(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 January 2007 (18.01.2007)

PCT

(10) International Publication Number
WO 2007/007344 A1

(51) International Patent Classification:
A61K 39/13 (2006.01)

(21) International Application Number:
PCT/IN2004/000267

(22) International Filing Date: 27 August 2004 (27.08.2004)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicant *(for all designated States except US)*: **PANACEA BIOTEC LTD.** [IN/IN]; B-1 Extn./A-27, Mohan Co-operative Industrial Estate, Mathura Road, New Delhi 110 044 (IN).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **JAIN, Rajesh** [IN/IN]; Panacea Biotec Ltd, B-1 Extn./A-27, Mohan Co-operative Industrial Estate, Mathura Road, New Delhi - 110 044 (IN). **CHAWLA, Anil** [IN/IN]; Panacea Biotec Ltd, B-1 Extn./A-27, Mohan Co-operative Industrial Estate, Mathura Road, New Delhi - 110 044 (IN). **KUMRAJ, Ganesh** [IN/IN]; Panacea Biotec Ltd, B-1 Extn./A-27, Mohan Co-operative Industrial Estate, Mathura Road, New Delhi - 110 044 (IN).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2007/007344 A1

(54) **Title:** INACTIVATED POLIOMYELITIS VACCINE DERIVED FROM SABIN STRAIN OF POLIO VIRUS

(57) **Abstract:** An inactivated Polio Vaccine derived from Sabin strain for safe and effective immunization against Poliomyelitis is provided. A process of preparation for such vaccine and formulations thereof are also provided. Administration of the vaccine of the present invention along with other antigens provides immunization not only against polio infection but also against other pathogens causing Hepatitis C. Hepatitis D. Hepatitis E. Meningitis A. Meningitis B. Meningitis C. Meningitis W. Meningitis Y. Pnemococcal (23 valent or more). Smallpox, Typhoid, Bacille Calmette Guerin, Tuberculosis. Human Immunodeficiency Virus. Anthrax or the like, to which children or adults not immunized earlier are susceptible, particularly to which children are susceptible.

INACTIVATED POLIOMYELITIS VACCINE DERIVED FROM SABIN STRAIN OF POLIO VIRUS

Field Of The Invention

The present invention relates to a Polio Vaccine, preferably in inactivated form, derived from Sabin strain for safe and effective immunization against Poliomyelitis, a process for the preparation of such vaccine, and formulation thereof.

The Polio Vaccine formulations of the present invention can be used along with one or more other antigens to provide immunization not only against polio infection but also against other pathogens such as those causing Hepatitis C, Hepatitis D, Hepatitis E, Meningitis A, Meningitis B, Meningitis C, Meningitis W, Meningitis Y, Pnemococcal (23 valent or more), Smallpox, Typhoid, Bacille Calmette Guerin, Tuberculosis, Human Immunodeficiency Virus, Anthrax, or the like, to which children or adults when not immunized earlier are susceptible, particularly to which children are susceptible.

The Polio Vaccine formulation prepared according to the present invention is very beneficial for primary immunization of children because it not only prevents polio infection but also other types of infection to which children or adults when not immunized earlier are susceptible, particularly to which children are susceptible.

Background Of The Invention

Poliomyelitis (also commonly referred to as 'Polio') is an acute infection that involves the gastrointestinal tract and, occasionally, the central nervous system. It is acquired by fecal-oral transmission. In the prevaccine era, infection with poliovirus was common, with epidemics occurring in the summer and fall in temperate areas. The incidence of poliomyelitis declined rapidly after the licensure of inactivated polio vaccine in 1955 and oral polio vaccine in the 1960s. The last cases of indigenously acquired polio in the United States occurred in 1979. Although a polio eradication program led to elimination of polio in the Western Hemisphere, where the last case associated with wild poliovirus was detected in 1991, outbreaks of

vaccine-derived poliovirus type 1 occurred in the Dominican Republic and Haiti in July 2000 and in the Philippines in 2001. In spite of these recent outbreaks, the global polio eradication initiative has reduced the number of reported polio cases worldwide by >99% since the mid-1980s, and worldwide eradication of the disease appears feasible in the near future. Clinical manifestations of poliovirus infection range from asymptomatic (the majority of infections) to symptomatic, including acute flaccid paralysis of a single limb to quadriplegia, respiratory failure, and, rarely, death.

Polio is caused by three types of quite stable viruses namely Type 1, Type 2, and Type 3 belonging to the family of enteroviruses. There are different polio vaccines available in the market. These vaccines are trivalent containing a mixture of all the three types of poliovirus so as to confer immunity against all of them.

