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PATENT SPECIFICATION

NO DRAWINGS

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COMPLETE SPECIFICATION

Vaccine Process and Product

We, MERCK & CO. INC., a corporation duly organised and existing under the laws of the State of New Jersey, United States of America, of Rahway, New Jersey, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention concerns a combined live measles virus and smallpox vaccine. Vaccines in accordance with the present invention have an appreciable storage life.

Measles (Rubelola) and smallpox, individually, are extremely important health problems to most of the peoples of the world. Highly effective vaccines against each of these diseases have been developed, but the vaccines are ordinarily administered separately. There would be considerable simplification and advantage in use of the two vaccines co-administered in a single dose. This would be especially true for the underdeveloped countries of the world in which medical facilities for administering vaccines are limited.

Early investigation efforts showed that measles and smallpox vaccines can be co-administered together in man using a jet gun as the means for injection. However, the procedure involved mixing the separate fluid measles and fluid smallpox vaccines immediately prior to administration. It is obvious that if the two materials could be premixed and stabilized in mixed form by drying, there would be considerable facilitation in handling the vaccines at the time of field use, especially in minimizing the chance for contamination and for error in manipulation.

According to the invention, a measles vaccine is mixed with a smallpox vaccine and the resulting mixture is then lyophilized to produce a stable dried combined measles and smallpox vaccine, the quantities of measles virus and smallpox in the uncombined vaccines being such that the product contains at least 10 $TCID_{50}$ units of measles virus and at least 10^2PFU of smallpox virus per 0.1 ml. of the intermediate mixture. The intermediate mixture may be dispersed into individual containers, preferably glass containers, and/or frozen, preferably at a temperature of −60° C, before it is lyophilized.

Preferably the resulting dried combined measles and smallpox vaccine contains at least 10^3TCID_{50} units of measles virus per 0.1 ml. of the intermediate mixture. It is also desirable that it should contain at least 10^5PFU of smallpox virus per 0.1 ml of the intermediate mixture.

The dried combined measles and smallpox vaccine may be diluted with a parenterally administrable liquid carrier to restore its volume to that of the intermediate mixture and produce a combined measles and smallpox vaccine suitable for administration. Preferably the parenterally administrable liquid carrier is sterile distilled water. The resulting vaccine is suitable for injection into human subjects by use of a jet gun having either an oblique or a vertical head. Intradermal or subcutaneous injection by hypoderic syringe is also acceptable. The oblique head of the jet gun allows 0.1 ml of the combined vaccine mixture to be injected intradermally. The vertical head, when fitted with a sleeve permitting an appropriate distance between nozzle and skin, permits dispensing 0.1 ml of combined vaccine intradermally and 0.4 ml into the deeper tissues. Use without the sleeve permits essentially all the vaccine to be deposited at greater depth than in the skin. Appropriate adjustment of the instrument permits a wide variation in these volumes.

The measles vaccine can be any one which is recognized as acceptable for human use, such as the Enders B-level live attenuated

[*Price 4s. 6d.*]

measles vaccine grown in chick embryo cell cultures. Such vaccine is commercially available from Merck and Co. Inc. under the trademark RUBEOVAX. Another measles vaccine which can be used is the Schwarz strain of live attenuated virus which also is commercially available.

If it is not desired to use a commercially available measles vaccine, the vaccine employed would be prepared by conventional techniques. A representative process is one using virus grown in cell culture preparations of chick embryos. The cell cultures are grown in a synthetic nutrient medium such as that designated Medium 199 containing bovine serum and appropriate antibiotics. The measles virus used to infect the cell cultures is a pure culture of the Edmonston or other acceptable strain of measles virus proven to be free of extraneous viruses. Virus which is harvested from the cell cultures is clarified by filtration to remove any extraneous cell debris. The virus preparation then is tested as prescribed by the Division of Biologic Standards of the National Institutes of Health, U.S. Public Health Service. Vaccine which conforms to all the regulatory standards is acceptable for use in man.

The smallpox vaccine can be any known one that is recognized as being acceptable for human use. It can be propagated in the calf, in chick embryos, in cell cultures of chick embryo or other acceptable tissue, or by any other acceptable procedure.

Procedures for preparation and control testing of smallpox vaccine have been described in the Minimum Requirements for smallpox vaccine published by the Division of Biologics Standards, the National Institutes of Health, U.S. Public Health Service. Most often, the smallpox vaccine consists of live vaccinia (cowpox) virus obtained from the vesicles in the ectoderm of the calf (or other suitable animal) at the height of vesiculation resulting from the artificial infection of the animal with a suitable seed preparation of vaccinia virus. The vesicles are removed from the calf or other animal in the form of a pulp or "lymph" and tested as prescribed by the Division of Biologics Standard of the National Institutes of Health. The pulp is then treated by a process which removes extraneous protein and markedly reduces or completely eliminates bacterial and fungal contamination. It is then subjected to tests for safety, purity and potency.

