WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTE

COURSE ON FREEZE-DRIED SMALLPOX VACCINE PRODUCTION

Yaba, Nigeria, 14-26 November 1960

WHO/Smallpox/16 ✓
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ORIGINAL: ENGLISH

FREEZE-DRIED SMALLPOX VACCINE PRODUCTION

by

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WHO Consultant, 1960

Introduction

From the 14th to the 26th November, 1960, a course was held at Yaba, Nigeria, on the production of freeze-dried smallpox vaccine. Eight participants, six of whom were awarded fellowships, drawn from the Eastern Mediterranean and African Regions, attended. Two fellows came from the United Arab Republic, and one each from Iran, the Sudan, Kenya and Ghana. There were two local participants, from Nigeria. Six were pathologists. and two were technicians. Of this total, six were currently active in smallpox vaccine production, or held positions with administrative responsibility for laboratories making vaccine. Only two had any real previous experience of freeze dried smallpox vaccine production: in both cases this knowledge was outdated.

The purpose of the course was to teach a method for the making of a stable smallpox vaccine, by desiccation of a partially purified vaccinia virus suspension. The method taught was that currently employed at the Lister Institute of Preventive Medicine, Elstree, Herts., England.

The host country, Nigeria, provided the laboratory facilities of the Federal Laboratory Service, which includes the Government Vaccine Production Units. The West African Council for Medical Research kindly lent a lecture theatre. A small bus was made available by the Medical Department.

Planning the Course

As it was felt that practical experience was of the first importance, the course was designed to give as much time as possible for each of the participants to carry out himself the techniques described. For this raason, the whole process was taken

stage by stage in lectures, demonstrations and practicals, in such a way that each participant was able to take away at the end of the course a sample of vaccine prepared by himself.

In order to cover all aspects of the production of dried vaccine as fully as possible, no formal teaching on the more traditional parts of vaccine production was given, though visits to watch various stages during the preparation of the raw vaccine pulps were arranged, and provision was made for those with queries in this field to make contact with persons able to help them.

The timetable for the course, and brief notes covering the range of matter taught, appear in Annex II to this report.

Administrative facilities

The WHO facilities available in Lagos were limited to the local representative and his secretary. Their time was fully occupied, however, in other directions, consequently private arrangement had to be made for secretarial assistance. This did not prove difficult, due to the writer's local knowledge, though the lack of an allotment to pay for this service might have proved embarrassing in other circumstances. The absence of the local representative throughout the greater part of the course meant that many of the day-to-day administrative details, such as arranging return air bookings, immigration formalities, and salaries of the participants had to be covered by the writer.

Laboratory facilities

These were not ideal, but by exercising a little ingenuity they proved adequate. Had the number of participants been larger, however, considerable difficulty would have been experienced. Not inconsiderable quantities of apparatus and space are required by even a small number of people engaged in practical work, and without the unstinted assistance given by local professional and technical staff these could not have been provided.

Conclusions

To sustain a course, particularly one involving extensive practical work, for four to five hours daily, for two weeks, without lengthy preparation, is difficult.

For one person to lecture, give demonstrations, supervise practical work, and, at the same time, arrange and prepare the next day's programme is only possible with the assistance of others. The fact that the writer is known to the people able to provide such assistance in Yaba made the present course possible, but in other circumstances the time available before the course for making local preparations would have been inadequate.

The intrinsic value of a course of this type is very difficult to assess. At least two of the participants in the present course did not have sufficient command of English to enable them to follow a lecture closely, though this loss was largely made up during demonstrations and practical work. It was evident that those with previous experience of preparing freeze-dried smallpox vaccine were able to benefit more than the others. It might be thought that it is in this respect that such a course has a valuable future application, as it enables a large number of vaccine producing centres to learn of recent advances and new techniques. Apart from this, the interchange of normally unpublished information between centres which occurs during such a course, is of the greatest value. For those with no previous experience of the technique, the course was probably of lesser value, except for those senior members of laboratory staffs with administrative and planning responsibility, who were able to form an accurate assessment of the possibility and value of starting freeze-dried vaccine production in their own units.