One type of Polio Vaccine available is Inactivated Polio Vaccine (IPV) based on Salk strain (Salk IPV). The vaccine contains all the three types of polio virus, inactivated by formalin. The major advantage of Salk IPV is that it can be incorporated with other childhood immunizations like DPT. However the Salk IPV has the several disadvantages such as the requirement of stringent standards for growing the Salk strain of poliovirus, and the potential risk posed due to accidental release of live virulent polio viruses into the environment while handling of virulent strain of polio viruses during its production process. Moreover, vaccine formulations comprising the Salk strain of poliovirus are costly, and have a comparatively lesser potency.

Another type of Polio Vaccine available is the Oral Polio Vaccine (OPV) based on Sabin strain. This orally administered Polio vaccine consists of live inactivated strains of polio virus. Such vaccine compositions comprising of live inactivated Sabin strains of polio virus are comparatively cheaper than Inactivated Polio-Vaccine (IPV) based on Salk strain.

The OPV is commonly used because of the ease of availability of monovalent bulks, larger number of manufacturing facilities, ease of administration, and most significantly, the manufacture of OPV does not pose any risk of accidental release of virulent polio viruses into the environment. However some of the disadvantages associated with the OPV are the perishability of OPV at about temperatures above minus 20°C, the reversal of attenuated

strains to virulent strains in vaccine composition, and vaccine associated paralytic poliomyelitis.

US patent number 5,639,649 by Almond et al relates to the construction of vaccines against rhinoviruses and enteroviruses, particularly polioviruses, by the introduction of defined mutations into their genomes. These mutations attenuate the virulence of wild type viruses and can further attenuate existing live attenuated vaccine virus strains, thereby making them less likely to revert to virulence.

US patent number 5,618,539 by Dorval et al pertains to stabilized viral vaccines, particularly live viral vaccines for poliomyelitis, comprising an aqueous solution of a live virus and a stabilizing amount of a compound containing at least two amino or imine groups, such as basic amino acids e.g. lysine.

The US patent number 4,525,349 by Montagnon et al describes a process for large-scale production of poliomyelitis vaccine. However, it does not mention the stabilization process of the polio virus prior to the inactivation stage.

Hence, there still exists an unmet need for developing cheaper vaccine compositions comprising live attenuated polio virus, which can be administered by injection and are devoid of toxicity or have reduced toxicity, without compromising the immunogenicity.

Moreover, although different types of polio vaccines are known in the art, there is no prior art disclosure of a stable and efficacious formulation comprising Sabin strain derived inactivated polio vaccine and other antigens effective against pathogens causing infections among children or adults, particularly children, which are devoid of toxicities or are less toxic, and are immunogenic.

Summary Of The Invention

It is an objective of the present invention to provide an Inactivated Polio Vaccine derived from Sabin strain for effective immunization against Poliomyelitis.

It is also an objective of the present invention to provide a process for the preparation of an Inactivated Polio Vaccine derived from Sabin strain for effective immunization against Poliomyelitis characterized in that the Sabin strain of the Polio virus are first stabilized by adding a stabilizer, and then inactivated by an inactivator.

According to a process of the present invention, the monovalent bulk of polio virus is initially stabilized by adding stabilizer followed by inactivation comprising of the following steps:

i) initial stabilization of bulk of polio virus by adding stabilizer,
ii) addition of inactivator such as formaldehyde (formalin) or beta-propiolactone to each of the filtered and purified monovalent pool or only heating the pool,
iii) incubation at about 37°C upto 48 hours and then at 2°C to 8°C upto 12 days in a single cycle before further processing,
iv) testing for free formaldehyde content or the inactivator concentration performed after every 12 hours during the inactivation process and the desired concentration level is maintained by intermittent readjustments,
v) optionally doing a second filtration after the process of inactivation.

According to another process of the present invention, the monovalent bulk of polio virus is initially stabilized by adding stabilizer followed by inactivation comprising of the following steps:

i) initial stabilization of bulk of polio virus by adding stabilizer,
ii) addition of inactivator such as formaldehyde (formalin) or beta-propiolactone or only heating,
iii) incubation at 37°C for 4 hours followed by chilling at 2°C to 8°C for 20 hours,
iv) repetition of the incubation and chilling cycle up to 12 times so that total exposure at 37°C does not exceed 48 hours during 12 days of incubation,
v) testing for free formaldehyde content or the inactivator concentration performed after every 12 hours during the inactivation process and the desired concentration level is maintained by intermittent readjustments,
vi) optionally doing a second filtration done after the process of inactivation.

It is another objective of the present invention to provide a formulation comprising an Inactivated Polio Vaccine derived from Sabin strain for immunization against Poliomyelitis characterized in that the strains of the Polio virus are first stabilized by adding a stabilizer, and then inactivated by an inactivator.

