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17567

DP/ID/SER.A/1206
19 May 1989
ORIGINAL: ENGLISH

HIGH-LEVEL ADVICE TO THE GOVERNMENT OF THE PHILIPPINES TO VALIDATE THE "INTERCARE STUDY ON THE ALABANG VACCINE COMPLEX" (MANILA, 4-6 APRIL 1989)

SI/PHI/89/801

PHILIPPINES

Technical report: Model programme for the production of vaccines in developing countries: manufacturing and control methods of the Hungarian freeze-dried intradermal BCG vaccine prepared from surface culture*

Prepared for the Government of the Philippines
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of Dr. L. Lugosi,
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* This document has not been edited.

V.89 55763

CONTENTS

I. INTRODUCTION

A. Abbreviations

BCG	Bacille Calmette-Guérin
i.d.	intradermal
VU	Viable Units
WHO	World Health Organization
L-J	Lőwenstein-Jensen medium
BOAA	Blood-Oleic-Acid-Albumin-Agar medium
M7H10*	Middlebrook 7H10 Agar medium

* BOAA can be replaced by M7H10

B. Historical Background

In line with the recommendations of the Second Consultation on the Pharmaceutical Industry, held in Budapest 1983, a survey was carried out in 1984 under the project No. UC/RAF/83/088 - Programme for Production of Vaccines in Africa (UNIDO/IO/R.148 dated 31 January 1985). The survey has covered 10 African countries as follows: Algeria, Chad, Ethiopia, Ghana, Kenya, Madagascar, Nigeria, Senegal, Tanzania, Tunisia. One of the follow up project of the survey, the rehabilitation of BCG vaccine production unit at the Pasteur Institute of Madagascar is under implementation (SI/MAG/84/801).

The Third Meeting of the Advisory Panel on Preventive Medicine(Bilthoven, The Netherlands, 6-7 June 1985) recommended the drafting of an explanatory memorandum to the "Model Programme for the Production of Vaccine in Developing Countries" including that there are alternative production systems and methods which may be covered in later UNIDO documentation. One of the worldwide used conventional methods for production of BCG vaccine against tuberculosis is the stationary cultivation. This method, that is the production of dried BCG vaccine from surface culture of SAUTON medium based on the "classical" technique of CALMETTE and GUERIN, is relatively simple and not expensive, therefore its transfer and application within the limited facilities of many developing countries seems to be both technically and economically feasible. For example, this method is used in Madagascar since 1964. The same technology is used in Hungary since 1959.

Because of the high risk of tuberculosis infection and the high incidence rate of tuberculosis in children population, the BCG primovaccination of newborns and the revaccination of tuberculin negative children and young adults up to 20 years of age has become compulsory in Hungary since 1953 and 1958, respectively (1,2). In order to meet the requirements of the WHO and to assist the National Programme for Control of Tuberculosis, the technology of production and quality control of the BCG vaccine was completely revised in the National Institute of Hygiene, Budapest in 1959 (3,4,5,6,7,8). The present document gives a detailed technological description of this technology.

It should be noted that this technology is regularly updated in order to meet the newest requirements of WHO. In the document the production and quality control of a single batch containing 10.000 vials of 10 or 20 doses, i.e. altogether 100,000 or 200.000 doses is described as an illustration (9,10,11). Costs of production and quality control are given in Annexes XVIII - XIX.

C. Definition

The freeze-dried i.d. BCG vaccine (Vaccinum Tuberculosis /BCG/ Cryodesiccatum) is an attenuated live vaccine used to prevent tuberculosis prepared from the Mycobacterium tuberculosis typus bovinus varietas Calmette Guérin (11).

Before delivery the batches are tested for sterility, safety and potency. According to the prescriptions, after reconstitution with the enclosed diluent 1.0 ml Aqua dest. pro. inj. the vaccine contains the appropriate number of culturable particles, $1\text{-}4.10^6$ VU/ml minimum 100,000 VU per 0.1 ml in one i.d. dosis.

D. Composition

The dried i.d. BCG vaccine seeled under vacuum or protective gas after lyophilisation in one vial of ten doses before reconstitution contains 1.0 mg (semi-dry weight) bacilli in stabilising medium containing 5.0% Sodium Glutamate, 5.0% Dextran and 7.5% Glucose.

E. Reconstitution

The enclosed diluent should be added to the dried BCG vaccine with a sterile sytinge. The resuspended vaccine should be shaken to avoid sedimentation before administration. The reconstituted vaccine should be used after reconstitution within four hours. Any reconstituted vaccine remaining unused after four hours should be discarded.

F. Administration (Annex XVI)

For new-borns and tuberculin negative individuals (children and adults after a previous tuberculin test) the BCG dose is 0.1 ml by i.d. injection. The superior and middle region surface of the left upper arm should be disinfected with alcohol or alcohol-ether mixture. From the reconstituted vaccine 0.1 ml should be injected strictly intradermally with a 1 cc. tuberculin syringe with a 26 gauge needle (special care must be taken of the exact dosage). Subcutaneous injection should be avoided.

G. Contraindications

BCG vaccination is forbidden for prematures under 2000,0 g weight, for new-borns suffering from obstetrical trauma, dermatological affections or alimentary disorders and for individuals suffering from acute or chronic infections or feverish diseases as well as for tuberculin positive children and adults.

H. Vaccination reactions

2-3 weeks after the intradermal BCG vaccination a small local lesion develops. The diameter of exulcered lesion reaches its maximum in the 5-8th weeks and generally heals after 12-16 weeks with a scar. The enlargement of the axillary lymph-nodes can be considered as normal regional reaction. In some cases, depending on the vaccination technique and on the age of the person vaccinated, a suppurative process may develop in the regional lymph-nodes with a longer spontaneous healing period. Surgical treatment is generally not necessary.

I. Expiration date

12 months after the delivery date if BCG vaccine at $+2^{\circ}C$ $+10^{\circ}C$ and protected from light.

