WORLD HEALTH ORGANIZATION
Regional Office
for the Eastern Mediterranean

SEMINAR ON SMALLPOX ERADICATION
Dacca, 29 October - 5 November 1969

EM/SEM.SE/38
ENGLISH ONLY

FREEZE-DRIED VACCINE FOR THE SMALLPOX ERADICATION PROGRAMME

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The use of freeze-dried smallpox vaccine of suitable quality is prerequisite to the success of the smallpox eradication programme. Therefore, at the beginning of the eradication programme in 1967, particular efforts were made to improve the quality and to increase the quantity of vaccine available. This paper describes WHO activities dealing with vaccine quality and summarizes certain aspects of the present status of vaccine production.

WHO Survey on Vaccine Production Status

In 1967, when WHO, in cooperation with its member countries, started the eradication programme on a world-wide basis, our first concern was to ensure an adequate supply of potent, heat stable vaccine to the programme. At that time, only limited information on the status of vaccine production in individual laboratories was available. Accordingly, early in 1967, questionnaires were sent to all member states where the production of freeze-dried vaccine was believed to be under way. The information provided by the laboratories was summarized early in 1968 and since then the data have been revised whenever additional information has been received. The data presented in Tables 1, 2 and 3 are based on this information and are believed to reflect fairly well the present status of vaccine production.

At present, 58 countries are participating in the production of freeze-dried vaccine (Table 1). In these countries, 81 laboratories are either producing or preparing to produce vaccine. Sixty-four laboratories are now in routine production.

Table 2 summarizes the types of strains being used in the 64 laboratories currently in routine production. The designation of the strains is provisional and respresents simply the names of strains as reported to us. The Lister strain is most frequently used. Of 64 laboratories, 20 use the Lister strain. The New York Board of Health strain is used in 5 laboratories in the Americas. In Europe, more than 10 strains are used in various laboratories, reflecting the very long history of vaccine development in this region. The Paravaccinar strain is used in

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virus laboratories in India; the EM63 strain, derived from the Ecuador strain, was selected for use in the Soviet Union after careful comparative studies of various vaccinia strains. It should be noted that there are several laboratories where the origin of the strain or the type of strain is unknown.

Among the animals and media available for production of vaccine, calves are most frequently employed (Table 3). Only three laboratories (one in the Americas and two in Europe) are producing vaccine on other than animal skin.

The WHO questionnaires in 1967 requested information regarding potency and stability of vaccine as tested in the individual laboratories. Only 16 of 45 laboratories recorded satisfactory results for both the potency and heat stability of their vaccines. During 1967, 16 laboratories in 16 countries submitted samples of vaccine to WHO for independent testing. Of these, vaccine from only 7 laboratories (43%) consistently met WHO requirements. Thus, the quality of the vaccine was generally unsatisfactory at that time and it was felt that if the eradication programme were to be successful, urgent measures were required to improve vaccine quality. Two principal approaches were taken. First, a Seminar on Vaccine Production was convened in March 1968 and, second, steps were taken to provide consultation, fellowship training, and independent testing of batches of vaccine.

Seminar on Vaccine Production

The purpose of this Seminar is best expressed in the introductory paragraph of its final report,[2] as quoted below:

"The various vaccine laboratories employ a wide variety of production methods evolved over many years through trial and error, experimentation, adaptation and arbitrary decision. Although the basic principles of smallpox vaccine production and testing have been elaborated, a detailed methodology has never been published. Accordingly, WHO convened a working group, composed of those with expertise in vaccine production and a broad [illegible] production particularly in the developing countries, to consider alternative methods for the production and testing of freeze-dried smallpox vaccine and to recommend the simplest, most practicable methods".

