2. THE PRESENT STUDY

The Vero cell-derived rabies vaccine for human use, utilized for this research work was prepared by propagating Vero cells (ATCC) in roux/roller bottles, infected with rabies virus PV-11. The viral harvests were concentrated by pellicon cassette system, inactivated with β-Propiolactone (BPL) and subjected to continuous flow centrifugation/column chromatography for purification. For the preparation of Vero cell derived rabies vaccine the criteria of purity are very stringent and clearly defined. The purified Vero cell rabies vaccine should not contain more than 100 pg residual cellular DNA and the protein of bovine serum origin should be below one part per million. The purified viral antigen was supplemented with 5% human serum albumin and 1% maltose as stabilizers to a potency of 2.5/ mL by NIH in vivo method and then filtered through a 0.45μ membrane. The bulk vaccine was distributed in 1-mL amounts into vials, freeze-dried and sealed. Simultaneously all inprocess and final quality control tests were conducted on each batch.

The present study has the following objectives

1. Optimization of zonal centrifugation runs for purification of Vero cell derived human rabies vaccine with minimal residual cellular DNA and calf serum protein well within the permissible level of less than 100 pg/dose and 1ppm, respectively.

2. Standardization of the column chromatography technique using DEAE–Sepharose CL–6B column for optimal viral antigen recovery, besides to maximal removal of substrate DNA and serum proteins in lieu of zonal centrifugation.

4. Validation of non-radioactive biotin method for the quantification of substrate DNA in place of radioactive labeling method.

5. Raising of specific immune serum against calf serum in rabbits and standardization of the technique of counter immuno-electrophoresis to quantify calf serum protein in the final human rabies vaccine.

6. Study of structural changes during lyophilization in the Vero cell-derived human rabies vaccine containing human albumin and maltose as stabilizers by Infrared (IR) spectroscopy.