J. Delivery (Annex XVII)

In boxes of 5 multidose (10 or 20) vials of dried BCG vaccine and 5 ampoules of distilled water (1.0 or 2.0 ml) enclosed as diluent for reconstituation.

II. GENERAL REQUIREMENTS

In order to avoid all risk of contamination during the preparation the production of the BCG vaccine takes place in a separate area with separate equipment and a staff responsible only for the BCG production.

A. Manufacturing establishment (Annex XX)

The facilities of the BCG production area are constructed so that their functions fulfil the requirements of the WHO (12, 13).

B. Control establishment

The facilities of the BCG control area are constructed so that the their functions fulfil the requirements of the WHO (12, 13).

C. Staff (Annex XXII)

The professional training, the state of health of the staff responsible for the BCG production and its control fulfil the requirements of the WHO (11).

D. Biosafety

With the continuous development of the technology of all biolcgical products and in order to ensure the condition for the security of the production all recent knowledge in biosafety is applied in the BCG manufacturing and control laboratories (14).

E. Biostatistics

To ensure a most extended level of the statistical quality control of the BCG vaccine exact biostatistical methods are used in the evaluation of the laboratory and field control data (15, 16, 17, 18, 19).

III. MANUFACTURING REQUIREMENTS (Annex I)

For the staff the details of the BCG manufacturing methods are described in Hungarian, recorded in the Hungarian Ministry of Health as National Control Authority of Biological Products and in Pharmacopoea Hungarica (20, 21, 22).

A. Seed-Lot System (Annex II)

The maintenance and the transfer of the French BCG strain 1173-P2 used for vaccine production in Hungary since 1960 are practised according to the principles of the Seed-Lot System (10, 11, 23, 24).

B. Strain transfer. Cultures (Annex III - VI)

Seven days 37°C incubated 60 cm^2 surface pellicles of BCG cultures on 100.0 ml Sauton liquid medium in Erlenmeyer bottles of 300.0 ml are used for bacterium production. One bottle contains 2.5-3.5 g semi-dry weight of BCG at seventh day (pH 6.5-7.0). For one batch 4-6 pellicles are harvested and pooled. To ensure sterility parallelly harvested-pools are maintained in independent thermostates also for security in case of short-circuit.

C. Harvesting. Stock suspension (Annex VII - VIII)

From Sauton medium the pellicles are harvested and separated in a steril device made of Büchner funnel and stainless steel filter. The collected BCG cake is weighted in covered steril glass-tube (80x30 mm) then transferred to the homogeneizing vessel (diameter: 250.0 mm, height: 60.0 mm) containing 2,500.0 stainless steel balls (diameter: 4.0 mm). The BCG cake is homogenised:

a) by hand without diluent during 1-1.5 minute,

b) with sterile Na-Glutamate of 1.5 % at a BCG concentration of 1.0 g/ml in a rotating mill with 40 rpm during 5 minutes,

c) as stock suspension of 100.0 mg/ml adjusted with Na-Glutamate of 1.5 % during 1-1.5 minute by hand. The stock suspension is kept at 4°C during 48 hrs in the homogeneizing vessel for testing sterility.

D. Liquid bulk

Before lyophilization the liquid bulk (volume total: 3,724.5 ml) of one batch (10,000 vials) is composed as follow:

a) 1,200.0 ml Na-Glutamate of 15 %,

b) 1,200.0 ml Dextran of 15 %,

c) 1,200.0 ml Glucose of 22.5 %,

d) 124.5 ml BCG stock suspension of 100.0 mg/ml

E. Liquid filling lot

The liquid bulk is divided in two (I and II) liquid filling lot of equal volume to fill the 10,000 vials distributed in 8 boxes (8 "sub-lots") of the freeze-drying machine (1250 vials/box). From the Ist filling lot sterilized vials of boxes no 1, 2, 3 and 4, from the IInd one 5, 6, 7 and 8 are filled. Each vial containes 0.3 ml liquid vaccine of the filling lot. After filling vials the boxes are stored at 4°C before freeze-drying.

F. Lyophilization (Annex IX)

USIFROID-SMRG (1960) freezing, drying and sealing machines are used for lyophilization of the vaccine.

F.1. Freezing

Boxes containing filled vials are sunk into alcool batch (-50°C) of the freezing machine. The freezing velocity is 4°C decrease/minute. To accelerate temperature transmission 300.0 ml steril distilled water is poured into boxes around vials. Temperature of the vaccine has to drop to -35°C. The total time has to be a minimum of 12 minutes.

Boxes containing the freezed vaccine are stored at -20°C -30°C in the freezer before drying.

F.2. Drying

The 4 shelves of the drying machine are prefreezed to -35°C -40°C. The 8 boxes containing the freezed vaccines are placed on the shelves within 3 minutes. Drying parameters are as follows:

a) Condensator tempepature: -55°C -60°C,

b) Vacuum: 0.01-0.001 Hgmm,

c) Heating:

i) primary drying +5C during 24 hrs,

ii) secondary drying +25°C during 18 hrs.

After finishing secondary drying the vacuum is broken with sterile filtered air.

F.3. Sealing of vials

Stoppering machine closes the vials under 0.01 Hgmm vacuum with special freeze-drying rubber stopper then vials are sealed with aluminium caps.

G. Final products, dried lot

The 8 boxes (8 "sub-lots") of the dried lot are considered as final product of the batch of 10,000 vials. The 8 "sub-lots" (1200 vials of each) are stored separately at +4°C during the control tests of sterility, safety and potency.

IV. CONTROL REQUIREMENTS (Annex XIII)

For the staff the details of the statistical quality control methods are described in Hungarian, recorded in the Hungarian Ministry of Health as National Control Authority of Biological Products and in Pharmacopoea Hungarica (20, 21, 22). Quality control tests are performed from the final products in the production laboratory and independently by the National Control Authority (25).

A. Identity

The identity of the acid fast BCG is verified by microscopical investigation after Ziehl-Neelsen staining and by the morphology of the colonies while viability determination on solid medium.