It is a further objective of the present invention to provide a stable and effective Polio Vaccine formulations with other antigens which upon administration provides immunization not only against polio infection but also against other pathogens causing Hepatitis C, Hepatitis D, Hepatitis E, Meningitis A, Meningitis B, Meningitis C, Meningitis W, Meningitis Y, Pnemococcal (23 valent or more), Smallpox, Typhoid, Bacille Calmette Guerin, Tuberculosis, Human Immunodeficiency Virus, Anthrax, or the like, to which children or adults are susceptible, particularly to which children are susceptible.

Detailed Description Of The Of The Invention

The present invention provides an Inactivated Polio Vaccine derived from Sabin strain for effective immunization against Poliomyelitis.

The present invention provides a Polio Vaccine derived from Sabin strain for effective immunization against Poliomyelitis prepared from Sabin seed strain or Sabin strain monovalent bulk suspensions used for blending of trivalent oral polio vaccine or by growing Sabin strain Polio viruses (from monovalent bulk used for trivalent OPV) in vero cell line or any suitable cell culture characterized in that the Polio virus is first stabilized by adding a stabilizer, as herein described, and then inactivated by adding formaldehyde (Formalin) according to a specific embodiment of the invention.

The Polio vaccine of the present invention eliminates the disadvantages, associated with known Polio vaccines, OPV and Salk IPV.

In an embodiment, the Sabin strain derived inactivated polio vaccine is trivalent containing a mixture of all the three types of polio virus namely type 1, Type 2 and Type 3.

In another embodiment, the Sabin strain derived inactivated polio vaccine is bivalent containing a mixture of any two types of polio virus selected from Type 1, Type 2 and Type 3.

In an essential embodiment of the present invention, the polio viruses are stabilized before inactivating the viruses to retain their shape, since the three-dimensional shape of the viruses are highly important for their antigenicity (immunogenicity). The stabilization process before inactivation protects the already weak Sabin strains from getting deformed or damaged during inactivation process.

The stabilization process of the monovalent bulk of poliovirus involves an addition of stabilizer(s) selected from but not limited to the group comprising of sucrose, trehalose, arginine hydrochloride, gelatin, magnesium chloride, aluminium chloride, and disodium EDTA, used either alone or in combination thereof, to retain the antigenicity of the vaccine during inactivation process.

In an embodiment, the inactivation process of each of filtered and purified monovalent pool involves addition of inactivator such as but not limited to formaldehyde (formalin) or beta-propiolactone, or mixture thereof preferably at about 0.001% to 0.1%, more preferably at 0.025% concentration, and/or heating and incubation at about 37°C for optimized number of days.

The present invention provides a process for the preparation of Inactivated Polio Vaccine derived from Sabin strain for effective immunization against Poliomyelitis

In an embodiment, the present invention provides a process for preparation of polio vaccine from Sabin seed strain or Sabin strain monovalent bulk suspensions used for blending of trivalent oral polio vaccine or by growing Sabin strain polio viruses in vero cell line or any suitable cell culture for the preparation of oral polio vaccine characterized in that the polio vaccine prepared is stabilized by a stabilizer and then inactivated by an inactivator.

In a preferred embodiment, the Polio vaccine prepared from Sabin strain, as stated above, may be stabilized and inactivated, according to present invention, by one of the following two processes.

Process –I:

Stabilization process: The monovalent bulk of poliovirus is initially stabilized by adding stabilizer like Sucrose, Trehalose or Arginine hydrochloride, to retain the antigenicity of the vaccine during inactivation process.

Inactivation process: The inactivation process is initiated immediately. Inactivation of each of the filtered and purified monovalent pool is carried out by adding formaldehyde (formalin) at 0.025% concentration or beta-propiolactone and incubation at about 37°C upto 48 hours and then at 2°C to 8°C upto 12 days in a single cycle before further processing. Either of the treatment could be used for inactivation. The test for free formaldehyde content is performed after every 12 hours during the inactivation process and the desired concentration level is maintained by intermittent readjustments. A second filtration is done after the process of inactivation. Consistent inactivation of the virus is monitored and verified.

Process –II:

Stabilization process: The monovalent bulk vaccines are stabilized using the same procedure as described for Process-I.

Inactivation Process: Inactivation is carried out by adding formaldehyde (formalin) at 0.025% concentration or beta-propiolactone and incubation at 37°C for 4 hrs followed by chilling at 2°C to 8°C for 20 hrs. This incubation & chilling cycle can be repeated up to 12 times so that total exposure at 37°C does not exceed 48 hrs during 12 days of incubation. The test for free formaldehyde content is to be performed after every 12 hrs during the inactivation process and the desired concentration level is maintained by intermittent readjustments. A second filtration is to be done after the process of inactivation. Consistent inactivation of the virus is monitored and verified.

