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WORLD HEALTH ORGANIZATION

REGIONAL OFFICE FOR THE EASTERN MEDITERRANEAN

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ORGANISATION MONDIALE DE LA SANTÉ

BUREAU RÉGIONAL POUR LA MÉDITERRANÉE ORIENTALE

REGIONAL COMMITTEE FOR THE EASTERN MEDITERRANEAN

Fourth Session

RC4/EM/8
16 August 1954

ORIGINAL : ENGLISH

DRIED SMALLPOX VACCINE

Glycerinated smallpox lymph vaccine has for long proved highly effective and dependable as an immunising agent. The success encountered in using it in cold countries prompted the health authorities to extend systematic vaccination to warm countries and territories where smallpox still causes great havoc among the people. It was found, however, that the glycerinated vaccine pulp loses its potency when carried to remote regions; hence came the idea of producing a substitute that resists long transportation at a high termperature without losing much of its potency.

In many parts of this region conditions of transportation and storage of vaccine lymph are very unfavourable and the liability of lymph to become ineffective after exposure to hot weather in remote rural areas in the region is well known. Expert opinion seems to be that the successful production of a dry vaccine will go a long way towards solving vaccination difficulties in rural areas, particularly in those tropical countries where smallpox epidemics are still frequent.

At its third session in Istanbul, the Regional Committee for the Eastern Mediterranean adopted a resolution (RC3/EM/38 Rev. 1, para 7.1.2.15 noting the possibility of using dry vaccine. The Regional Office promised, in that session, to collect information on dried smallpox vaccine and the trials of it carried out in different countries.

Since the discovery of the smallpox vaccine it has been realized that vaccine matter when dried remains potent for a long time.

Jenner himself (1798) sent specimens of his vaccine, dried on thread, to be tried in other countries by foreign observers.

In 1836, a law was issued in Austria prescribing the drying of the vaccine on ivory splinters. It is claimed that this vaccine gave positive results after four to six months or even more.

Mueller (1869) claimed to have used with excellent results, vaccine lymphs which he dried and preserved between glass plates for ten years.

The Reissner Vaccine (1881) aroused great interest. He dried in the dessicator the vaccinal lymph of a heifer, ground it into very fine powder and kept it in small boxes. Reissner claimed that his vaccine conserved its properties for seven months.

Formerly, it was a common practice that physicians preserved the dried crust from a typical take.

Professor Wurtz was able in 1897 to inoculate calves and prepare vaccine fro them from some dried vaccine which he took with him to Abyssinia and which was originally prepared by Chambon and St. Yves Ménard.

Since the beginning of this century many workers have devised different techniques for the preparation of dried lymph vaccine. The results obtained by using dried lymph vaccine proved, in general, according to these works, to be mor favourable than those obtained through the usual glycerinated lymph.

Achalme and Phisalix (1909) prepared a dried vaccine which, after storage for one year at a temperature of 37°C to 38°C, was still active. A temperature of 41°C to 42°C did not have any influence on the potency of their dried pulp, a temperature of 45°C reduced it very slightly and a temperature of 57°C, a littl more.

Camus continued the researches on dried vaccine, and in 1909 devised a technique for its preparation. Joyeux tried Camus' vaccine in Kan-Kan with 71 - 98% positive takes. In 1911 - 1912 a new type of dried vaccine was prepared by Camus.

The results claimed to have been obtained by a number of investigators are shown in the following tables:

Investigator	Dried vaccine prepared by	Place and date of trial	Positive results
Joyeux	Camus	French Guinea in 190[illegible]	89.5% after o month 50% after two months
Ross	Achalme and Phisalix	East Africa 1911	91.5% after 6 months
Léger	Institut de vaccine animale, Paris	~~French Africa~~ Indochina 1911	12% after 2 months
Ross	Achalme and Phisalix	Nairobi, 1911	72% after 14 months
Sorel and Arlo	Camus (new type)	Ivory Coast, 1912	66% - 100%
Manteuffel	(non-pulverised vaccine)	German East-Africa, 1912	60% after 6 months
Ross	Achalme and Phisalix	Forte Hale, 1913	81% vaccine preserved at Nairobi for o year 82% vaccine preserved for 2 years 70% vaccine preserved for 3 years