B. Sterility (Annex X)

Number of vials ($0.4\sqrt{N}$) for sterility tests are random selected separately from the 8 "sub-lots". Tests for absence of bacterial or mycotic contamination are performed according to the WHO Requirements (26).

C. Safety (Innocuity) (Annex XII)

C.1. Absence of virulent mycobacteria

6 guinea-pigs (tuberculin negative, same sex, 250-300 g weight) are s.c. inoculated with 50 human i.d. doses from the liquid bulk (III. D.). The observation, examination and evaluation are performed according to the WHO Requirements (11).

C.2. Test for toxic products

0.5 ml (0.5 mg) reconstituted final products are s.c. inoculated to 2 mices (12-18 g) to exclude the presence of toxic products in substances used in liquid bulk and in diluent. Observation: 10 days.

C.3. Test for tetanus

5.0 ml (5.0 mg) reconstituted final product is s.c. inoculated to 2 guinea-pigs (250-300 g) to exclude tetanus contamination. Observation: 10 days.

D. Residual virulence

D.1. Skin reactivity in guinea-pigs (Jensen test)

The test is optional and performed according to the WHO Requirements (11).

D.2. Relative persistence capacity in mice spleen

The test is optional and performed as described in reference (27).

D.3. Virulence test in hamsters

The test is optional and performed as described in reference (28).

E. Tuberculin sensitivity

The test of the development of BCG induced allergy in guinea-pigs is optional and performed as described in reference (34).

F. Protective effect

The test of the protective effect in animals (mice, guinea-pigs, bank-volves) is optional and performed as described in reference (34).

G. Viability. In vitro potency (Annex XI)

The test is performed as described in references (29, 30, 31).

H. Stability of the viability

The test is performed as described in references (32, 33, 34).

I. Residual moisture

The residual moisture content of the lyophilized BCG should be between 0.5 and 2.0 %.

J. Vacuum test of vials

The vacuum of vials is tested as described in reference (11).

K. Production consistency

The test is performed as described in references (11, 35, 36).

L. Field-trial

Field-trial for clinical surveillance of the vaccine in man are conducted by the National Control Authority according to the WHO Requirements (11).

V. RELEASE (Annex XIV - XV)

When all quality control tests have been performed in the production and in the National Control Laboratory and then fulfil all requirements, the National Control Authority of the Biological Product delivers the certificate as an official national release document of the tested dried BCG batch.

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Annex I

BCG Laboratory. National Institute of Hygiene. H-1097 Budapest, Gyáli ut 2.
Daily control data of laboratory and machines.

19.. month .... day	contr. hrs	incubators temperature C° no 1	no 2	no 3	no 4	refrigators temperature C° no 1	no 2	no 3	no 4	laboratory temp. C°	bar. p.	drying machine manometer p low. press	kompr.	high press	freezing mach. temp. C° freez-er	con-serv.	contr. by sign.	observ.
mo 1																		
tu 2																		
we 3																		
th 4																		
fr 5																		
sa 6																		
su 7																		

BCG Laboratory. National Institute of Hygiene. H-1097 Budapest, Gyáli ut 2.
BCG strain maintaining and passage SEED-LOT system.

pass. date 19..	culture					seeded medium				sterility test						observ.
	previous definition	flask no.	inoc. dat.	med. pH	descendant definition	name	ser. no.	nbs inoc. flask	by sign.	ref. no.	media Db	Sa	NaS	result date	sign.	

BCG Laboratory. National Institute of Hygiene. H-1097 Budapest, Gyáli ut 2.
Sauton medium preparation records.

Sauton ser.no.: .... date 19..			Sauton ser.no.: .... date 19..		
compounds	origin, fabric.no.	quantity	compounds	origin, fabric.no.	quantity
Fe amm. citr.	.....	.....	Fe amm. citr.	.....	.....
MgSO4.7H2O	.....	.....	MgSO4.7H2O	.....	.....
K2HPO4	.....	.....	K2HPO4	.....	.....
Acid. citr.	.....	.....	Acid. citr.	.....	.....
L-Asparagin	.....	.....	L-Asparagin	.....	.....
H2O dest	.....	.....	H2O dest	.....	.....
Glycerin	.....	.....	Glycerin	.....	.....
ZnSO4 0.01% sol.	.....	.....	ZnSO4 0.01% sol.	.....	.....
NH4OH 10% sol.	.....	.....	NH4OH 10% sol.	.....	.....
pH before sterilization	.....		pH before sterilization	.....	
distribution	.....		distribution	.....	
	.....			.....	
	.....			.....	
	.....			.....	
	.....			.....	
sterilization	.....		sterilization	.....	
pH after sterilization	.....		pH after sterilization	.....	
prepared by	.....		prepared by	.....	
observations:			observations:		

BCG Laboratory. National Institute of Hygiene. H-1097 Budapest, Gyáli ut 2.
Löwenstein-Jensen (Löw.-Jens.) medium preparation records.

Löw.-Jens. ser.no.: .... date 19.. .....

compounds	origin, fabric.no.	quantity
$MgSO_4.7H_2O$	.....	.....
KH_2PO_4	.....	.....
L-Asparagin	.....	.....
Mg.citr.	.....	.....
H_2O dest.	.....	.....
Eggs	.....	.....
Malachitgreen 2% sol.	.....	.....

distribution in Legroux flask 20.0 ml ..... pcs

coagulation: 85-90°C/60-90'

data of coagulation: time and temperature

t	°C	t	°C	t	°C

prepared by: .....

observations:

Löw.-Jens. ser.no.: .... date 19.. .....

compounds	origin, fabric.no.	quantity
$MgSO_4.7H_2O$	.....	.....
KH_2PO_4	.....	.....
L-Asparagin	.....	.....
Mg.citr.	.....	.....
H_2O dest.	.....	.....
Eggs	.....	.....
Malachitgreen 2% sol.	.....	.....

distribution in Legroux flask 20.0 ml ..... pcs

coagulation: 85-90°C/60-90'

data of coagulation: time and temperature

t	°C	t	°C	t	°C

prepared by: .....

observations:

Eggs cleaning: soak in 1% - Na_2CO_3 sol. washing with soap, rincing in water.