The maintenance of potency/antigenic integrity of highly heat labile Sabin viruses is ensured. The inactivation processes are standardized in such a way that live inactivated virus of each type is completely inactivated and the process ensures intact antigenic structure of Sabin strain. Inactivated vaccine is able to impart adequate immunity in vaccines when compared with standard vaccine. The Sabin strain poliovirus, in present invention, is stabilized before inactivation using stabilizer like sucrose, trehalose, arginine hydrochloride, magnesium chloride, aluminium chloride, and disodium EDTA to avoid degradation of nativeness and antigenic structure. The inactivated Polio Vaccine, prepared according to the present

invention, in monovalent, bivalent or trivalent form has required amount of equivalent D-antigen or antigen form which could be compared with an established reference standard of inactivated polio vaccine and/or provide sero-conversion and required protection.

The Sabin strain derived Inactivated Polio Vaccine (SIPV) prepared according to the present invention, possesses the advantages of both OPV and Salk IPV. The process of manufacture does not require growing of virulent strains of polio virus and hence the chances of accidental release of live virulent polio viruses into the environment is very less. Also the strains of Polio virus retain their shape even in the formulations which prevents a compromise on the immunogenicity, since the three-dimensional shape of the viruses are highly important for their effectiveness. Moreover, the SIPV are devoid of disadvantages like vaccine associated paralytic poliomyelitis. Also, the SIPV of the present invention can be effectively combined with other antigens thus providing effective immunization against several diseases at the same time.

The Sabin strain derived Polio vaccine used in the formulations according to the present invention may be trivalent containing a mixture of all the three types namely Type 1, Type 2 and Type 3 of Polio virus or may be a mixture of any two of the said Types.

The administration of the Polio Vaccine formulations prepared according to the present invention would overcome or at least mitigate the problems associated with multiple injections of the different antigens. The formulations provided by the present invention are stable and highly immunogenic which are suitable for administration to children or adults, particularly suitable for administration to children.

In an embodiment of the present invention, the Polio vaccine obtained by the standardized methods is used to provide a multivalent vaccine formulation comprising Sabin strain derived inactivated Polio vaccine (SIPV).

In another embodiment, the Polio vaccine obtained by the standardized methods is used to provide a multivalent vaccine formulation comprising Sabin strain derived inactivated Polio vaccine (SIPV) and a number 'n' of other antigens, optionally in combination with an adjuvant comprising one or more aluminum salts, in which the value of 'n' is 1 or greater than 1. Preferably the value of 'n' is about 1 to 30. More preferably the value of 'n' is 2,3,4,5

or 6. The term 'multivalent' used herein means vaccine formulation comprising at least two or more antigens.

In yet another embodiment of the present invention, a formulation is provided, wherein the Sabin strain derived inactivated polio vaccine is trivalent containing a mixture of all the three types of polio virus namely Type 1, Type 2 and Type 3, or bivalent containing a mixture of any two types of polio virus selected from Type 1, Type 2 and Type 3.

In yet another embodiment of the present invention, a formulation is provided wherein the vaccine is multivalent.

The other antigens present in the Polio Vaccine formulations according to the present invention are selected from but not limited to those which provide immunity against one or more of the pathogens causing infections like Hepatitis C, Hepatitis D, Hepatitis E, Meningitis A, Meningitis B, Meningitis C, Meningitis W, Meningitis Y, Pnemococcal (23 valent or more), Smallpox, Typhoid, Tuberculosis, Human Immunodeficiency Virus, Anthrax or the like, to which children or adults are susceptible, particularly to which children are susceptible.

The adjuvant(s) used in the present invention include but not limited to aluminium hydroxide, aluminium phosphate, calcium phosphate, oil emulsions such as Freund's emulsified oil adjuvants (complete and incomplete), Arlacel A, Mineral oil, Emulsified peanut oil adjuvant (adjuvant 65), products from bacteria (their synthetic derivatives as well as liposomes) or gram-negative bacteria, endotoxins, cholesterol, fatty acids, aliphatic amines, paraffinic and vegetable oils, Algammulin, and QS-21. Preferably, aluminium hydroxide and aluminium phosphate is used as adjuvant either alone or in combination thereof.

In an embodiment, the formulations of the present invention additionally comprises of but not limited to preservatives or tissue fixatives or both.

The preservatives used in the formulations of the present invention are selected from but not limited to ethyl mercury, thimerosal, merthiolate, and 2-phenoxy ethanol, used either alone or in combination thereof.

The tissue fixative used in the formulations of the present invention is but not limited to , formaldehyde.