Staff from five laboratories participated in the Seminar: Rijks Instituts voor de Volksgezondheid, Netherlands; Wyeth Laboratories, Inc., USA; Research Institute of Virus Preparations, Moscow, USSR; Connaught Medical Research Laboratories, Canada; and Research Institute of Immunology, Prague, Czechoslovakia. The group, after discussions in Geneva, visited two laboratories, the Research Institute of Virus Preparations in Moscow and the Wyeth Laboratories, USA, to observe actual production processes and to discuss further the proposed methods. A manual termed "Methodology of Freeze-dried Smallpox Vaccine Production" was prepared during the Seminar and this has been made available to producers on request. It might be useful to mention briefly several important points noted in this manual. First, the group recommended the establishment of a seed virus system with a high potency seed lot of more than $10^{8.7}$ p.f.u./ml. to ensure consistency in the vaccine strain and a high concentration of virus in the pulp. It was noted that with good technique, the pulp should contain 10^{10} p.f.u./ml. of virus. Copious cleansing of the animals and subsequent appropriate use of phenol, in addition to the usual precautions to avoid contamination, should result in a very low or nil bacterial count in the final bulk material The choice of freeze-driers, problems of sealing, types of final containers such as ampoules, vials, and vampoules are discussed and illustrated in the manual. A vaccine fill of 0.25 ml. was recommended in view of the universal use of the bifurcated needle in the eradication programme. For diluent, 25% glycerol was suggested since it is less virocidal than the customarily used higher concentrations. For egg testing, the use of a 0.1 ml. inoculum on CAM was recommended as being more sensitive than the use of 0.2 ml. It was noted that some producers are not certain about the history of their seed virus and some normally observe different morphological types of pocks from seed virus inoculated on CAM, indicating that their seed virus may not be homogenous. While it is difficult to translate the significance of such observations into practical terms of reactogenicity and immunogenicity, the group was of the opinion that it would be more practical and realistic to replace questionable strains with strains such as the Lister or EM63, which have proven to be satisfactory both with respect to immunogenicity and reactogenicity.

Provision of Consultant Services, Fellowship Training and Vaccine Testing by WHO.

It was felt most desirable to establish the closest possible contact between producers and consultant laboratories. In the Americas, since 1967, the Connaught Medical Research Laboratories, Toronto, Canada, have assumed responsibility, under contract with WHO, for the provision of consultation, fellowship training and vaccine testing to vaccine producers in South and Central America. Thirteen South and Central American laboratories have now established close communication with the Connaught laboratories. For laboratories in other parts of the world, the National Institute of Public Health, Utrecht, Netherlands, has undertaken to provide a vaccine testing service as well as consultative assistance; additional help is provided also by special consultants from the United Kingdom, Czechoslavakia, the Soviet Union, Austria, France and Sweden. Vaccine testing includes determination of initial potency, heat stability (both at 100°C for 1 hour and at 37°C for 4 weeks), bacteriological testing, phenol content, moisture content and degree of vacuum in the final containers.

These laboratories are also prepared to provide seed lot virus, ready for inoculation, to producers who wish to replace their current strain. During the Seminar on Vaccine Production, the availability of reference vaccine was of concern since some laboratories in developing countries are not able initially to produce a national reference vaccine for purposes of routine vaccine testing. For such laboratories, the two testing laboratories agreed to provide their own national reference preparation until producers could develop their own reference standard. Developmental studies, related to vaccine production problems have also been carried out in these two laboratories. In the Utrecht laboratory, a bank of vaccinia strains has also been established. In 1969, these two laboratories were officially designated as the WHO Regional Reference Centre for Smallpox Vaccine (Connaught Medical Research Laboratories) and the WHO International Reference Centre for Smallpox Vaccine (Rijks Institute).

Potency and Heat Stability of Vaccines Presently in Use

With the assistance particularly of the two WHO Reference Laboratories, closer contact with producers throughout the world has been established. Recent results of vaccine testing, as performed by these Reference

Laboratories suggest that substantial improvements in vaccine quality have been made although further improvements would be desirable.

Table 4 presents the results of 201 lots tested during 1968 and 1969 to date. Twenty-two producers submitted samples. Of the 201 lots tested, 150 lots met WHO recommended standards for potency, heat stability and purity. Of 51 lots which showed unsatisfactory results, 45 lots were unsatisfactory with respect to heat stability. Only four lots revealed unsatisfactory bacterial counts. Of the lots with satisfactory results, about 40% had nil bacteria by plate count assay.