ANNEX I

Names and addresses of participants

Fellows

1. Dr Bouthaina Shafik
   Serum and Vaccine Institute
   Agouza, Cairo, Egypt, UAR

2. Dr Bouik Seydian
   Institut Pasteur
   Teheran, Iran

3. Dr Haidar Samman
   Ministry of Health
   Damascus, Syria, UAR

4. Dr C. A. Linsell
   Medical Research Laboratory
   Box 30141, Nairobi, Kenya

5. Dr W. N. Laing
   Medical Research Institute
   P.O. Box 300, Accra, Ghana

6. Mr A. K. Sorig
   Stack Medical Research Laboratory
   Khartoum, Sudan

Local participants, not awarded fellowships

7. Dr H. S. O. Ahimie
   Federal Laboratory Service
   Yaba, Nigeria

8. Mr M. S. O. Nwachukwu
   Federal Laboratory Service
   Yaba, Nigeria

Medical Consultant

Major P. D. Meers, R.A.M.C.

17, The Dene, Cheam, Surrey, England

ANNEX II

WORLD HEALTH ORGANIZATION

COURSE ON THE PRODUCTION OF

DRIED SMALLPOX VACCINE

HOST GOVERNMENT:

THE FEDERAL GOVERNMENT OF NIGERIA

HOST ORGANIZATIONS:

FEDERAL LABORATORY SERVICE

WEST AFRICAN COUNCIL FOR MEDICAL RESEARCH

Held at

YABA, NIGERIA

14-26 November 1960

Annex II

Time-table

Transport will arrive at the hotels to collect fellows every morning between 8.00 and 8.15. Fellows are particularly requested not to delay the transport.

Date	Time	Location	
14 November Monday	8.00	Hotels	Fellows taken to bank
	11.00	W.A.C.M.R.	Coffee
	11.30	W.A.C.M.R.	Lecture - Introduction
			Tour Federal Laboratory Service, especially the Smallpox Vaccine Section
	2.00		Return to hotels
15 November Tuesday	8.30	W.A.C.M.R.	Lecture The preparation of the "10% extract"
	9.30	Rabies Lab.	Demonstration do
	11.00	W.A.C.M.R.	Coffee
	11.30	Rabies Lab.	Practical do
	2.00		Return to hotels
16 November Wednesday	8.30	W.A.C.M.R.	Lecture The preparation of an E.B.S.
	9.30	Smallpox Lab.	Demonstration do
	11.00	W.A.C.M.R.	Coffee
	11.30	Smallpox Lab.	Practical do
	2.00		Return to hotels
17 November Thursday	8.30	W.A.C.M.R.	Lecture Freeze-drying
	9.30	Y.F.V.L.	Demonstration Freeze-drying 1. The structure of the primary machine 2. Putting the machine into use after delivery
	11.00	Y.F.V.L.	Coffee
	11.30	Y.F.V.L.	Practical Using the primary freeze-dryer - I
	2.00		Return to hotels
18 November Friday	8.30	Y.F.V.L.	Practical Using the primary freeze-dryer - II
	9.30	Y.F.V.L.	Demonstration The parts of the freeze-dryer
	11.00	Y.F.V.L.	Coffee
	11.30	Y.F.V.L.	Demonstration Servicing and care of the machine
	2.00		Return to hotels

Time-table (continued)

Annex II

Date	Time	Place	Activity
19 November Saturday	8.30 12.00		Reserved for repeat of any part of course to date, at request of fellows
20 November Sunday			Free
21 November Monday	8.30	Y.F.V.L.	Demonstration The secondary freeze-dryer
	9.30	Y.F.V.L.	Practical The use of the secondary freeze-dryer
	11.00	Y.F.V.L.	Coffee
	11.30	Y.F.V.L.	Practical continued
	2.00		Return to hotels
22 November Tuesday	8.30	W.A.C.M.R.	Lecture Potency testing the vaccine
	9.30	Rabies Lab.	Practical Freeze-drying of fellows' own vaccine - I
	11.30	Rabies Lab.	Demonstration and Practical Titration of vaccine
	2.00		Return to hotels
23 November Wednesday	8.30	W.A.C.M.R.	Lecture Bacteriology of vaccine
	9.30	Rabies Lab.	Practical Freeze-drying of fellows' own vaccine-II
	11.00	W.A.C.M.R.	Coffee
	11.30	W.A.C.M.R.	Visit to W.A.C.M.R. laboratories
	2.00		Return to hotels
24 November Thursday	8.30	W.A.C.M.R.	Lecture Testing, storing and issuing vaccine Reconstituting fluid
	9.30	Rabies Lab.	Practical Freeze-drying of fellows' own vaccine - III
	11.00	W.A.C.M.R.	Coffee
	11.30	Rabies Lab.	Demonstration and practical Harvest of eggs, pock-counting
	2.00		Return to hotels
25-26 Nov. Friday-Saturday		W.A.C.M.R. and elsewhere	Recapitulation of complete method Discussion of laboratory facilities and apparatus Discussion of difficulties experienced or expected by fellows Repeat of any practical work as dictated by experience during the course
26 November	12.00	W.A.C.M.R.	Course closes