In another aspect, the invention provides a vaccine formulation comprising Sabin strain derived inactivated Polio vaccine (SIPV) adsorbed to Aluminium Phosphate (AP) and an antigen adsorbed to AP or to Aluminium Hydroxide (AH) or any other suitable adjuvant selected from an antigen providing immunity against one or more of the following pathogens namely Hepatitis C, Hepatitis D, Hepatitis E, Meningitis A, Meningitis B, Meningitis C, Meningitis W, Meningitis Y, Pnemococcal (23 valent or more), Smallpox, Typhoid, Bacille Calmette Guerin, Tuberculosis, Human Immunodeficiency Virus, Anthrax or the like.

In a further aspect of the invention, there is provided a stable and effective vaccine formulation directed to the prevention of at least two diseases, comprising SIPV and at least one other antigens selected from Hepatitis C, Hepatitis D, Hepatitis E, Meningitis A, Meningitis B, Meningitis C, Meningitis W, Meningitis Y, Pnemococcal (23 valent or more), Smallpox, Typhoid, Bacille Calmette Guerin, Tuberculosis, Human Immunodeficiency Virus, Anthrax or the like.

In another embodiment of the present invention, the formulation also includes SIPV compatible antigens like antigens against Meningitis B, Meningitis A and C, or otitis media.

In a further embodiment, the inactivated monovalent viruses are mixed with but not limited to stabilizer or/and preservative to make monovalent, bivalent, trivalent or multivalent inactivated vaccine. The final vaccine may be spray dried & resuspended in ready-to-inject liquids like perfluorocarbons, requiring no refrigeration for storage or preparation before injection.

The formulations prepared according to the present invention may be administered through but not limited to the routes such as oral, transdermal, parenteral, nasal, mucosal, and the like.

In a preferred embodiment of the present invention, the formulation is administered as an injection through the parenteral route, particularly through subcutaneous or intramuscular route.

The example provided below illustrates the embodiments of the present invention. However, it does not intend to limit the scope of the present invention.

Example-1

Seed strain of the same type and passage as used for poliomyelitis vaccine (oral): Oral Polio Vaccine (OPV) is propagated to produce monovalent suspension for inactivation.

CELL CULTURE:

i) Different cell substrates for propagation of virus include Human diploid cell line, Monkey kidney cells, Vero cell line or any other suitable cell culture.

ii) Large scale production of cells:

1. Preparation of the Manufacturer's working cell bank (MWCB): MWCB in case of primary cell cultures (cells derived from normal tissue and stored frozen at minus 70°C) or from any other permitted continuous cell line are prepared from the cells & the cells pooled after serial subculture within the specified number of cell cultures.

2. Any of the following systems can be used which ever is suitable, for stepwise increase in the volume of cells.

- Roller bottles: Growth in roller bottles differs from that in stationary cultures in distribution of cells on glass surface and maximum attainable cell density. Roller bottle cultures do not deteriorate less rapidly as they can tolerate a density nearly two times higher than that of stationary cultures. Mammalian cells grown in monolayer culture will adapt best to roller bottle technique.

- Cell factories: These are stack of culture trays that share a common inlet and outlet port. These provide cells with a growing surface as large as inside of roller bottle, but take up less space inside incubator. These are ideal for adherent cell cultures, have low contamination risk and are compact.

- Roux flasks

- Micro-carrier system

3. Samples for control cell cultures are incubated separately for at least two weeks and these are examined for evidence of cytopathic changes.

PROPAGATION OF POLIO VIRUS IN CELL CULTURES:

i) Seed lot system:

a) Passage level as followed for Oral Polio Vaccine OR

b) Its next passage level as derived from monovalent bulk suspension that could be used to manufacture oral polio vaccine.

1. The vaccine is manufactured on the basis of virus seed lot system. Virus seed lot is a quantity of virus processed together and of uniform composition prepared from seed lot.

2. Sub-culture of the seed virus should not be done more than 10 times, counted from a seed lot used for the production of the vaccine on which original laboratory and field tests were done.

SINGLE HARVEST AND MONOVALENT POOLS:

The virus is propagated in cell cultures and harvested from cell cultures derived from a single batch of cells and processed together. This is known as single harvest. The single harvests of one type of virus suspension are processed at the same time to get crude form of monovalent pool.

FILTRATION OR CLARIFICATION OF MONOVALENT POOL:

Crude virus suspension of each monovalent pool is purified stepwise through filters of decreasing porosity. The purpose of filtration step is to remove particulate material and other substance that may affect inactivation process as such aggregates tend to increase on standing.

CONCENTRATION OF THE MONOVALENT POOL:

Each filtered monovalent pool is concentrated by Ultra-filtration.

PURIFICATION OF THE MONOVALENT POOL:

Chromatography with DEAE sepharose / immobilized DNA-ase column / immuno-adsorption column is employed for the purification of the monovalent pool. The purification process reduces consistently the level of cellular DNA from that of initial virus harvest by a factor of at least 10^8. The D-antigen concentration is determined by ELISA and display of comparable immunogenicity in rats. Accordingly the potency is adjusted. The monovalent pools of Poliovirus are blended to form the final trivalent bulk product.