Since the heat stability of vaccine remains the most significant problem, a further analysis was made of the results of heat stability testing obtained in tests of up to five successive lots from 20 different producers (Table 5). With the exception of two producers (one in Africa and one in Australasia), the average loss of titre after incubation of the vaccine for 4 weeks at 37°C was less than 0.67 log. In 10 laboratories the average loss of titre was less than 0.3 log.

Rapid Heat Stability Test (after 1 hour at 100°C)

In some endemic countries, the pressing demands for vaccine supply and the lack of storage space recommended the evaluation of a test for vaccine stability which would take less time than the conventional 4 week stability test. During the Seminar on Vaccine Production, further evaluation of the test requiring incubation at 100°C for 1 hour was proposed. Since 1968, the WHO Vaccine Reference Laboratory in Utrecht, has conducted in parallel this rapid heat stability test and the conventional heat stability test.

Table 6 shows a comparison of the average loss of titre between the conventional and rapid heat stability tests for several successive sample lots produced in 12 laboratories. With the rapid heat stability test, the loss in titre is greater; vaccine produced by 6 of 12 producers showed a titre reduction of more than 1.0 log after incubation. The correlation in results obtained with these two tests is at best approximate; results obtained with [illegible] provided by producer 5 are particularly at variance with the others.

A further examination of the data was made to determine the possible application of the rapid heat stability test as a screening test for lots of vaccine - in other words, to ascertain if vaccine which contained a defined minimum titre of virus and lost nor more than a defined maximum amount of virus after incubation at 100°C for 1 hour, could be assured of passing the conventional stability test involving incubation for 4 weeks at 37°C. A comparison of results obtained for 139 lots from 24 producers tested during 1968 and 1969 is shown in Table 7[2]. Of the 139 lots examined, 106 passed the usual heat stability test. As can be seen in the figure, all lots except one, which passed the conventional heat stability test had an initial titre of $10^{8.5}$ p.f.u./ml. or more and retained a titre of $10^{7.5}$ or more after incubation at 100°C for one hour

With regard to the exceptional lot, it should be noted that there was a substantial difference between titres obtained before incubation when this lot was tested on two occasions, suggesting that the lot of vaccine itself may not have been homogenous.

On the basis of these observations, it is proposed to accept as satisfactorily stable, without further testing, lots of vaccine which have an initial titre of $10^{8.5}$ p.f.u./ml. and which retain a potency of $10^{7.5}$ p.f.u./ml. or more after incubation for 1 hour at 100°C. Of the 106 lots which were satisfactorily stable by the conventional stability test, 68 (64%) conformed with these criteria, and could be immediately released for use without further stability testing.

In summary, considerable overall improvement in vaccine quality has been noted since the beginning of the intensified global eradication programme. Further improvement is certainly necessary. Close cooperation and communication among all the laboratories concerned will be most important.

References

1) Methodology of Freeze-Dried Smallpox Vaccine Production, World Health Organization, SE/68.3 Rev. 1.

2) Testing results provided by Dr A. Hekker, Rijks Institute, WHO International Reference Centre for Smallpox Vaccine, Utrecht, Netherlands.

Table 1. Geographical Distribution of Producers of Freeze-dried Smallpox Vaccine

	Routine Production	Developing
AFRICA		
Angola	1	-
Algeria	1	-
Congo (D.R.)	-	1
Ethiopia	1	-
Guinea	-	1
Kenya	1	-
Mozambique	1	-
Nigeria	1	-
Rwanda	-	1
Senegal	-	1
Tunisia	1	-
Union of South Africa	1	-
United Arab Republic	1	-
Total: (13)	9	4
Australiasia		
Australia	-	1
Burma	1	-
Cambodia	1	-
Ceylon	-	1
India	4	-
Indonesia	1	-
Iran	1	-
Iraq	-	-
Japan	6	-
Jordan	-	1
New Zealand	1	-
Pakistan	1	1
Philippines	1	-
Taiwan	1	-
Syria	-	-
Thailand	1	-
Vietnam	-	1
Total: (17)	19	5