Annex II

THE PREPARATION OF DRIED SMALLPOX VACCINE - AN OUTLINE

1. The problem

It is required to convert the raw material, or "pulp", containing the vaccinia virus, obtained from whatever source animal is used, into a relatively purified form, suitable for freeze drying. The final material should be able to pass the suggested International and/or National requirements as regards bacterial and virus content. (The suggested international standards may be found in Wld Hlth Org. techn. Rep. Ser., 1959, 178 and 180.)

In the preparation of dried smallpox vaccine, the initial problems are two in number, namely:

1.1 Removal of as much as possible of the contaminating animal host material, without damage to, or excessive loss of, virus.

1.2 Reduction of the bacterial content to an acceptable level, with as little damage as possible to the virus.

If the amount of virus in the raw pulp is too low to meet the requirement as to titre directly, a third problem arises, namely:

1.3 Concentration of the virus.

It must be stressed that the nature of the initial virus containing material prepared in different centres varies widely. For this reason, the greatest possible flexibility in approach to the problems enumerated above must be maintained: the method to be outlined below, and amplified during this course must, for this reason, be accepted with reservation. The commencement of dried smallpox vaccine production for the first time in a laboratory should be regarded as an experimental problem, the solution presented here being taken as an indication of one method which may be followed, and not as a set of hard and fast rules.

The technique to be described during this course is due to workers at the Vaccine Lymph Department, the Lister Institute, Elstree, Herts., England.

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2. Brief details of one technique for the production of dried smallpox vaccine

2.1 The preparation of a "10% extract"

2.1.1 The raw pulp is homogenized with 10 times its weight of McIlvaine's buffer (pH 7.2, 0.004M), with added phenol to 0.4%, and "Arcton 113" to about 12%.

2.1.2 The suspension is centrifuged for about five minutes at an R.C.F. of 500G, and the supernate decanted.

2.1.3 This supernate (the 10% extract) is kept for about 18 hours at a temperature of about 22°C, to reduce the viable bacterial count.

2.1.4 At the end of the period of incubation, the 10% extract is stored at between 0°C and 4°C, to await titration of the virus and bacterial contents (if required at this point) and until it is convenient to proceed to the next stage.

2.2 The use of the 10% extract

2.2.1 If the degree of bacterial contamination found is acceptable, and the virus titre is high enough (say over 10^9 P.U.'s per ml), the 10% extract may be diluted with an equal volume of 10% peptone solution (or more, depending on the titre), distributed into ampoules, and freeze dried. If the bacterial examination is satisfactory, but the titre lies between 10^8 and 10^9 P.U.'s per ml, the 10% extract may be converted into an E.B.S. (section 2.3), thereby concentrating the virus. If the result of the bacterial examination is unsatisfactory, further exposure at 22°C may be attempted, though it must be realised that even one overnight exposure at this temperature reduces the vaccinial titre by upwards of half a log., and that, therefore, two such exposures might render the suspension useless.

2.2.2 A 10% extract may be stored, without significant loss of titre, for upwards of one month.

2.3 The preparation of an E.B.S.

2.3.1 The 10% extract is centrifuged at an R.C.F. of between 2300 and 10 000 G, to sediment the virus. To reduce the time necessary at any given R.C.F., an angle head centrifuge should be used. At 10 000 G, with an angle head of 28°, half an hour more than suffices. At an R.C.F. of 2300, over two hours may be required. The use of a machine capable of giving 10 000 G will avoid the necessity for a refrigerated centrifuge, as long as cold-room or air-conditioned facilities are available.