BCG Laboratory, National Institute of Hygiene, H-1097 Budapest, Gyáli út 2.
Blood-Oleic-Acid-Albumin (BOAA) medium preparation records.

BOAA ser.no.: .... date 19..		
compounds	**origin, fabric.no.**	**quantity**
KH_2PO_4	.....	.....
$Na_2HPO_4.2H_2O$	.....	.....
L-Asparagin	.....	.....
Agar	.....	.....
H_2O dest.	.....	.....
Trace elements:		
Fe amm.citr. 1.0% sol.	.....	.....
$MgSO_4.7H_2O$ 1.0% sol.	.....	.....
$CaCl_2$ 0.05% sol.	.....	.....
$ZnSO_4$ 0.01% sol.	.....	.....
$CuSO_4$ 0.01% sol.	.....	.....
sterilization:		
cooling: 55-58°C		
add:		
Bovine alb. fract.V. 5% sol.	.....	.....
n/20 NaOH dil. oleic acid. 1.2% sol.	.....	.....
human transfusion blood	.....	.....
distribution: 15.0 ml/Petri dishes ..... pcs		
prepared by:		
observations:		

BOAA ser.no.: .... date 19..		
compounds	**origin, fabric.no.**	**quantity**
KH_2PO_4	.....	.....
$Na_2HPO_4.2H_2O$	.....	.....
L-Asparagin	.....	.....
Agar	.....	.....
H_2O dest.	....	.....
Trace elements:		
Fe amm.citr. 1.0% sol.	.....	.....
$MgSO_4.7H_2O$ 1.0% sol.	.....	.....
$CaCl_2$ 0.05% sol.	.....	.....
$ZnSO_4$ 0.01% sol.	.....	.....
$CuSO_4$ 0.01% sol.	.....	.....
sterilization:		
cooling: 55-58°C		
add:		
Bovine alb. fract.V. 5% sol.	.....	.....
n/20 NaOH dill oleic acid. 1.2% sol.	.....	.....
human transfusion blood	.....	.....
distribution: 15.0 ml/Petri dishes ..... pcs		
prepared by:		
observations:		

RCG Laboratory. National Institute of Hygiene. H-1097 Budapest, Gyáli ut 2.
Formulae of media for sterility tests. Preparation records.

Media Bch. no. -fabr. date	Semi-fluid Sodium hydrosulfite ...-.....		Fluid Saboureaud ...-.....		Solid Beef extract agar ...-.....	
compounds	origin, fabric. no.	quantity	origin, fabric. no.	quantity	origin, fabric. no.	quantity
Sodium chlorid (NaCl)	.....	2.5 g	.....	2.5 g	.....	3.0 g
Na2HPO4. 12HO	.....		.....		.....	4.0 g
Dextrose (C6H12O6. H2O)	.....	5.0 g	.....	20.0 g	.....	
Yeast extract	.....	50.0 ml	.....	50.0 ml	.....	
Trypcasin	.....	16.0 g	.....	16.0 g	.....	
Refined powder agar	.....	0.75 g	.....	0.75 g	.....	17.0 g
Sod. hydrosulfite (Na2S2O4. 2H2O)	.....	0.5 g	.....		.....	
Methylene blue (sol 0.05‰)	.....	4.0 ml	.....		.....	
Pepton	.....		.....		.....	10.0 g
Beef extract	.....		.....		.....	15.0 g
H2O dest.	.....	1,000.0 ml	.....		.....	
pH	7.1 (8/n NaOH)	1.2 ml	6.0 (25% HCl)		.....	

BCG Laboratory, National Institute of Hygiene, H-1097 Budapest, Gyáli út 2.
Freeze-dried BCG vaccine cryoprotective agents preparation.

Bch no.: .... date 19..			Bch no.: .... date 19..		
compounds	origin, fabric.no.	quantity	compounds	origin, fabric.no.	quantity
Dextran pulvis /DE/	.....	.....	Dextran pulvis /DE/	.....	.....
H2O dest.	.....	.....	H2O dest.	.....	.....
pH before sterilization	.....		pH before sterilization	.....	
distribution	.....		distribution	.....	
sterilization	.....		sterilization	.....	
pH after sterilization	.....		pH after sterilization	.....	
Na L-Glutamat /NG/	.....	.....	Na L-Glutamat /NG/	.....	.....
H2O dest.	.....	.....	H2O dest.	.....	.....
pH before sterilization	.....		pH before sterilization	.....	
distribution	.....		distribution	.....	
sterilization	.....		sterilization	.....	
pH after sterilization	.....		pH after sterilization	.....	
D-Glucose /DG/	.....	.....	D-Glucose /DG/	.....	.....
H2O dest.	.....	.....	H2O dest.	.....	.....
pH before sterilization	.....		pH before sterilization	.....	
distribution	.....		distribution	.....	
sterilization	.....		sterilization	.....	
pH after sterilization	.....		pH after sterilization	.....	
DE+NG+DG before sterilization .... after sterilization .... prepared by:			DE+NG+DG before sterilization .... after sterilization .... prepared by:		
observations:			observations:		

Freeze-dried BCG vaccine preparation and control records.
Bch no.-date: ... - ..... - .. dos .... mg .... ml/amp.

National Institute of Hygiene.
BCG Laboratory. H-1097 Budapest, Gyáli ut 2.