	Routine Production	Developing
AMERICAS		
Argentina	1	-
Bolivia	-	1
Brazil	2	2
Canada	1	1
Chile	1	-
Colombia	1	-
Cuba	-	1
Ecuador	-	1
Peru	1	-
USA	2	-
Venezuela	-	1
Total: (11)	9	7
Europe		
Austria	1	-
Belgium	1	-
Bulgaria	1	-
Czechoslovakia	1	-
France	3	-
Germany	3	-
Hungary	1	-
Italy	3	-
Netherlands	1	-
Portugal	1	-
Spain	1	-
Sweden	-	1
Switzerland	1	-
Turkey	1	-
United Kingdom	1	-
USSR	6	-
Yugoslavia	1	-
Total: (17)	27	1
GRAND TOTAL: (58)	64	17

Table 2. Vaccinia Strains Used in Laboratories Presently in Routine Production

	No. of Laboratories	Lister	New York Board of Health	Institut Pasteur	Berne	Patwadanger	EM 63	Other*	Unknown
AFRICA	9	3	0	1	0	0	0	2	3
AMERICAS	9	2	5	0	0	0	0	1	1
Australasia	19	7	0	1	0	3	0	7	1
Europe	27	8	0	1	2	0	4	10	2
Total	64	20	5	3	2	3	4	20	7

* Includes Institut Chambon (Paris), Ikeda, Budapest, Bohemia, Hamburg, Bordeaux, Aosta, Minsk.

Table 3. Medium Used for Production of Vaccinia Virus in Laboratories Presently in Routine Production of Freeze-Dried Vaccine

	No. of Laboratories	Calves	Sheep	Eggs	Tissue Culture	Unknown
AFRICA	9	4	5	0	0	0
AMERICAS	9	8	0	1	0	1
Australasia	19	13	5	0	0	1
Europe	27	21	6*	0	2**	1
Total	64	46	16	1	2	3

* One laboratory uses both calves and sheep

** Two laboratories use bovine embryo muscle tissue cell cultures, in addition to animals

Table 4. Testing Results of Vaccines from 22 Producers by WHO Reference Laboratories - 1968 and 1969 (to June)

	No. of Lots Tested	No. of Lots Satisfactory*	No. of Lots Unsatisfactory	No. of Lots Unsatisfactory when Tested		
				Initial Potency	Heat Stability	Bacterial Count
AFRICA Producer						
No. 1	23	23	-	-	-	-
2	5	-	5	5	5	-
3	2	2	-	-	-	-
4	4	-	4	4	4	-
5	3	3	-	-	-	-
AMERICA Producer						
No. 1	2	2	-	-	-	-
2	5	5	-	-	-	-
3	3	-	3	1	3	-
4	10	-	10	-	10	-
5	2	-	2	2	2	-
6	1	1	-	-	-	-
Australasia Producer						
No. 1	12	3	9	-	9	-
2	3	3	-	-	-	-
3	60	54	6	2	2	3
4	5	5	-	-	-	-
5	6	3	3	-	3	-
6	11	10	1	-	-	1
7	2	2	-	-	-	-
8	16	16	-	-	-	-
Europe Producer						
No. 1	2	2	-	-	-	-
2	2	2	-	-	-	-
3	22	14	8	1	7	-
TOTAL	201	150	51	15	45	4

* Vaccine with potency of over 1×10^8 p.f.u./ml., with heat stability maintaining over 1×10^8 p.f.u./ml after 4 weeks at 37°C and with bacterial count less than 500/ml.