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2.3.2 After centrifugation, the supernate is discarded and the pellet resuspended in McIlvaine's buffer. The volume used will vary with experience: as a starting point, twice the weight of the pulp used in the preparation of the 10% extract may be tried. Resuspension of the virus is effected in some suitable homogenizer. This suspension is the E.B.S. and it is stored between $0^{\circ}C$ and $4^{\circ}C$, to await titration and bacteriological examination, and further processing.

2.4 Use of the E.B.S.

2.4.1 If the bacteriological examination is satisfactory, and the virus titre high enough (say over 5×10^9 P.U.'s per ml), the E.B.S. may be diluted 10-fold with 5.5% peptone solution, distributed into ampoules, and freeze-dried. Dilutions of less than 10-fold may be used, as dictated by circumstances.

2.5 Ampouling and freeze-drying

2.5.1 The 10% extract, or the E.B.S., diluted with peptone solution as described, is ampouled in 0.25 ml amounts, this representing a nominal 25 doses.

2.5.2 Primary drying by the centrifugal technique is advised. Eighteen hours of primary drying is usually sufficient. The ampoules are then transferred to the secondary desiccating machine, where they are exposed to a high vacuum over phosphorus pentoxide, for a further 18 to 24 hours. At the end of this time, dry, oxygen-free nitrogen is admitted to the system, and the ampoules are sealed with a blow-torch. They are then tested for leaks, examined for imperfections, and are stored in the refrigerator, to await issue.

3. Bacteriological control, and potency testing

3.1 Bacteriological control

3.1.1 This will depend on existing local practice, and regulations. Total bacterial counts will be required at the 10% extract and/or at the E.B.S. stage. Detailed bacteriological examination for the special classes of pathogenic organism may be done at the same time, or on the final dried product.

3.2 Potency testing

3.2.1 Potency testing will be required at the stages indicated above, and also on the final product, to comply with national requirements.

4. Storage and issue

4.1.1 Long-term storage of the dried vaccine should be at sub-zero temperatures. At -10°C, vaccine which has been properly made has a virtually unlimited life.

4.1.2 Due to the excellent keeping qualities of the vaccine at high ambient temperatures, it may be distributed unrefrigerated, by post. Users should be advised to store it in a domestic refrigerator.

5. Formulae

5.1 McIlvaine's phosphate - citric acid buffer

Anhydrous Na_2HPO_4 . . . . 5.6808 g
or: $Na_2HPO_4.12H_2O$ . . . . 14.32 g
In 200 ml distilled water (= 0.2M)

Citric acid . . . . 2.1008 g
In 100 ml distilled water (= 0.1M)

pH required	ml 0.1M citric acid	ml 0.2M Na_2HPO_4
5.0	9.70	10.30
6.0	7.37	12.63
7.0	3.53	16.47
7.2	2.61	17.39
7.4	1.83	18.17
7.6	1.27	18.73
8.0	0.55	19.45

Used at pH 7.2. The mixture shown underlined above is the "concentrated buffer" (0.187M), pH 7.2

Buffer ready for use is 0.004M, and is prepared by adding 1.0 ml of concentrated buffer to each 46 ml distilled water.

Sterilized in the autoclave - 5 lb pressure for 30 minutes. Store at 4°C.

5.2 Concentrated phenol solution

100 g crystalline phenol melted over low flame. Pour into heated measuring cylinder, and allow to cool. Make up to 120 ml with sterile distilled water. (Wear gloves)

1.2 ml = 1 g phenol

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5.3 Peptone solution

Evans bacteriological peptone dissolved to either 10% or 5.5% in distilled water. Adjust pH to 8.0 with 40% NaOH. Heat to 90°C, and filter hot. Correct pH to 7.2-7.4 with 40% HCl. Autoclave 15 lb. pressure for 15 minutes.

5.4 Reconstituting fluid

102 ml concentrated (0.187M) buffer, pH 7.6

498 ml distilled water

400 ml glycerol

Autoclave for 20 minutes at 15 lb. pressure

Ampouled in 0.35 ml amounts, in double ended ampoules

Re-autoclave

Test for leaking ampoules by the vacuum method, with a dye in the water