BCG strain, culture, definition, seed date, harvest date, hrs												bact. semi-dry weight g	homog. diluent ml	Ist dil. ser. no. ..... ml	IInd dilution, liq. vacc. bulk cryoprotective agents ser. no. ml
cult. flask no.															
Sauton ser. no.															
Sauton pH												hrs	hrs	hrs	hrs .....

bulk flask no. pH	drying lot box no.	drying lot vial nbs	liquid vacc. filling lot date: ..... ml vial	filled by	hrs min	lab. t C	sterility test inoc. vol. ... ml/tube ref. no.	result by	result date	media Bd	media Sa	media NaS	vial stopp. date: ..... by	hrs min	vial seeling date: ..... by	hrs min	reject nbs	packing date: ..... by	hrs min
Final liquid vaccine bulk prepared by:							Ist dil						Observations						
Preparation data recorded by:							10 mg ml												
							10 mg ml												

Annex IX

BCG Labor. Natl Inst. Hyg. H-1097 Bp. Gyáli ut 2.
BCG vaccine freeze-drying records.
Bch no.-date: ... - ...... - .. dos .... mg .... ml/amp.

shelf /s/	**2**	**4**	**6**	**8**	**10**	**12**
vacc. /v/	**3**	**5**	**7**	**9**	**11**	**13**
boxes	**1/2**	**3/4**	**5/6**	**7/8**	**0/10**	**11/1**

hrs	**temperature tC°**												**machine: Usifroid SMRG**	
	trap	s_2	s_4	s_6	s_8	s_{10}	s_{12}	v_3	v_5	v_7	v_9	v_{11}	v_{13}	**operations**	**by**

Annex X

Dried BCG vaccine VU determination and sterility tests.
Bch no.: ... - ...... - .. dos .... mg .... ml/amp.
reconstituted by: ..-.. fluid ser.no. -date: ....
diluted by: ..-.. fluid ser.no. -date: ....
inoculated by: ..-.. date: ....
counted by: ..-.. date: ....

Natl Inst. Hyg. BCG Lab.
H-1097 Budapest, Gyáli ut 2.

bch lot amp. heat	rec. hrs dil. hrs inoc. hrs	sterility test inoc. vol. .... ml/tube result		media			dil. lev.	colony counts on .... media medium ser.no.: ... - .... container ...... inoc.vol. = ...ml										cont. ....
		by	date	Bd	Sa	NaS	$4 \cdot 10$	01	02	03	04	05	06	07	08	09	10	co-de

vacc.dil.after reconst. before seeding, hrs .....-..... diluent meas. by, hrs ..-.. .....-.....

dilution levels 00E										
diluent ml										
vaccine ml										
seeded medium										

Remarks

BCG Laboratory. National Institute of Hygiene. H-1097 Budapest, Gyáli ut 2.
Freeze-dried BCG vaccine Viable Units (VU) x10^6/ml in vitro laboratory control results and moisture content.
Bch. no. -date/lot/dos: ...-...../.-./... .... mg .... ml/amp. Nbs of amp./lot .... total/bach .....
BCG strain, culture, definition: ..... Cryoprotective agents composition: .....

Testing laboratory			BCG Laboratory											Nat.Contr.	Moisture	
Investigation date days after drying			....			 30			 90		 180		 360	....	....	....
Heat exp. +C°			4			4	20	37	4	20	4	20	4	4	4	4
Medium ser. no.			BOAA ...		L-J ...	BOAA ...			BOAA ...		BOAA ...		BOAA ...	BOAA ...	resid. % in amp.	
Amp. pool			1+2	3+4	$\bar{x}$	1+2	1+2	1+2	1+2	1+2	1+2	1+2	1+2	1+2+3	1	2
before drying from bulk no.	after drying from lot no.	1														
		2														
		3														
		4														
		5														
		6														
A1 ...		7														
A2 ...		8														
		$\bar{X}$														
VU % before lyoph. / after lyoph.																
VU % after heat exp. / after lyoph.																

Observations:

BCG Laboratory. National Institute of Hygiene. H-1097 Budapest, Gyáli ut 2.
Freeze-dried BCG vaccine control on laboratory animals tests records.
Bch no.-date: ...-..... - .. dos .... mg .... ml/amp.

Tests	Safety			Jensen			Tuberculin			tetanus	chem. poison
guinea pigs no / sex	1	2	3	1	2	3	1	2	3	1	1
guinea pigs color											
preliminary tuberculin test, TU dose date, result											
BCG vaccination date, dose											
animal weight in g control date											
results of autopsy local reaction allergy observations											

Annex XIII

BCG Laboratory. National Institute of Hygiene.
H-1097 Budapest, Gyáli ut 2.

SUMMARY PROTOCOL FOR DRIED BCG VACCINE PRODUCTION AND STATISTICAL QUALITY CONTROL

(Based on the Requirements for Biological Substances, No. 11- Requirements for Dried BCG Vaccine, WHO Technical Report Series, No. 638. 1979.

Identification of Final Lot

Name of manufacturer _____
Address of manufacturer _____
_____
_____
Telephone no. Telex No. _____
Lot No. of vaccine _____
Date of manufacture of final lot _____
Type of vaccine, Intradermal/ /Percutaneous/Other _____
Vol. (ml) of recommended single human dose _____
No. of containers in final lot for each filling volume _____

Information and tests on Seed Lot

1. Seed lot

Identity of seed lot strain used in vaccine _____
Origin of seed lot _____
Date of preparation of seed lot _____
Date of receipt of seed lot _____
Date of reconstitution of seed lot ampoule _____

2. Tests on seed lot (If these data on the same seed lot have been submitted before, completion of this paragraph is not necessary.)

(a) Identity test

Identified as BCG: Yes/No _____

Annex XIII (continued)

BCG Lab. NIH. Budapest. Summary, dried BCG vacc. contr. p.2.

(b) Absence of contamination. Sterility

Medium (Media) _____

Date of start of test(s) _____

Date of end of test(s) _____

Results _____

(c) Safety test. Innocuity. Absence of virulent mycobacteria

No. of human dose injected _____

No. of guinea-pigs given injection _____

Weight range (gramme) and sex of guinea-pigs _____

Date of start of test _____

Date of end of test _____

Health of animals during test _____

Mean weight gain in gramme and in % _____

Results: Passed/Failed _____

Seed lot approved: Yes/No _____

Date of approval _____

Information and tests on Manufacture, on Final Liquid Bulk.