Table 5. HEAT STABILITY AFTER 4 WEEKS AT 37°C OF MOST RECENT SUCCESSIVE FIVE LOTS PRODUCED BY 20 LABORATORIES AND TESTED BY WHO REFERENCE LABORATORIES

Titre of S.F.I./Aa expressed as Log

	Producers	Initial Titre		After 4 weeks at 37°C		Average Loss of Titres (A)-(B)
		(A) Average	(Range)	(B) Average	(Range)	
AFRICA	A	8.78	(9.04-8.57)	8.57	(8.84-8.25)	0.21
	B	8.26	(8.55-8.04)	7.73	(8.38-7.36)	0.53
	C****	7.83	(8.51-7.17)	6.80	(7.47-6.04)	1.03
AMERICAS	A	8.40	(8.63-8.25)	8.19	(8.30-8.08)	0.21
	B	8.05	(8.17-7.93)	7.45	(7.60-7.30)	0.60
AUSTRALASIA	A	9.43	(9.51-9.34)	9.28	(9.39-9.20)	0.15
	B	8.63	(8.75-8.50)	8.42	(8.61-8.27)	0.21
	C	7.97	(8.02-7.74)	7.72	(7.91-7.56)	0.25
	D	8.89	(9.11-8.80)	8.63	(8.95-8.27)	0.26
	E	8.59	(8.76-8.46)	8.33	(8.41-8.17)	0.26
	F**	8.82	(8.88-8.77)	8.48	(8.53-8.43)	0.34
	G***	8.78	(8.95-8.51)	8.39	(8.60-8.00)	0.39
	H	9.00	(9.60-8.51)	7.79	(8.75-6.98)	1.21
EUROPE	A	9.02	(9.11-8.82)	8.81	(9.14-8.55)	0.21
	B	8.43	(8.63-8.25)	8.21	(8.36-8.14)	0.22
	C*	8.07	8.07	7.80	7.80	0.27
	D	8.92	(9.11-8.65)	8.56	(8.81-8.23)	0.36
	E**	8.60	(8.73-8.46)	8.22	(8.30-8.14)	0.38
	F	8.95	(9.39-8.79)	8.56	(8.79-8.32)	0.39
	G*	8.74	8.74	8.07	8.07	0.67

* Only one test lot

** Two test lots

*** Three test lots

**** Four test lots

Table 6. Comparison of Loss of Titres between Heat Stability Tests after [illegible] at 100°C and after 4 weeks at 37°C

Serial No. of Producers	No. of Test Lots	Average loss of titre after 4 weeks at 37°C	Average loss of titre after 1 hour at 100°C
1	5	0.15	0.97
2	5	0.21	0.61
3	5	0.21	0.46
4	[illegible]	0.21	0.41
5	5	0.25	>3.56
6	5	0.26	0.68
7	5	0.26	1.39
8	1	0.27	1.04
9	2	0.34	1.63
10	5	0.39	0.89
11	3	0.39	1.19
12	5	1.21	>4.36

Conventional and Rapid Heat Stability Tests
(Titre of p.f.u./ml. expressed as Log)

o: Lot passed conventional test (4 weeks at 37°C).
x: Lot failed conventional test (4 weeks at 37°C).

Initial Titre	Titre after 1 hour at 100°C: 9.00 or greater	8.50 - 8.99	8.00 - 8.49	7.50 - 7.99	7.00 - 7.49	6.50 - 6.99	6.49 or less
9.00 or greater		ox[1] oooo oo	ooooooo ooooooo	ooooo	o		ooxo
8.90 - 8.99		o	ooooooo	o			
8.80 - 8.89			ooooooo ooo	o	o	oo	o
8.70 - 8.79			oooooo	o	o	o	ooo
8.60 - 8.69			ooooo oo	ooo	xo		xo
8.50 - 8.59			oo	o	xo	o	x
8.40 - 8.49			o	oooox		o	
8.30 - 8.39			o	ooxxox	xoxo		x
8.20 - 8.29				ox	xx		xox
8.10 - 8.19			o	ooxx	xoo		xxox
8.00 - 8.09				xxx	xox	xx	xx

[1]The titre of this vaccine is:

	100°C 1 hour			37°C 4 weeks		
	Before	After	Loss	Before	After	Loss
	9.25	8.53	0.72	8.72	7.65	1.07