An appropriate stabilizer is preferably added to the two vaccines to prevent or to minimize loss of potency on drying. The final product may also contain neomycin sulfate or other acceptable antibiotic or preservative.

The mixing of the two vaccines is carried out under aseptic conditions. After dilution to restore it to its original volume, the mixed vaccine intended for human use preferably contains at least 10^3 $TCID_{50}$ per 0.1 ml, (50% tissue culture infective doses) of measles virus and about 10^5 plaque forming units (PFU) per 0.1 ml, of vaccinia virus. The virus titer obtained by plaque method usually equals that obtained by observation of cytopathic effect ($TCID_{50}$). These amounts of virus will evoke antibody against both viruses in essentially all nonimmune persons. Also, essentially all individuals will develop a primary smallpox "take" with scar formation.

The following Examples illustrate the invention.

EXAMPLE 1

Measles vaccine with a pre-pool titer of $10^{3.4}$ $TCID_{50}$/0.1 ml was used.

Vaccinia had a calculated titer of $10^{8.4}$ PFU/ml. The smallpox vaccine was mixed with the measles vaccine in a ratio of one part smallpox to 59 parts measles.

The combined vaccine was filled in 11 ml amounts, frozen at −60° C. and dried by lyophilization. For testing or for use, the contents of the vial was reconstituted with 11 ml of sterile distilled water.

An assay of this mixture gave the following results:

Vaccinia plaque assay:	$10^{5.6}$/0.1 ml
Vaccinia tube assay:	$10^{5.4}$ $TCID_{50}$/0.1 ml
Measles assay:	$10^{2.9}$ $TCID_{50}$/0.1 ml

The following examples illustrate variations of Example 1 in the dilution of smallpox vaccine in measles vaccine.

Example 2—1 part smallpox—9 parts measles.

Example 3—0.1 part smallpox—9.9 parts measles.

Example 4—0.01 part smallpox—9.99 parts measles.

Example 5—0.001 part smallpox—9.999 parts measles.

Tests on these mixtures assayed as follows:

The smallpox virus was assayed for both the undried frozen vaccine and the dried product in order to determine any loss of potency due to lyophilization.

Example	Descript.	Smallpox vaccine $TCID_{50}$/0.1 ml	Smallpox vaccine PFU/0.1 ml	Measles vaccine $TCID_{50}$/0.1 ml
2	Frozen	$10^{5.6}$	$10^{5.9}$	
	Dried	$10^{5.6}$	$10^{5.9}$	$10^{2.9}$
3	Frozen	$10^{4.7}$	$10^{5.1}$	
	Dried	$10^{4.6}$	$10^{5.1}$	$10^{2.8}$
4	Frozen	$10^{3.6}$	$10^{3.8}$	
	Dried	$10^{3.8}$	$10^{3.8}$	$10^{2.6}$
5	Frozen	$10^{2.6}$	$10^{2.9}$	
	Dried	$10^{2.4}$	$10^{3.0}$	$10^{2.9}$

EXAMPLE 6

A mixture was prepared in the identical manner as Example 1 with the exception that the smallpox and measles vaccines were taken from different sources.

Virus	Test Results $TCID_{50}$/0.1 ml	Test Results PFU/0.1 ml
Smallpox (frozen mixture)	$10^{4.7}$	$10^{5.2}$
Smallpox (dried mixture)	$10^{4.6}$	$10^{5.1}$
Measles (dried mixture)	$10^{2.7}$	

Essentially all of the susceptible children vaccinated to date with the combination vaccine in Example 1 developed a primary smallpox vaccination "take," developed hemagglutination-inhibiting antibody against vaccinia virus, and these individuals were immune to smallpox as shown by challenge with smallpox vaccine given by the ordinary multiple puncture method at a later date. Essentially all children responded serologically to the measles vaccine with the development of antibody at titer levels which approximate those obtained when measles virus vaccine is given alone.

These examples illustrate that the two live virus vaccines are compatible with each other, and that by mixing and drying it is possible to produce a stable product which is highly effective for immunizing human subjects.

WHAT WE CLAIM IS:—

1. The process that comprises mixing a measles vaccine with a smallpox vaccine and then lyophilizing the resulting mixture to produce a stable dried combined measles and smallpox vaccine, the quantities of measles virus and smallpox virus in the uncombined vaccines being such that the product contains at least 10 $TCID_{50}$ units of measles virus and at least 10^2PFU of smallpox virus per 0.1 ml. of the intermediate mixture.