STABILISING THE SABIN VIRUS BEFORE INACTIVATION:

The antigenicity of the vaccine is closely associated with the stability of the native virus antigens during inactivation process. The poliovirus antigenic structure is to be initially stabilized by adding stabilizer like sucrose, trehalose, or arginine hydrochloride.

INACTIVATION USING FORMALDEHYDE:

i) Inactivation process is initiated preferably within 24 hours and not later than 72 hours after filtration.

ii) Inactivation of each of filtered and purified monovalent pool is carried out by adding formaldehyde (formalin) at 0.025% and incubation at 37°C for specific time period as described earlier. The test for free formaldehyde is performed at intervals and the desired concentration level is maintained by intermittent readjustments.

iii) A second filtration is done after the process of inactivation.

iv) Consistent inactivation of the virus is monitored and verified.

v) For testing the extent of inactivation, formaldehyde is neutralized by adding sodium bisulfite and subsequently dialyzing it out.

FORMULATION AND MANUFACTURING OF THE VACCINE:

The purified inactivated bulk is filtered, preferably through 0.22 micron or 0.45-micron filter. The inactivated monovalent viruses is optionally mixed with stabilizer and/or preservative to make monovalent, bivalent, trivalent or multivalent inactivated vaccine.

TESTING THE EFFICACY OF THE VACCINE:

The efficacy of the vaccine as manufactured above, as compared with established reference vaccine, is found to be similar in in-vitro and/or in-vivo method.

Various other tests based on the standard accepted methods were carried on the vaccine manufactured above such as pH determination, sterility testing, test for effective inactivation, abnormal toxicity study, volume measurement, in vitro potency determination, endotoxin test, identity test, protein nitrogen content estimation, and determination of stabilizer content and formaldehyde content to ensure its effectiveness, safety and potency.

The in vitro potency of the SIPV manufactured above was determined using Sandwich ELISA. Two primary antibodies raised in different species against polio virus were utilized

for the same and a second antibody conjugated with HRP is used against the second primary antibody. The results were analyzed by comparison with reference standard.

The identity test for the vaccine was carried using direct ELISA. Antigen was coated well in 96 ELISA plates and type specific anti polio sera is employed for determining the presence of all the three types of polio virus in the vaccine.

CLAIMS

1. An Inactivated Polio Vaccine derived from Sabin strain for effective immunization against Poliomyelitis.

2. A polio vaccine according to claim 1, wherein the Sabin strain derived inactivated polio vaccine is trivalent containing a mixture of all the three types of polio virus namely type 1, Type 2 and Type 3.

3. A polio vaccine according to claim 1, wherein the Sabin strain derived inactivated polio vaccine is bivalent containing a mixture of any two types of polio virus selected from Type 1, Type 2 and Type 3.

4. A polio vaccine according to claim 1, prepared from Sabin seed strain or Sabin strain monovalent bulk suspensions used for blending of trivalent oral polio vaccine or by growing Sabin strain polio viruses in vero cell line or any suitable cell culture for the preparation of oral polio vaccine characterized in that the polio vaccine prepared is stabilized by stabilizer and then inactivated by an inactivator.

5. A polio vaccine according to claim 4, wherein the stabilizer is selected from the group comprising of sucrose, trehalose, arginine hydrochloride, gelatin, magnesium chloride, aluminium chloride, and disodium EDTA, and the like used either alone or in com bination thereof.

6. A polio vaccine according to claim 4, wherein the inactivator is selected from the group comprising of formaldehyde or beta-propiolactone or mixture thereof.

7. A process for the preparation of Inactivated Polio Vaccine derived from Sabin strain for effective immunization against Poliomyelitis.

8. A process for preparation of polio vaccine according to claim 7, from Sabin seed strain or Sabin strain monovalent bulk suspensions used for blending of trivalent oral polio vaccine or by growing Sabin strain polio viruses in vero cell line or any suitable cell culture

for the preparation of oral polio vaccine characterized in that the polio vaccine prepared is stabilized by a stabilizer and then inactivated by an inactivator.

9. A process according to claims 7 and 8, wherein the monovalent bulk of polio virus is initially stabilized by adding stabilizer followed by inactivation comprising of the following steps:

i) initial stabilization of bulk of polio virus by adding stabilizer,
ii) addition of inactivator such as formaldehyde or beta-propiolactone to each of the filtered and purified monovalent pool or only heating the pool,
iii) incubation at about 37°C upto 48 hours and then at 2°C to 8°C upto 12 days in a single cycle before further processing,
iv) testing for free formaldehyde content or the inactivator concentration performed after every 12 hours during the inactivation process and the desired concentration level is maintained by intermittent readjustments,
v) optionally doing a second filtration after the process of inactivation.