Investigator	Dried vaccine prepared by	Place and date of trial	Positive results
Ringenbach	Degive, Bruxelles	Belgian Congo, 1913	32% after 4 months under tropical temperature
Kersten	Pondorf, Weimar	New-Guinea, 1914	60% after 2,5 months
Martin	Institute of animal vaccine	Cameroun 1917	85% after 4 months, at a temperature reaching up to 50°C
Rosario and Rizal	Rudiger	Philippines, 1913	58% after one month
	O.Schobl	Philippines, 1918-19	65%
Lasnet	Vaccine Institute Academy of Medicine, Paris	French Western Africa 1926	Very satisfactory

Paschen was able to have a positive result in a child vaccinated with 9 years old dried vaccine prepared at the Vaccine Institute of Hamburg.

Legendre had excellent results with dried vaccine in 1928 in tropical Africa in the High-Volta far districts.

Dried smallpox lymph vaccine was used with satisfactory results in Belgian Congo, Mozambique, Kenya, Angola, French Somaliland, French India, Dutch East Indies, Indonesia, French Indochina (Vietnam), the Philippine Islands and China.

Otten (1927) reviewed the history of dried lymph vaccine production and reported on its successful use in Batavia. He insists more particularly on the use of dried glycerinated lymph which is far weaker and the duration of its activity is limited.

Through the dessication of the vaccine and its conservation in vacuum, Otten obtained a very resistant virus: under a temperature of 36° it resisted for several months; under 41° - 45°, for many weeks; under 58°, it could still resist for some days. This dried vaccine, kept at room temperature for 9 months and a half, including 4 months at Samarang (Java) and one month incubated at 36°, gave one hundred per cent positive. According to Otten, vacuum is an indispensable condition for the conservation and efficacy of the virus. An inconvenience of this dried lymph is the necessity, once the tube is open, to use it on the same day or a few days later, as it rapidly loses its efficacy.

According to Gaide and Bodet (1931), dry vaccine for thirty or forty thousand persons is prepared every year in Indochina, for Cambodia and Laos; Laos also receives dry vaccine sent directly from France.

In 1932-1933, Otten summarized the results of 16,000 vaccinations performed with dried vaccine. The vaccine remained stable for days at 58°C, for months at 37°C - 42°C and for a year at average temperature in the tropics.

In 1932 - 33, during a smallpox epidemic in Karikal, French Indies, 8400 vaccinations were made with dry vaccine with 95.5% positive results.

A purified dried vaccine containing few secondary germs was tested in 1939 by Prof. Henri Bernard and was later used in Dakar, giving successful results.

Other workers have achieved a fair measure of success using dried lymph and it may maintain potency for over a year if dried in vacuo from the frozen state (Boulnois, 1936, 1937; Kaiser, 1938; Morosov et al, 1943).

The dried vaccine made by Otten's method at the Institute Pasteur, Bandung, has been used in Indonesia since 1931. The number of doses of vaccine supplied rose from 147,000 in 1931 to 801,700 in 1941 and to more than 26 million in 1949. The use of this vaccine rapidly became general throughout Indonesia, even in the most distant islands of the archipelago. During the 1948-50 epidemic, the vaccine was of great service.

TECHNIQUES OF PREPARATION

Blaxall (1900)

The pustules are collected from heifer at the one hundred and twentieth hour, then placed in a sterilized porcelain box and dried in vacuum over sulfuric acid (H_2SO_4). After twenty-four-twenty-eight hours, the pulp derived from the pustule is completely dry, and it is then put in small sealed glass tubes.

Carini (1906)

Desiccation over sulfuric acid or potassium hydroxide (KOH), *in vacuum* at room temperature (18° - 20°C). After twenty-four-forty-eight hours, the vaccine, which has lost two-thirds of its initial weight, is pulverised in a sterilized mortar. It is then kept inside vacuum glass tubes.

Achalme and Phisalix (1909)

Desiccation in vacuum over sulfuric acid. Preservation inside sealed glass tubes, air-emptied or not. The virulence is maintained longer if the pulp is kept in vacuum and not pulverised.

