PRODUCTION OF A THERMOSTABLEvero CELL-ADAPTED RINDERPEST VACCINE

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SUMMARY: The method of vaccine virus production, chemical stabilization, and lyophilization of a thermostable Vero cell-adapted rinderpest vaccine is presented. The production method results in a vaccine which can be used in the field without refrigeration for up to 30 d.

Key words: rinderpest; vaccine; Vero cells; thermostable; lyophilization.

I. INTRODUCTION

A thermostable Vero cell-adapted rinderpest vaccine (TVRPV) was developed to address several problems relating to the production and delivery of the cell culture rinderpest (RP) vaccine developed by Plowright and Ferris (5) from the Muguga modification of the Kabete “O” strain (RBOK) of RP virus. The attenuated RBOK vaccine is used extensively in Africa and Asia for the control of RP and peste des petits ruminants (PPR). It traditionally has been produced with bovine kidney cells and lyophilized with a variety of chemical stabilizers. Thirty years of extensive use has shown this vaccine to be safe, effective, and inexpensive to produce (6). However, as with many modified-live lyophilized vaccines, the attenuated RBOK vaccine must be stored under refrigeration to prevent thermal inactivation. This “cold chain” requirement is the most costly component of RP control and eradication programs. In addition, the fetal bovine kidney cell production system is prone to adventitious viral contaminants, and calf fetuses are frequently unavailable in developing countries, leading to the interruption of RP vaccine production.

We have recently completed a detailed comparison of the stability of RP vaccines produced using a variety of methods of chemical stabilization and lyophilization (3). The most stable preparation was designated TVRPV and was found to have sufficient thermostability for use in the field without refrigeration for up to 30 d. This vaccine is produced in Vero cells, stabilized with 5% lactalbumin hydrolysate and 10% sucrose, and lyophilized using a 72-h cycle with vacuum level regulation and high final shelf temperatures. This paper provides a detailed outline of production and the complete lyophilization protocol required for the production of TVRPV.

II. MATERIALS

A. Equipment
1. Autoclave, Eagle 2021 series, Amsco
2. Balance, model PC4400, Mettler
3. Capping machine, 224465, Wheaton
4. Cornwall syringe, 2-ml, 3526, Becton Dickinson
5. Freezer, −70°C ultra 1786, Revco
6. Laminar flow cabinet, model 408, NU Aire Inc
7. Lyophilizer, SRCMS, Virtis
8. Lyophilizer trays, removable bottom
9. Lyophilizer tray covers
10. Lyophilizer, vacuum level controller 10-424
11. Magnetic stirrer-hot plate PC101, Corning
12. Magnetic stirring bars, Fisher Scientific
13. pH Meter model 63, Beckman
14. Incubator, Bellco Glass
15. Roller apparatus
16. Inverted microscope, Diavert, Leitz
17. Seitz filter, Hormann
18. Water bath, 37°C dual chamber, model 1250, VWR
19. Desiccator

B. Biologics
1. Certified Vero cells NVSL
2. Vero cell-adapted RP vaccine production seed

C. Chemicals and reagents
1. Eagle’s minimum essential medium (MEM), 8830, Whittaker
2. Fetal bovine serum
3. Gentamicin, 2331-41, Elkins-Sinn
4. Glutamine, 17-605A

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5. Amphoteracin B, 43760, Squibb
6. Lactalbumin hydrolysate, 102131, ICN
7. Potassium hydroxide (KOH)
8. Calf serum, HyClone
9. Sucrose
10. Disodium EDTA (Versine) $311-500
11. Trypsin, 1:250; 0152-15, Difco

D. Glassware and plasticware
1. Beakers, 1000 ml
2. Erlenmeyer flask, 1000 ml
3. Erlenmeyer flask, 2000 ml
4. Roller bottles, 850 cm², 30274
5. Pipettes, 1 ml, 75304
6. Pipettes, 10 ml, 75064
7. Serum bottles, 500 ml
8. Weigh dishes, plastic
9. Vials, lyophilizer, 5 ml
10. Stoppers, grey butyl
11. Seals, aluminum