10. A process according to claims 7 and 8, wherein the monovalent bulk of polio virus is initially stabilized by adding stabilizer followed by inactivation comprising of the following steps:

i) initial stabilization of bulk of polio virus by adding stabilizer,
ii) addition of inactivator such as formaldehyde or beta-propiolactone or only heating,
iii) incubation at 37°C for 4 hours followed by chilling at 2°C to 8°C for 20 hours,
iv) repetition of the incubation and chilling cycle up to 12 times so that total exposure at 37°C does not exceed 48 hours during 12 days of incubation,
v) testing for free formaldehyde content or the inactivator concentration performed after every 12 hours during the inactivation process and the desired concentration level is maintained by intermittent readjustments,
vi) optionally doing a second filtration done after the process of inactivation.

11. A process according to claims 8 to 10, wherein the stabilizer is selected from the group comprising of sucrose, trehalose, arginine hydrochloride, gelatin, magnesium chloride,

aluminium chloride, and disodium EDTA, and the like used either alone or in combination thereof.

12. A process according to claims 8 to 10, wherein the inactivator is selected from the group comprising of formaldehyde or beta-propiolactone or mixture thereof.

13. A process according to claims 7 to 12, which comprises mixing Sabin strain derived inactivated polio vaccine adsorbed on conventional adjuvant with a number 'n' of other antigens.

14. A process according to claim 13, wherein the other antigens are selected from a group comprising of Hepatitis C, Hepatitis D, Hepatitis E, Meningitis A, Meningitis B, Meningitis C, Meningitis W, Meningitis Y, Pnemococcal (23 valent or more), Smallpox, Typhoid, Bacille Calmette Guerin, Tuberculosis, Human Immunodeficiency Virus, Anthrax, or the like used alone or in combination thereof.

15. The process as according to claim 13, wherein the value of 'n' is about 1 to 30.

16. The process as according to claim 13, wherein the value of 'n' is 2,3,4,5, or 6.

17. The process according to claims 7 to 16, wherein the Sabin strain derived inactivated polio vaccine is trivalent containing a mixture of all the three types of polio virus namely type 1, Type 2 and Type 3.

18. The process according to claims 7 to 16, wherein the Sabin strain derived inactivated polio vaccine is bivalent containing a mixture of any two types of polio virus selected from Type 1, Type 2 and Type 3.

19. The process according to claims 7 to 16, wherein the vaccine is multivalent.

20. A Sabin strain derived Inactivated Polio Vaccine formulation for effective immunization against Poliomyelitis.

21. A Sabin strain derived Inactivated Polio Vaccine formulation according to claim 20 for effective immunization against Poliomyelitis, wherein the vaccine is adsorbed on adjuvant optionally with a number 'n' of other antigen(s).

22. A formulation according to claim 21, wherein the antigens are selected from a group comprising of Hepatitis C, Hepatitis D, Hepatitis E, Meningitis A, Meningitis B, Meningitis C, Meningitis W, Meningitis Y, Pnemococcal (23 valent or more), Smallpox, Typhoid, Bacille Calmette Guerin, Tuberculosis, Human Immunodeficiency Virus, Anthrax, or the like used alone or in combination thereof.

23. A formulation according to claim 21, wherein the value of 'n' is about 1 to 30.

24. A formulation according to claim 21, wherein the value of 'n' is 2,3,4,5,or 6.

25. A formulation according to claims 20 to 24, wherein the Sabin strain derived inactivated polio vaccine is trivalent containing a mixture of all the three types of polio virus namely Type 1 , Type 2 and Type 3.

26. A formulation according to claims 20 to 24, wherein the Sabin strain derived inactivated polio vaccine is bivalent containing a mixture of any two types of polio virus selected from Type 1, Type 2 and Type 3.

27. A formulation according to claims 20 to 24, wherein the vaccine is multivalent.

28. A formulation according to claims 20 to 27, which comprises of adjuvant(s) selected from but not limited to aluminium hydroxide, aluminium phosphate, calcium phosphate, oil emulsions such as Freund's emulsified oil adjuvants (complete and incomplete), Arlacel A, Mineral oil, Emulsified peanut oil adjuvant (adjuvant 65), products from bacteria (their synthetic derivatives as well as liposomes) or gram-negative bacteria, endotoxins, cholesterol, fatty acids, aliphatic amines, paraffinic and vegetable oils, Algammulin, and QS-21 used either alone or in combination thereof.

29. A formulation according to claim 28, wherein the adjuvant is aluminium hydroxide or aluminium phosphate or mixtures thereof.

30. A formulation according to claims 20 to 29, which additionally comprise of preservatives or tissue fixatives or both.