Camus (1909)

Desiccation in vacuum under 15°C temperature, the pulp being laid down in thin layer. Preservation in vacuum. In order to increase the stability of the emulsion, a solution of 10% of gum-arabic is added to the pulp.

Degive (1913)

Desiccation in draught. Pounding by means of a special mill, then filtration and preservation inside cork-stopped tubes placed in another tube filled with glass flakes and air-tight sealed. No vacuum is made in the tubes.

Wurtz and Camus (1919)

Before desiccation, the pulp is congealed in order to facilitate dehydration. Thus prepared, it is pulverised and cleared of foreign substances. It is dried by means of the air-pump, at the room temperature, using sulfuric acid or phosphoric acid. The filling of the tubes takes place under a tent-shaped apparatus (thus enabling the operator to be in the open air, with his arms inside) containing an atmosphere dried by means of sulfuric or phosphoric acid. The main idea is to obtain dryness quickly and to maintain it.

Schöbl (1920)

The pulp obtained from animals is pounded in a sterilized mortar, laid on a wide surface under perfectly aseptic conditions, quickly dried in vacuum over a hygroscopical chemical product, pulverised, then kept in a desiccator, under the room-temperature.

The dry vaccine is put inside glass phials provided with rubber plugs, paraffin sealed. Another phial of the same type contains the glycerine to be mixed with the pulverised vaccine immediately before use.

Otten (1927)

a) "Room lymph" - The freshly obtained pulp is pounded in a "Chalybaus" mill, then it is laid in a thin layer on a glass slide and dried in vacuum, over sulfuric acid. After forty-eight hours, the vaccine is pounded in a mortar, then kept in vacuum, with or without phosphoric anhydride (P205) under the room-temperature.

b) "Frigilo-lymph" - Vacuum is made under room-temperature, but desiccation is obtained at -15°C.

c) "Congealed lymph" - The lymph is congealed under -15°C; one hour later, vacuum is made and desiccation is carried out, both operations taking place at -15°C temperature.

The loss of weight resulting from the desiccation of the pulp is about 80%. When using the dried lymph for vaccination, and in order to obtain the same dilution as for the glycerinated lymph (i.e. one part of pulp for two parts of glycerine), 1.4 gr. of glycerine should be added for 100 millgr. of dried pulp.

Institut Pasteur, Saigon (1929)

The vaccine is dried in vacuum as soon as obtained. A mercury steam pump which is able to create a vacuum at 1/1000 mm. Hg. is used for desiccation. Further, by using liquid air refrigerators, desiccation is much quicker than previous techniques.

Office National de Lutte contre les Epidémies (1929) - (National Office for the Control of Epidemics).

Once gathered from heifers, the pulp is pounded three times in a vaccine vertical grinder, then it is collected in a sterilized glass container. The pasty substance thus obtained is laid in thin layers in sterilized Petri dishes.

The dishes are then put in the desiccator, after which their lids are taken off. The desiccator has the shape of a small horizontal autoclave provided on each face with an aperture, one of which is connected by means of rubber tubes to two bottles of sulfuric acid and one bottle of calcium chloride, and the other to an aspiration pipe. The dessicator is then closed and a slight dry draught is continuously maintained in it. The next morning, the vaccine, thus dried, is hand-pounded in a porcelain mortar. The powder is then put in another sterilized Petri dish, and kept in the dessicator during the following night.

The next morning, the pulverised vaccine is put in sterilized glass phials, approximately one gram in each phial. The phials, thus filled, are kept uncovered in the dessicator during the next night. They are taken out of the dessicator the next morning in order to be provided with cork-stoppers and sealed with sealing wax. To each phial containing this pulverised vaccine is joined a similar phial containing about 1 cm3 of 70% glycerine water (without phenol).

For the bacteriological test, one takes three phials of pulverised vaccine and pours in each of them the contents of a phial of glycerine; after a careful mixing, each of the three mixtures is poured in a fermentation Smith tube containing glucose broth. The tubes are incubated during nine days. If no gas is formed, the vaccine is considered utilizable. If, on the contrary, gas is formed in one of the three tubes, 4 cm3 of the broth contained in this tube are subcutaneously injected into a guinea-pig. If the animal shows symptoms of tetanus or gas gangrene, the vaccine is turned down; otherwise, it is considered utilizable. No counting of bacteria is carried out.