3. Single harvest

No. of passages from reconstitution of seed lot _____

No. and size (ml) of vessel inoculated _____

Medium, volume in ml/vessel and pH _____

Date of inoculation _____

Date of harvest _____

pH of medium after harvest _____

Weight of harvested BCG mass in gramme _____

Visual inspection of culture and results _____

4. Final liquid bulk

Date of preparation _____

No. of single harvests included _____

Annex XIII (continued)

BCG Lab. NIH. Budapest. Summary, dried BCG vacc. contr. p. 3.

(a) Absence of contamination. Sterility

Medium (Media) for bacteria, fungi specific _____

Quantity tested; inoculated ml/medium _____

Temperatures, selected ... °C _____

Date of start of test _____

Date of end of test _____

Results:* _____

(b) Safety test. Innocuity.
Absence of virulent mycobacteria

No. of human doses injected _____

No. of guinea-pigs given injection _____

Weight range (gramme) and sex of guinea-pigs _____

Date of start of test _____

Date of end of test _____

Health of animals during test _____

Mean weight gain in gramme and in % _____

Results: Passed/Failed _____

(c) Substances added to final liquid bulk

Excipient(s), concentration of BCG: mg/ml _____

5. Freeze-drying

Type (amp., vial) and size (ml) of containers _____

No. of doses per container _____

No. of containers of each size in the filling liquid lot _____

Method of sealing the containers, vacuum, flame, rubber, under .. gas _____

* Records enclosed according to WHO-TRS 1973. No. 530. Part A. 6.

Annex XIII (continued)

BCG Lab. NIH. Budapest. Summary, dried BCG vacc. contr. p.4.

Information and Tests on Final Dried Product

Recommended reconstitution fluid _____

Volume (ml) of reconstitution fluid per final container _____

6. Identity test of BCG

Type of test _____

Results _____

7. Absence of contamination. Sterility

No. of containers tested (% of total) _____

Medium (Media) for bacteria, fungi, specific _____

Temperatures, selected ... °C _____

Date of start of test _____

Date of end of test _____

Results:* _____

8. Safety test. Innocuity.

(a) Absence of virulent mycobacteria (if test not performed on final bulk)

No. of human doses injected _____

No. of guinea-pigs given injection _____

Weight range (gramme) and sex of guinea-pigs _____

Date of start of test _____

Date of end of test _____

Health of animals during test _____

Mean weight gain in gramme and in % _____

Results: Passed/Failed _____

(b) Mouse test for toxic products

No. of human doses injected _____

---

* Records enclosed according to WHO-TRS 1973. No. 530. Part A.6.

Annex XIII (continued)

BCG Lab. NIH. Budapest. Summary, dr 1 BCG vacc. contr. p. 5.

No. of mice given injection _____

Date of start of test _____

Date of end of test _____

Results: Passed/Failed _____

(c) Guinea-pig test for tetanus

No. of human doses injected _____

No. of guinea-pigs given injected _____

Date of start of test _____

Date of end of test _____

Results: Passed/Failed _____

9. Residual virulence tests of BCG strain

(a) Skin reaction on guinea-pigs (Jensen test)

	Test vacc.				Ref. vacc.			
Date of start of test								
Date of end of test								
No. of guinea-pigs given injection								
Vaccine dose (0.1 ml) injected dilutions ...x	1	10	100	1000	1	10	100	1000
Mean diameter in mm								
95% conf. limits in mm								
95% conf. limits in %								

Relative potency _____

95% conf. limits of relativ potency _____

95% conf. limits of relativ potency in % _____

(b) Relative persistence capacity in mice spleen

	Test vacc.	Ref. vacc.
Date of start of test		
Date of end of test		
No. of mice given injection		
Vaccine dose (VU) injected dilutions ...x		
Sacrifice days (1, 60, 120, 360) of mice		
Mean Relativ Persistence Capacity at each sacrifice days		

Annex XIII (continued)

BCG Lab. NIH. Budapest. Summary, dried BCG vacc. contr. p.6.

	Regression coefficients		
	95% conf. limits of regr. coeff.		
	VU in % estimated at day 360		
	Validity of regression, Linearity: p<or>0.05		
(c)	Virulence test in hamsters	Test vacc.	Ref. vacc.
	Date of start of test		
	Date of end of test		
	No. of hamsters given injection		
	Vaccine dose (VU) injected dilutions ...x		
	Mean survival time (day)		
	95% conf. limits of survival time		
	95% conf. limits in %		
10.	Total bacterial content		
	Method of estimation		
	Results		
11.	Test for viability		
(a)	Viable Units (VU) determination	Test vacc.	Ref. vacc.
	Date of start of test		
	Date of end of test		
	Medium		
	No. of containers tested (% of total)		
	Mean VU 10^6/ml		
	95% conf. limits of VU 10^6/ml		
	95% conf. limits in %		
	Relative in vitro VU potency		
	95% conf. limits of rel. potency		
	95% conf. limits of rel. potency in %		
(b)	A.T.P. content (optional)	Test vacc.	Ref. vacc.
	Results		

Annex XIII (continued)

BCG Lab. NIH. Budapest. Summary, dried BCG vacc. contr. p.7.

(c) Stability of viability Accelerated degradation test	Test vacc.	Ref. vacc.
Date of start of heat exposure		
Date of end of heat exposure		
Temperature(s) of heat exposure: ... ^{o}C		
Sampling days of exposure		
No. of containers tested (% of total)		
Mean VU 10^{6}/ml		
95% conf. limits of VU 10^{6}/ml		
95% conf. limits in %		
Regression coefficients		
95% conf. limits of regr. coeff.		
Validity of regression, Linearity: p< or >0.05		
Percentage of survival of VU at day 30		

Information on Release

Does the batch fulfil the requirements _____

Has the lot been released by the National Control Authority ?: Yes/No _____

If no, why _____

Can a certificate be supplied by the National Control Authority ?: Yes/No _____

Which laboratory would supply such a certificate ? _____

Signature of head of the National Control Laboratory (Authority) _____

Name typed _____

Date _____

Annex XIII (continued)

BCG Lab. NIH. Budapest. Summary, dried BCG vacc. contr. p. 8.

