

We claim:

1. A method for producing Enteroviral (D-Antigen) particles useful for preparation of vaccine compositions, wherein the method comprises the steps of:
 - a. producing Enteroviral particles in Vero cells;
 - b. purification of the Enteroviral particles either by Single Step Chromatography or Two Step Chromatography;
 - c. stabilization of purified Enteroviral particles; and
 - d. inactivation of the Enteroviral particles to obtain Enteroviral (D-Antigen) particles;wherein the said method comprises addition of one or more excipient including stabilizers and adjuvants before or after the inactivation step, and wherein the D-antigen recovery is in the range of 80% to 99%.
2. The method as claimed in claim 1, wherein the Single Step Chromatography is carried by using a single column prepared by mixing one or more ion exchange resin with one or more hydrophobic resin.
3. The method as claimed in claim 1, wherein the Two Step Chromatography is carried by first running Size Exclusion Chromatography or Gel Filtration Chromatography followed by ion exchange chromatography.
4. The method as claimed in claim 1, wherein the inactivation process is selected from heat inactivation, chemical inactivation, irradiation or UV treatment.
5. The method as claimed in claim 4, wherein the chemical inactivation is carried by using one or more chemicals selected from a group of formalin, beta-propiolactone (BPL), hydrogen peroxide, glutaraldehyde, N-acetyl ethyleneimine, binary ethyleneimine, tertiary ethyleneimine, ascorbic acid, caprylic acid, psolarens or a detergent; and an inactivation buffer.

6. The method as claimed in claim 5, wherein formalin inactivation involves addition of inactivation buffer selected from a group comprising phosphate buffer, TRIS, Sodium Acetate, TBS, MOPS, HEPES or bicarbonate buffer in presence or absence of salt.
7. The method as claimed in claim 6, wherein the inactivation buffer comprises a buffer selected from a group of phosphate buffer, combinations of phosphate buffer and TRIS, sodium acetate, salts with sodium acetate, TBS, MOPS, HEPES and bicarbonate buffers; M-199; glycine and tween 80.
8. The method as claimed in claim 7, wherein the phosphate buffer is used at a concentration in the range of 1 mM-10 mM, preferably 2 mM with or without NaCl in the pH range of 6.0 to 7.5, preferably at 7.2.
9. The method as claimed in claim 7, wherein the phosphate buffer is used at a concentration in the range of 1 mM-10 mM, preferably 2 mM along with saline at a concentration of 10mM in the pH range of 6.0 to 7.5, preferably at 7.2.
10. The method as claimed in claim 7, wherein the sodium acetate buffer is used at a concentration in the range of 20 mM- 50 mM, preferably 25 mM with or without NaCl in the pH range of 6.0 to 7.5, preferably at 7.2.
11. The method as claimed in claim 10, wherein the sodium acetate buffer is used at a concentration of 25 mM having the pH of about 7.2.
12. The method as claimed in claim 1, wherein the stabilizer is selected from the group of sucrose, maltose, trehalose, human serum albumin, and M199.
13. The method as claimed in claim 1, wherein the excipient is added before the inactivation step.
14. The method as claimed in claim 1, wherein inactivation is carried by an inactivation buffer comprising a buffer selected from a group of phosphate buffer, combinations of phosphate buffer and TRIS, sodium acetate, salts with sodium acetate, TBS, MOPS,

HEPES or bicarbonate buffers; M-199 or M-199 in combination with HEPES; glycine and tween 80.

15. The method as claimed in claim 14, wherein the phosphate buffer is used at a concentration in the range of 1 mM-10 mM, preferably 2 mM at the pH range of 6.0 to 7.5, preferably at 7.2 with one or more stabilizer.
16. The method as claimed in claim 15, wherein the stabilizer is selected from a group comprising sucrose in 5% w/v concentration, M199 in 9.9 g/L concentration, trehalose in 5% w/v concentration, maltose in 5% w/v concentration and human serum albumin in 5% w/v concentration.
17. The method as claimed in claim 15, wherein stabilizer is M199 in 9.9 g/L concentration and trehalose in 5% w/v concentration.
18. The method as claimed in claim 1, wherein the Enteroviral particles are of polioviruses.
19. The method as claimed in claim 18, wherein the polioviruses are Sabin serotypes 1, 2 and 3.
20. The method as claimed in claim 19, wherein the polioviruses are Sabin serotypes IPV type 1 (Sabin-1 strain), IPV type 2 (Sabin-2 strain), and/or IPV type 3 (Sabin-3strain).
21. A method for preparing a dose reduced inactivated Polio vaccine containing Sabin polioviruses, said method comprising the steps of:
 - a. producing polioviruses in Vero cells;
 - b. purification of the polioviruses;
 - c. stabilization of purified polioviruses by addition of M-199 medium containing glycine and tween 80;
 - d. inactivation of the polio viruses by using formaldehyde 0.025% at 37°C for 13 days in presence of Phosphate buffer with saline at a concentration between 2mM to 10mM or Sodium acetate (20mM-30mM) to reduce aggregation of the

- poliovirus particles thereby reducing the D-antigen losses post inactivation by greater than 90% as compared to other buffers; and
- e. formulating the said polio vaccine.