E. Miscellaneous supplies
1. Aluminum foil, Reynolds Metals
2. Tray, stainless steel

III. PROCEDURE

A. Cell culture
1. Prepare cryopreserved seed stocks of Vero cells certified for purity and characterized according to the United States Department of Agriculture Guidelines (1).
2. Maintain cells in liquid nitrogen vapor phase or in a −70°C freezer. Derive working cell culture stocks from this cryopreserved stock and maintain by weekly passage.
3. Use Eagle’s MEM supplemented with 7% calf serum, 3% fetal bovine serum, glutamine (2 mM), gentamicin (30 µg/ml), and amphotericin B (2.5 µg/ml) as a growth medium.
4. Passage cells by removing them from culture vessels with a solution of 0.5% Trypsin, 0.2% Versine, and resuspend in growth media at 200 000 to 300 000 cells/ml.
5. Seed roller bottles (850 cm²) with 200 ml of cell suspension and place on roller apparatus at 37°C.
6. Use up to 60% of the working stock cell cultures for vaccine production per week.

B. Virus production
1. Prepare a virus stabilizer consisting of 5% lactalbumin hydrolysate (LAH) and 10% sucrose.
   a. Weigh out 50 g of LAH, and place it in a 1000-ml Erlenmeyer flask with 700 ml of distilled water.
   b. Place the flask on the magnetic stirrer-hot plate and begin gentle stirring and heating. Do not boil.
   c. Discontinue heating once the LAH is dissolved and allow to cool while stirring for 15 min.
   d. Weigh out 100 g of sucrose and add it to the solution.
   e. Add distilled water to bring the volume to 1 liter, and cool the solution to room temperature.
   f. Adjust the pH to 7.2 using a pH meter and 1.0 Molar KOH.
   g. Pass the liquid through a sterile Seitz filter with a D10 pad into 500-ml sterile glass serum bottles and refrigerate. LAH stabilizer may be stored up to 1 yr at 4°C.
2. Inoculation of Vero cell cultures with RP virus.
   a. Examine the monolayers for confluency and absence of contamination using an inverted microscope.
   b. Inoculate cultures when they are 90% confluent (at 2 to 4 d after seeding).
   c. Inoculate Vero cell-adapted RP production seed into roller bottle cultures using a sterile 1-ml pipette at the rate of 1 TCID₅₀/2000 cells (generally 0.1 ml production seed per 850-cm² roller bottle).
   d. Maintain at least one noninoculated cell culture per 20 inoculated cultures as a control.

C. Lyophilization
1. Load lyophilizer trays with clean 5-ml vaccine vials.
2. Place covers on the trays and wrap in aluminum foil.
3. Place clean stoppers and aluminum seals in 1-liter beakers; cover with aluminum foil. Sterilize trays, stoppers, and seals in an autoclave at 121°C for 45 min; follow with a drying cycle.
4. Inspect the lyophilizer to ensure that the chamber is clean and dry, the water coolant system is functioning, and the vacuum pump has adequate oil.
5. Turn on the freeze cycle and chill shelves to −45°C or lower.
6. Place frozen bulk vaccine in a 37°C water bath and agitate every 5 to 10 min during thawing.
7. Remove vaccine just before a complete thaw, dry the containers, and allow the thawing process to be completed inside the laminar flow cabinet.
8. Fill the vials in a laminar flow cabinet using a sterile technique.
   a. Pool the contents of the bulk vaccine containers in a 2000-ml Erlenmeyer flask, and place a sterile magnetic stirring bar in the suspension.
   b. Place the flask with pooled vaccine in a crushed ice bath on the magnetic stirrer (no heat), and stir gently.
   c. Insert the hose of a sterile 2-ml Cornwall syringe into the vaccine pool and calibrate the syringe to 1 ml.
   d. Uncover the lyophilizer tray inside the cabinet and dispense 1 ml of vaccine per vial.