The purified antigen is then filter through 0.22 micron filter. PRA prepared by this method is found to be meeting all quality attributes. In this study, our focus is on the core manufacturing process in the preparation of rabies vaccine.

Materials

- Rabies virus strain used in this study is Pitman Moore (PM 3218).
- Vero cell ATCC CCL-81 is used as substrate.
- Minimum Essential Medium (MEM)
- Minimum Essential Medium (MEM) with bovine serum
- Beta-propiolactone (BPL)
- Stabilizer
- CellCube Module
- Cell Factory (6320 cm?)
- Tissue culture flask (175 cm?)

Methodology in the Preparation of Rabies Vaccine

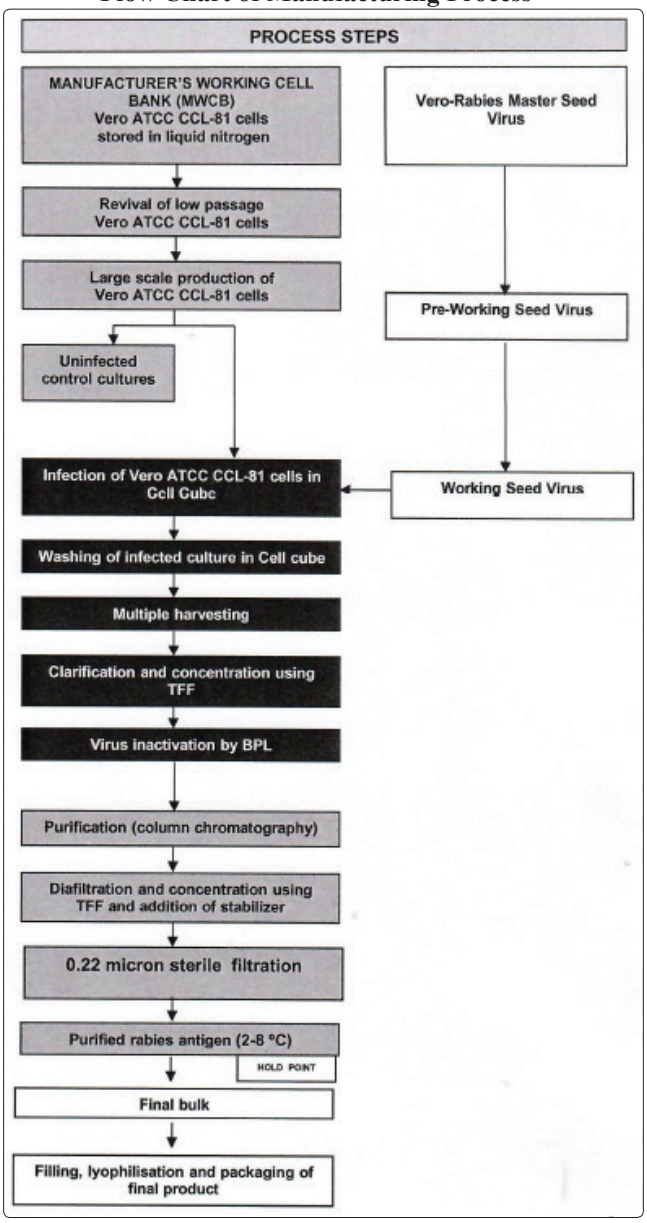
Preparation of Purified Rabies Antigen

The preparation of purified rabies antigen is proceed in a stepwise manner with the large-scale production of Vero cells, rabies virus propagation, virus inactivation, purification of inactivated antigen and antigen stabilization. Initially low passage of vero cells is revived. Large-scale production of Vero ATCC CCL81 cells were seeded in CellCube system. Then cells are infected with Pitman Moore (PM 3218) strain of rabies virus. After 48-72 hrs of incubation infected cells are washed. The multiple harvests are collected from one Cell Cube system and clarified by filtration. Clarified harvest is then concentrated using tangential flow filtration system to reduce working volume of live rabies virus for further processing. Inactivation of rabies virus is done using beta-propiolactone (BPL). In the next step, purification is done by affinity column chromatography with cellufine sulfate resin in order to remove BSA, host cell DNA and host cell protein. The column-eluted antigen is diafiltered by TFF system to remove high salt concentration. Purified rabies antigen is then prepared by adding stabilizer to diafiltered antigen in 1:1 v/v ratio. Finally, antigen is filtered by 0.22-micron filter and collected in media bags. These bags then stored at 2- 8°C.

Preparation of Rabies vaccine

The Process of formulation involves blending of Purified Rabies Antigen and diluent to give a final batch volume with set antigen content (SRID). The 1 ml liquid vaccine is filled in clear and tubular vial and lyophilized. With the lyophilized vial of rabies vaccine 1.0 ml of sterile water for injection is supplied as a diluents.

Flow Chart of Manufacturing Process



Results and Discussion

Study gives well-developed new purified vero cell rabies vaccine, following all the GMP requirements. This vaccine is good option among the available modern WHO pre-qualified rabies vaccine. The new things about Rabies vaccine is that the PM 3218 strain was for the first time successfully adapted on Vero ATCC CCL 81. The adaptation worked very well. Rabies virus is inactivated by using BPL at 1:3500 at 2 to 8°C within 24 hours after addition of BPL. During purification optimal reduction in HCP, residual DNA, BSA and other impurities is observed. Study also gives optimal recovery of rabies glycoprotein during purification process.

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