## CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>TITLE</th>
<th>PAGE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1.</td>
<td>RABIES – A DREADFUL DISEASE</td>
<td>1</td>
</tr>
<tr>
<td>1.2.</td>
<td>THE VIRUS</td>
<td>2</td>
</tr>
<tr>
<td>1.2.1.</td>
<td>Morphology</td>
<td>2</td>
</tr>
<tr>
<td>1.2.2.</td>
<td>Structure</td>
<td>3</td>
</tr>
<tr>
<td>1.2.3.</td>
<td>Transcription, replication and budding</td>
<td>3</td>
</tr>
<tr>
<td>1.3.</td>
<td>VIRAL PROTEINS AND THE HOST IMMUNE RESPONSE</td>
<td>7</td>
</tr>
<tr>
<td>1.3.1.</td>
<td>Glycoprotein (G)</td>
<td>7</td>
</tr>
<tr>
<td>1.3.2.</td>
<td>Nucleoprotein (N)</td>
<td>8</td>
</tr>
<tr>
<td>1.3.3.</td>
<td>RNA core-associated phosphoprotein (M1)</td>
<td>9</td>
</tr>
<tr>
<td>1.3.4.</td>
<td>Matrix protein (M2)</td>
<td>9</td>
</tr>
<tr>
<td>1.3.5.</td>
<td>RNA dependent RNA polymerase (L)</td>
<td>9</td>
</tr>
<tr>
<td>1.4.</td>
<td>DEVELOPMENT OF VACCINE</td>
<td>10</td>
</tr>
<tr>
<td>1.4.1.</td>
<td>Nervous tissue vaccines (NTV)/ Suckling mouse brain vaccines</td>
<td>10</td>
</tr>
<tr>
<td>1.4.2.</td>
<td>Embryo vaccine</td>
<td>11</td>
</tr>
<tr>
<td>1.4.3.</td>
<td>Cell culture vaccines</td>
<td>11</td>
</tr>
<tr>
<td>1.4.3.1.</td>
<td>Primary cell culture derived vaccine</td>
<td>12</td>
</tr>
<tr>
<td>1.4.3.2.</td>
<td>Diploid cell line derived vaccine</td>
<td>12</td>
</tr>
<tr>
<td>1.4.3.3.</td>
<td>Continuous cell culture derived vaccine</td>
<td>13</td>
</tr>
<tr>
<td>1.5.</td>
<td>VERO - A CONTINUOUS CELL LINE FOR VACCINE PRODUCTION</td>
<td>14</td>
</tr>
</tbody>
</table>
2. THE PRESENT STUDY

3. MATERIALS AND METHODS

3.1. VIRUS SEED PREPARATION, STORAGE AND USE

3.2. VERO CELL LINE

3.2.1. Cryostorage of Vero cells

3.2.2. Media used for cell culture

3.2.3. Revival of cells

3.2.4. Propagation

3.3. INFECTION (VIRUS CULTIVATION)

3.3.1. Viral harvests

3.3.2. Titration of virus infectivity

3.3.3. Pooling

3.3.4. Concentration

3.3.5. Inactivation

3.4. VIRUS PURIFICATION

3.4.1. Zonal centrifuge purification

3.4.1.1. System description

3.4.1.2. Control panel

3.4.1.3. Rotor and core

3.4.1.4. Operating procedure

3.4.1.4.1. System preparation

3.4.1.4.2. Rotor assembly and installation

3.4.1.4.3. Zonal centrifuge purification run

3.4.2. Column purification

3.4.2.1. Criteria of purification

3.4.2.2. Purification process

3.4.2.3. Column techniques

3.4.2.4. Elution conditions

3.4.2.5. Checking the process of purification
3.4.2.6. Regeneration and cleaning
3.5. HAEMAGGLUTINATION TEST
3.6. OD VALUE
3.7. SDS-PAGE
3.8. POTENCY ASSAY METHODS
3.8.1. SRD assay \textit{(in vitro method)}
3.8.1.1. Reference vaccines
3.8.1.2. Immune serum
3.8.1.3. Procedure
3.8.1.4. Calculation of potency
3.8.2. NIH mouse protection test \textit{(in vivo method)}
3.8.2.1. Reference vaccine
3.8.2.2. Challenge virus standard (CVS)
3.8.2.3. Preparation of the working CVS
3.8.2.4. Determination of the LD_{50} of the working CVS
3.8.2.5. Immunization of mice
3.8.2.6. Challenge of control and test mice
3.8.2.7. Calculation of potency
3.8.2.8. Minimum potency requirements
3.9. PURITY ANALYSIS
3.9.1. Residual DNA assay
3.9.1.1. Isolation of Vero DNA from cells grown in tissue culture (for control and probe preparation)
3.9.1.2. DNA extraction from test samples
3.9.1.3. Blotting
3.9.1.4. PROBE PREPARATION
3.9.1.4.1. Radioactive labeling method
3.9.1.4.1.1. Nick translation method
3.9.1.4.1.2. Random priming method
3.9.1.4.1.3. Probe purification (spun-column chromatography)
3.9.1.4.1.4. Hybridization of filters and autoradiography
3.9.1.4.2. Non-radioactive labeling method (Biotin method)