2. A process as claimed in claim 1, in which the smallpox vaccine is a live vaccinia virus vaccine.

3. A process as claimed in claim 1 or 2, in which the product contains at least 10^5 $TCID_{50}$ units of measles virus per 0.1 ml of the intermediate mixture.

4. A process as claimed in any preceding claim, in which the product contains at least 10^5PFU of smallpox virus per 0.1 ml. of the intermediate mixture.

5. A process a claimed in any preceding claim, in which the mixture is frozen before it is lyophilized.

6. A process as claimed in claim 5, in which the mixture is frozen at −60° C.

7. A process as claimed in any preceding claim including the step of diluting the product with a parenterally administrable liquid carrier to restore its volume to that of the intermediate mixture and produce a combined measles and smallpox vaccine suitable for administration.

8. A process as claimed in claim 7, in which the parenterally administrable liquid carrier is sterile distilled water.

9. A process as claimed in claim 1 substantially as hereinbefore described in any one of the Examples.

10. A dried combined measles and smallpox vaccine when prepared by a process as claimed in any one of claims 1 to 6.

Example	Descript.	Smallpox vaccine $TCID_{50}$/0.1 ml	Smallpox vaccine PFU/0.1 ml	Measles vaccine $TCID_{50}$/0.1 ml
2	Frozen	$10^{5.6}$	$10^{5.9}$	
	Dried	$10^{5.6}$	$10^{5.9}$	$10^{2.9}$
3	Frozen	$10^{4.7}$	$10^{5.1}$	
	Dried	$10^{4.6}$	$10^{5.1}$	$10^{2.8}$
4	Frozen	$10^{3.6}$	$10^{3.8}$	
	Dried	$10^{3.8}$	$10^{3.8}$	$10^{2.6}$
5	Frozen	$10^{2.6}$	$10^{2.9}$	
	Dried	$10^{2.4}$	$10^{3.0}$	$10^{2.9}$

EXAMPLE 6

A mixture was prepared in the identical manner as Example 1 with the exception that the smallpox and measles vaccines were taken from different sources.

Virus	Test Results $TCID_{50}$/0.1 ml	Test Results PFU/0.1 ml
Smallpox (frozen mixture)	$10^{4.7}$	$10^{5.2}$
Smallpox (dried mixture)	$10^{4.6}$	$10^{5.1}$
Measles (dried mixture)	$10^{2.7}$	

Essentially all of the susceptible children vaccinated to date with the combination vaccine in Example 1 developed a primary smallpox vaccination "take," developed hemagglutination-inhibiting antibody against vaccinia virus, and these individuals were immune to smallpox as shown by challenge with smallpox vaccine given by the ordinary multiple puncture method at a later date. Essentially all children responded serologically to the measles vaccine with the development of antibody at titer levels which approximate those obtained when measles virus vaccine is given alone.

These examples illustrate that the two live virus vaccines are compatible with each other, and that by mixing and drying it is possible to produce a stable product which is highly effective for immunizing human subjects.

WHAT WE CLAIM IS:—

1. The process that comprises mixing a measles vaccine with a smallpox vaccine and then lyophilizing the resulting mixture to produce a stable dried combined measles and smallpox vaccine, the quantities of measles virus and smallpox virus in the uncombined vaccines being such that the product contains at least 10 $TCID_{50}$ units of measles virus and at least 10^2PFU of smallpox virus per 0.1 ml. of the intermediate mixture.

2. A process as claimed in claim 1, in which the smallpox vaccine is a live vaccinia virus vaccine.

3. A process as claimed in claim 1 or 2, in which the product contains at least 10^5 $TCID_{50}$ units of measles virus per 0.1 ml of the intermediate mixture.

4. A process as claimed in any preceding claim, in which the product contains at least 10^5PFU of smallpox virus per 0.1 ml. of the intermediate mixture.

5. A process a claimed in any preceding claim, in which the mixture is frozen before it is lyophilized.

6. A process as claimed in claim 5, in which the mixture is frozen at −60° C.

7. A process as claimed in any preceding claim including the step of diluting the product with a parenterally administrable liquid carrier to restore its volume to that of the intermediate mixture and produce a combined measles and smallpox vaccine suitable for administration.

8. A process as claimed in claim 7, in which the parenterally administrable liquid carrier is sterile distilled water.

9. A process as claimed in claim 1 substantially as hereinbefore described in any one of the Examples.

10. A dried combined measles and smallpox vaccine when prepared by a process as claimed in any one of claims 1 to 6.

* C L M S *

11. A combined measles and smallpox vaccine when prepared by a process as claimed in claim 7, 8 or 9.

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