For the virulence test, a small quantity of the contents of a phial of glycerine is poured into a phial of pulverised vaccine; after careful mixing, the mixture is laid on small scarification fields prepared on rabbits and also on partially immunised heifers. If well-developed pustules are formed, the vaccine is considered utilizable.

Institut du Vaccin (Vaccine Institute), Lourenço-Marquès (1929)

The vaccine to be dried is collected on a sterilised gauze drum, so that the pustules, when exposed to a draught, get quickly dry. The drum is then put over sulfuric or phosphoric acid in a dessicator which is kept in a refrigerator until the pulp is congealed. The dessicator is then connected to an air-pump, and as soon as vacuum is made inside, it is replaced in the refrigerator; the vaccine which, thus, becomes dry and friable, is then easy to pound.

For this purpose, a special apparatus is used; it consists of a bell containing a glass flask. The apparatus is driven by a motor and works in vacuum. Among other advantages, this apparatus allows the operators to avoid inhaling the pulverised vaccine. A sterilised mortar, cooled in a refrigerator, may also be used. Before being put in tubes, the pulverised vaccine must be dried again, as dampness facilitates putrefaction.

The dry vaccine may also be prepared in tablets. It is hygroscopic enough to be made into pills, even without adding gum or other excipients; however, in order to avoid compression or any other manipulation which might reduce its virulence, it is preferable to produce it as a powder, if possible, in opaque vacuum containers.

Dried vaccines in use at present

At present, there are four methods for the preparation of dried lymph vaccine namely :

1. Otten's method at the Pasteur Institute, Bandoeng ;
2. Kaiser's method at the Lymph Institute, Vienna ;
3. Fasquelle St. Yves Ménard at the Institute of Animal Vaccine, Paris ;
   and
4. Lyophilisation method at the Division of Laboratories at the Michigan State Department of Health.

According to the technique employed at the Institut Pasteur, Bandoeng, the pulp collected from the buffalo is ground, dried in vacuo at laboratory temperature, pulverized in a mortar, and then filled into ampoules, which are sealed after evacuation of air.

The lyophilisation method which appears to be very efficient in maintaining the viability of virus strains is essentially a method of rapid freezing at a very low temperature and rapid dehydration from the frozen state under high vacuum; the process is continuous and conducted in the final container in which the material is to be sealed under the original vacuum, stored and distributed.

The cryochem process is a new process for the preservation and dessication in vacuum from the frozen state. It is said to be more economical than the yophile process. It is simpler to carry out, faster in drying and requires no storage of dry-ice.

Difficulties experienced

Three major difficulties have been encountered in the preparation and use of dried smallpox vaccine, and these probably account for its not being widely used:

1. It has been difficult to obtain preparations in which the number of contaminating bacteria have been reduced to an acceptable level;
2. It is more expensive to produce dried vaccine than to produce glycerinated lymph;
3. It is more difficult to reconstitute a dried vaccine at the time of use than to open a capillary liquid vaccine.

A major problem in the use of dried vaccine is a convenient and safe method to be followed by the unqualified vaccinators who usually administer the vaccine in remote districts in the tropics. A definite hazard is involved in opening the vials of vaccine because there is a tendency for a small amount of the powdered pulp to be blown from the evacuated tube when it is broken. This pulp may cause ocular or respiratory infection, or a deep vaccinia infection may be produced from accidental cuts.

It was found that rigid attention to the details of sanitation during the quarantine and handling of animals, together with the treatment of operative surface with Roccal solution, made possible the production of vaccine containing very low numbers of viable organisms.

J.W. Hornibrook and W.H. Gerhard were able to produce smallpox vaccine with only 600 organisms per ml., well below the maximum number allowed (USA). They obtained this result by adding penicillin to the carefully prepared vaccine before drying.

Kreshnamurthy, in trying the effects of penicillin and streptomycin on vaccine lymph found that while both antibiotics do not affect the potency of vaccine lymph, only streptomycin successfully brings down the bacterial flora of the vaccine lymph to the required extent. On the other hand penicillin was found to be ineffective in reducing the bacterial contaminants of vaccine lymph.