Certification by person taking overall responsibility for production of the vaccine:

I certify that lot No. _____ of BCG vaccine satisfies Part A of the WHO Requirements for BCG Vaccine.

Signature _____

Name typed _____

Date _____

The protocol must be accompanied by a sample of the label, a copy of the leaflet, and a copy of the national control release certificate, if issued.

Information on the Manufacturer's product

When was the vaccine last tested in humans? Date		
Which area: country, dispensary, school, etd. ?		
Name of Head of testing group		
Summary of results	Test vacc.	Ref. vacc.
No. of children examined		
Age group		
Vaccination-testing interval, days		
Mean (mm) post-vaccination i.d. (Mantoux)* tuberculin reaction with ... TU		
95% conf. limits in mm		
95% conf. limits in %		
Mean (mm) vaccination lesion size		
95% conf. limits in mm		
95% conf. limits in %		

* Cf.: WHO Standard Tuberculin Test. WHO/TB/TG/3/1963.

Annex XIV

Control Department for Medico-Biological Preparations.
National Institute of Hygiene. H-1097 Budapest, Gyáli ut 2.
Approbation of the National Control authority for freeze-dried BCG vaccine.
Dried BCG vaccine national license no.: 60096/1964.V.5./821/V/55/64.OKI.Ell.
Certificate no.: ..... Reference no.: .....

Manufacturing laboratory: BCG Laboratory, 1097 Budapest, Gyáli u 2. Hungary.
Product, proper name: Freeze-dried BCG vaccine for Intradermal Injection.
descriptive definition: Prepared from the living Bacillus Calmette-Guérin Pasteur Institute Paris BCG Strain.
International name: Vaccinum tuberculosis (BCG) exsiccatum.
Bch.no.-fabrication date - dose - mg/ml/amp. .....
Package: ampoules, vials/box ..... Expiration date: .....
Manufacturing laboratory control results:
Results of test for number of culturable particles: $x10^6$/ml = ... date: .....
Results of test for absence of contaminating micro-organisms: ..... date: .....

Results of National Control Laboratory:
Samples arrived in the National Control Laboratory, date: .....
Potency: requirements by National Standard: .....
Potency: determined in National Control Laboratory: .....
Results of microscipic test: .....
Results of physical and chemical control .....
Results of sterility test: aerob: 20C ..... 37C .....
anaerob: 20C ..... 37C .....
Results of safety test:
Mice test for toxice products: ..... date: .....
Guinea-pig test for tetanus: ..... date: .....
Guinea-pig test for absence of virulent mycobacteria: ..... date: .....
Details of tests:

drying lot no.	total amp. nbs	ampoules sample nbs	in vitro potency VU/ml	sterility test	safety test	approved by	observ.

Budapest, ...

.....
Chief, National Control Laboratories

Annex XV

ORSZÁGOS KÖZEGÉSZSÉGÜGYI INTÉZET
NATIONAL INSTITUTE OF HYGIENE
INSTITUT NATIONAL DE LA SANTÉ
STAATLICHES INSTITUT FÜR HYGIENE
ГОСУДАРСТВЕННЫЙ ИНСТИТУТ ГИГИЕНЫ

H-1097 BUDAPEST, Gyáli út 2–6.
Tel: 142-250 Tlx: 22-5349 oki h, Telegr.: BUDOKI

.....19... OKI.
Object: Certificat for Hungarian Freeze-Dried BCG Vaccine issued by the Control Department for Medico-Biological Preparations of National Institute of Public Health.
Certif. No.:

The Hungarian BCG Laboratory of the National Institute of Public Health delivers of FREEZE-DRIED BCG VACCINE FOR INTRADERMAL INJECTION; Vaccinum tuberculosis /BCG/ exsiccatum; Prepared from the living BACILLUS CALMETTE-GUERIN PASTEUR INSTITUTE PARIS BCG STRAIN for

.....
.....
..... vials of ..... dosis Bch. No.:

Control tests carried out before delivery according to the PHARMACOPOEIA HUNGARICA and to the Requirements for Biological Substances 6, General Requirements for the Sterility of Biological Substances WHO Techn. Rep. Ser. 1973. No. 530 and to the Requirements for dried BCG vaccine WHO Techn. Rep. Ser. 1979. No. 638.

(1) In vitro sterility test. Control for absence of contaminating microorganisms on Semi-fluid sodium hydrosulfits medium and on Saboureaud medium: RESULT = NEGATIV

(2) Viability control of the final product. In vitro test for the estimation of the number of culturable particles on Blood Oleic Acid Albumin Medium: RESULTS = Fill the requirements.

(3) Innocuity tests:
(a) mouse test for toxic products: RESULT = NEGATIV
(b) guinea-pig test for tetanus: RESULT = NEGATIV
(c) guinea-pig test for virulent Mycobacteria: RESULT = NEGATIV

(4) Test of skin tuberculin reactivity in guinea-pigs. Tuberculin sensitivity test with 10 TU after 28 days on the vaccinated animals; 0.5 mg BCG by i.m. route: RESULTS = Fill the requirements.

Budapest, ...

.....
Chief, BCG Laboratory

Annex XVI

HUNGARIAN BCG LABORATORY
NATIONAL INSTITUTE
OF PUBLIC HEALTH
Budapest, IX., Gyáli út 2. Hungary

FREEZE—DRIED BCG VACCINE FOR INTRADERMAL INJECTION

Vaccinum tuberculosis (BCG) exsiccatum

Prepared from the living

BACILLUS CALMETTE—GUERIN
PASTEUR INSTITUTE PARIS BCG STRAIN

DESCRIPTION OF VACCINE AND PREPARATION

The Hungarian freeze-dried BCG vaccine is prepared from the PASTEUR BCG STRAIN. The harvested and homogenised BCG culture is freeze-dried in a sodium-glutamate and dextran medium in vials rubber-stoppered under vacuum after lyophilisation. Before delivery the batches are tested for sterility, safety and potency. According to the prescriptions, after reconstitution with the enclosed diluent the vaccine contains appropriate number of culturable particles, minimum [illegible] viable units per 0.1 ml.