3.9.1.4.2.1. Nick translation method

3.9.1.4.2.2. Hybridization of filters and visualization

3.9.1.4.2.3. Saran wrap method

3.9.1.5. Inference of the result

3.9.2. Residual serum quantification

3.9.2.1. Quantification of calf serum

3.9.2.1.1. Antigens (BSA, Calf serum and Test vaccine)

3.9.2.1.2. Antisera (Anti calf serum)

3.9.2.1.3. Procedure

3.9.2.1.4. Inference of the result

3.10. IMMUNOGENICITY STUDY

3.10.1. Immunization in human

3.10.2. Immunization of Guinea pigs

3.10.3. Rapid fluorescent focus inhibition test (RFFIT)

3.10.3.1. Principles of the test

3.10.3.2. Preparation of test samples

3.10.3.2.1. Inactivation of sera

3.10.3.2.2. Dilution of serum samples

3.10.3.2.3. Challenge virus dilutions

3.10.3.2.4. Addition of neuro-2A cells

3.10.3.2.5. Virus titration

3.10.3.2.6. Cell control

3.10.3.2.7. Acetone fixation and staining by immunofluorescence

3.10.3.2.8. Virus – neutralizing antibody titres

3.10.3.2.9. Relative potency of test sera (IU/mL)

3.11. FINAL BULK VACCINE PREPARATION

3.12. LYOPHILIZATION

3.13. FTIR SPECTRUM ANALYSIS
4. RESULTS AND DISCUSSION

4.1. ZONAL CENTRIFUGE - VIRUS PURIFICATION

4.1.1. Potency assay for the zonal purified material
(SRD and NIH unitage- a comparative study)

4.2. COLUMN CHROMATOGRAPHY PURIFICATION

4.2.1. DEAE - Sepharose CL-6B as column packing material

4.2.2. DEAE - Sephacel as column packing material

4.2.3. Standardization of DEAE Sepharose CL-6B column purification

4.2.4. Process optimization for optimal virus recovery using Sepharose CL-6B column

4.2.5. Potency assay for the column purified material

4.3. IDENTITY TEST - VIRUS PROTEIN STRUCTURAL ANALYSIS

4.4. RESIDUAL DNA ASSAY

4.4.1. Analysis of residual cellular DNA in the zonal purified rabies vaccine (Radioactive and Non-radioactive labeling - a comparative study)

4.4.2. Analysis of residual cellular DNA in the column purified rabies vaccine

4.5. QUANTIFICATION OF RESIDUAL CALF SERUM PROTEINS

4.5.1. Analysis of residual serum content in the zonal purified rabies vaccine

4.5.2. Analysis of residual serum content in the column purified rabies vaccine

4.6. FINAL BULK VACCINE PREPARATION

4.7. LYOPHILIZATION-FTIR SPECTRUM ANALYSIS OF THE FINAL VACCINE

4.8. IMMUNOGENICITY ANALYSIS BY RFFIT TEST

4.8.1. In human volunteers
4.8.2. In Guinea pigs 77
4.9. ZONAL AND COLUMN PURIFICATION-
A COMPARITIVE STUDY 77

5. SUMMARY AND CONCLUSION 78

REFERENCES

APPENDIX 1: REAGENTS USED FOR HAEMAGGULITINATION TEST (HA)
APPENDIX 2: REAGENTS USED FOR SINGLE RADIAL IMMUNO
DIFFUSION TEST (SRD)
APPENDIX 3: REAGENTS USED FOR COUNTER IMMUNO
ELECTROPHORESIS TEST (CIE)
APPENDIX 4: REAGENTS USED FOR SDS-PAGE
APPENDIX 5: REAGENTS USED FOR DNA TEST