LABORATORY INVESTIGATIONS AND FIELD TRIALS OF DRIED SMALLPOX VACCINE SPONSORED BY WHO

In 1948 a joint OIHP/WHO Study-Group on Smallpox recommended that further studies and observations be made on the means of preparing an active but pure dry vaccine. This work progressed during 1949 at the Animal Vaccine Institute, Paris, where it was found possible, through treatment with an antiseptic "Roccal", to improve the purity of the dry vaccine formerly produced at the Institute. The pure lymph vaccine appeared to be somewhat less potent when obtained in this way, and methods for removing the antiseptic and thereby enhancing its potency had to be studied. The vaccine was tried out in the field in India with satisfactory "takes".

In accordance with the resolution of the World Health Assembly (Resolution WHA3.18 Off. Rec. World Health Organization 28) the WHO Expert Committee on Biological Standardization (1950) recommended that an investigation be made of the value of dried smallpox vaccine.

It suggested that the dried vaccines to be investigated should be made from lymph of a potency which, on vaccination of children over six months of age who have not previously been vaccinated nor recently exposed to infection with variola or vaccinia, will produce a typical primary vaccinal reaction. The material should be free from anaerobic and aerobic pathogenic bacteria and should contain not more than 1,000 non-pathogenic bacteria per ml. If the dried material is intended for injection it must before drying be bacteriologically sterile.

During 1951, WHO continued to encourage research on the immunizing properties of dried smallpox vaccine. Several outstanding specialists in the preparation of dried smallpox vaccine were requested to submit descriptions of their respective methods and reports on the results obtained with dried smallpox vaccine.

Experiments were begun at the Vaccine Institute in Belgaum, Bombay Province to determine, in monkeys, the protective potency against virulent smallpox virus of dried vaccines prepared from various strains of vaccinia. The Indonesian dried vaccine in endemo-epidemic conditions was tested in field trials in India; similar trials were conducted in Peru by the Pan American Sanitary Bureau with vaccine produced by the Michigan Department of Health.

To obtain information on the keeping qualities of dried smallpox vaccine under field conditions, WHO sponsored a series of detailed laboratory experiments to determine the rate of loss of potency of four dried smallpox vaccines kept at different temperatures.

The four "producing" laboratories supplied samples of their dried vaccine for testing, together with a "wet" preparation from the same strain of vaccinia virus:

1. Institut de Vaccine Animale, Paris ;
2. Division of Laboratories, Department of Health, Michigan (USA) ;
3. Staatliche Impfanstalt und Staatliche Serumprufungsinstitut, Vienna
4. Institut Pasteur, Bandoeng (Java).

The directors of five laboratories in Copenhagen, Michigan, Paris, London and New York met in Geneva in June 1952 and agreed upon a standardized procedure for testing the four dried vaccines.

Early, in 1953, three laboratories - one in Copenhagen, one in Paris and one in London - started subjecting to standardized tests the four dried smallpox vaccines prepared in Paris, Michigan, Vienna and Bandoeng. The purpose of these laboratory tests was to determine the rate of loss of potency of dried smallpox vaccines when stored at adverse temperatures, such as are likely to be met under field conditions.

The long series of tests are not yet sufficiently complete to allow statistical analysis of the final results to be carried out.

The vaccine which proves by laboratory investigations to be the best of the four chosen will be used in human field trials both in endemic areas and in non-endemic areas.

Plans are already well advanced to commence the field trials on man in a non-endemic area at an early date. It is envisaged that a field trial in an endemic smallpox area will commence sometime later.

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Fourth Session

RC4/EM/8 Corr. 1
21 September 1954

ENGLISH ONLY

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Corrigenda

Page 2 line 28	-	delete "French Africa" and substitute "Indochina"
" 3 " 16	-	insert "Africa,1926" after "Western"
" 3 " 27	-	delete "glycerinated" and insert after "lymph" the following : "in tropical countries, due to its superiority to glycerinated lymph"
" 5 " 15	-	"Frigilo lymph" should read "Frigololymph"
" 5 " 16, 17 and 19	-	"15° C" should read "-15° C"