31. A formulation according to claim 30, wherein the preservatives are selected from ethyl mercury, thimerosal, merthiolate, and 2-phenoxy ethanol used either alone or in combination thereof.

32. A formulation according to claim 30, wherein the tissue fixative used is formaldehyde.

33. A Polio vaccine derived from Sabin strain substantially as described and illustrated herein.

34. A process for the preparation of Polio vaccine derived from Sabin strain substantially as described and illustrated herein.

35. A Polio vaccine formulation derived from Sabin strain substantially as described and illustrated herein.

INTERNATIONAL SEARCH REPORT

Int nal Application No
PCT/IN2004/000267

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/13

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, LIFESCIENCES, SCISEARCH, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ABE S ET AL: "EFFECTS OF ARILDONE ON THE IMMUNOGENICITY OF FORMALIN-INACTIVATED POLIOVIRUSES" JAPANESE JOURNAL OF MEDICAL SCIENCE AND BIOLOGY, vol. 40, no. 1, 1987, pages 1-14, XP001203965 ISSN: 0021-5112	1,4,6-8, 12,20, 30,31
Y	page 2, paragraph 3 page 3, paragraph 6 - page 4, paragraph 2 tables 1,2	5
	-/--	

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 December 2004	28/12/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Voigt-Ritzer, H

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JIANG S D ET AL: "INACTIVATION OF POLIOVIRUS WITH BETA PROPIOLACTONE" JOURNAL OF BIOLOGICAL STANDARDIZATION, vol . 14, no. 2, 1986, pages 103-110, XP001203964 ISSN: 0092-1157	1,7,20, 21,28, 30-32
Y	page 104, paragraph 6 - paragraph 7 page 105, paragraph 2	13-16, 22-24
X	MARTIN JAVIER ET AL: "Characterization of formaldehyde-inactivated poliovirus preparations made from live-attenuated strains." JOURNAL OF GENERAL VIROLOGY, vol . 84, no. 7, July 2003 (2003-07), pages 1781-1788, XP002308423 ISSN: 0022-1317 page 1782 page 1787, column 1, paragraph 2	1,7,20, 21,28-32
X	MURPH J R ET AL: "SABIN INACTIVATED TRIVALENT POLIOVIRUS VACCINE FIRST CLINICAL TRIAL AND SEROIMMUNITY SURVEY" PEDIATRIC INFECTIOUS DISEASE JOURNAL, vol . 7, no. 11, 1988, pages 760-765, XP009040788 ISSN: 0891-3668 abstract page 761, column 1, paragraph 5 - column 2, paragraph 1	1-3,7, 18,20, 25,26, 30,31
X	DOI Y ET AL: "Progress with inactivated poliovirus vaccines derived from the Sabin strains." DEVELOPMENTS IN BIOLOGICALS. 2001, vol . 105, 2001, pages 163-169, XP009040789 ISSN: 1424-6074 table 1 page 164, paragraphs 1,2	1-3,7, 17-20, 25-27, 30,31
X	KERSTEN G ET AL: "Antigenic and immunogenic properties of inactivated polio vaccine made from Sabin strains" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol . 17, no. 15-16, 9 April 1999 (1999-04-09), pages 2059-2066, XP004165056 ISSN: 0264-410X	1-3,7, 17-20, 25-27, 30,31
Y	page 2060, column 1, paragraphs 2,3 page 2061, column 1, paragraph 2 page 2065, column 2, paragraph 1	13-16, 22-24

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCHMITT H J ET AL: "PRIMARY VACCINATION OF INFANTS WITH DIPHTHERIA-TETANUS-ACELLULAR PERTUSSIS-HEPATITIS B VIRUS- INACTIVATED POLIO VIRUS AND HAEMOPHILUS INFLUENYAE TYPE B VACCINES GIVEN AS EITHER SEPARATE OR MIXED INJECTIONS" JOURNAL OF PEDIATRICS, MOSBY-YEAR BOOK, ST. LOUIS, MO, US, vol. 137, no. 3, September 2000 (2000-09), pages 304-312, XP009023955 ISSN: 0022-3476 abstract	13-16, 22-24
Y	MIRCHAMSY H ET AL: "Stabilizing effect of magnesium chloride and sucrose on Sabin live polio vaccine." DEVELOPMENTS IN BIOLOGICAL STANDARDIZATION. 1978, vol. 41, 1978, pages 255-257, XP001203962 ISSN: 0301-5149 page 255, paragraph 1	5

INTERNATIONAL SEARCH REPORT

national application No.
PCT/IN2004/000267

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.: 33-35
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 33-35
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claim s Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 33-35

Claims 33-35 refer to a vaccine "substantially as described and illustrated herein". This is not a technical features.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.