RECONSTITUTION

The enclosed diluent should be added to the dried vaccine with a sterile syringe. The resuspended vaccine should be shaken to avoid sedimentation before administration. The reconstituted vaccine should be used after reconstitution within four hours. Any reconstituted vaccine remaining unused after four hours should be discarded.

ADMINISTRATION

For new-born, and tuberculin negative individuals (children and adults after a previous tuberculin test) the dose is 0.1 ml. by intradermal injection. The superior and middle region surface of the left upper arm should be disinfected with alcohol or alcohol-ether mixture. From the reconstituted vaccine 0.1 ml. should be injected strictly intradermally with a 1 cc. tuberculin syringe with a 26 gauge needle (special care must be taken of the exact dosage). Subcutaneous injection should be avoided.

CONTRAINDICATIONS

Vaccination is forbidden for prematures under 2000 g. weight, for new-born suffering from obstetrical trauma, dermatological affections or alimentary disorders and for individuals suffering from acute or chronic infections or feverish diseases as well as for tuberculin positive children and adults.

VACCINATION REACTIONS

2—3 weeks after the intradermal vaccination a small local lesion develops. The diameter of exulcered lesion reaches the maximum in the 5—8th weeks and is generally healing after 12—16 weeks with a scar. The enlargement of the axillary lymph-nodes can be considered as normal regional reaction. In some cases, depending on the vaccination technique and on the age of the person vaccinated, a suppurative process may develop in the regional lymph-nodes with a longer spontaneous healing period. Surgical treatment is generally not necessary.

EXPIRATION DATE

Protected from light, after the delivery date:
12 months if stored at + 2°C + 10°C

DELIVERY

In boxes containing 5 multidose ampoules (10, 30, 50 or 100 doses) of dried vaccine and 5 ampoules of distilled water (1.0, 3.0, 5.0 or 10.0 ml) enlosed as diluent for reconstitution.

Annex XVII

HUNGARIAN BCG LABORATORY
LABORATORIO HUNGARO DEL BCG
LABORATOIRE DU BCG DE HONGRIE
Nat. Inst. Publ. Hlth. —
Inst. Nat. Hig. — Inst. Nat. Santé Publ.
Hungary Hungrie Hongrie
Budapest IX., Gyáli út 2.

5 vials (1 vials = 10 dosis)
FREEZE-DRIED BCG VACCINE
FOR INTRADERMAL INJECTION
PREPARED FROM THE LIVING
BACILLUS CALMETTE-GUERIN
PASTEUR INSTITUTE PARIS BCG STRAIN

VACUNA BCG LIOFILIZADA
INYECCION INTRADÉRMICA
PREPARADO DE GÉRMENES VIVOS ATENUADOS
DE BACILOS CALMETTE-GUERIN
CEPA BCG INSTITUTO PASTEUR PARIS

VACCIN BCG CONGELE-DESSECHE
POUR INJECTION INTRADERMIQUE
PREPARE A PARTIR
DU BACILLE CALMETTE-GUERIN VIVANT
SOUCHE BCG DE L'INSTITUT PASTEUR PARIS

Vaccinum tuberculosis (BCG) exsiccatum
Hung. Lic. No.: 60096
Batch — Lote — Lot No.:
Expir. date Fecha caduc, Date exp:
Storage-Conservation-Conservation +2°C+10°C
Protected from light — Protegida de la luz —
Protégé de la lumière

Reconstitution: the enclosed diluent should be added to the dried vaccine with a steril syringe. One vial contains: after reconstitution appropriate number of culturable particles minimum 100.000 viable units per 0,1 ml freeze dried in a medium containing Na-Glutamate. **Administration:** the resuspended vaccine should be shaken to avoid sedimentation and used after reconstitution within four hours.

Reconstitucion: mediante una jeringuilla esterilizada se anade del diluyente a la vacuna liofilizada. Un vial contiene: después, de la reconstitucion los suficientes organismos como minimo 100.000 bacilos vivos en 0,1 ml liofilizados en un medio conteniendo Glutamato de Sodio. **Administracion:** la suspension de vacuna obtenida debe emplearse dentro de las 4 horas siguientes, para evitar su contaminación y sedimentación.

Reconstitution: le diluant doit être ajouté au vaccin sec avec une seringue stérilisée. Un vial contient: après la reconstitution des particules cultivables appropriées, minimum 100.000 unités vivantes dans 0,1 ml lyophilise dans un milieu contenant du Na-Glutamate. **Administration:** le vaccin resuspendu doit être agité pour éviter la sédimentation avant l'administration, et utilisé dans les quatre heures qui suivent la reconstitution.

Any reconstituted vaccine remaining unused after four hours should be discarded.
La vacuna reconstituida no utilizada 4 horas despues debe ser desechada.
Le vaccin reconstitué restant non utilisé au delà de quatre heures doit être jeté.

Diluent: Diluyente: Diluant:
5 x 1,0 ml Aqua dest. pro inj.
Dose: 0,1 ml by intradermal injection.
Dosis: 0,1 ml en inyección intradérmica.
Dose: 0,1 ml par voie intradermique.

Annex XVIII

BCG Laboratory. National Institute of Hygiene.
H-1097 Budapest, Gyáli ut 2.

Materials for the production 150 000 vials a 10 or 20 doses of freeze-dried BCG vaccine during in 15 batches one year in a laboratory of 250 sq meter with 15 persons.

Description	Defin.	Quant.
Vial (Trident)	2.5 ml	150,000
Rubber stopper (Pharma)	188 type	150,000
Aluminium cap (Pharma)	188 type	150,000
Pipette	1 ml	200
Pipette	2 ml	100
Pipette	5 ml	50
Pipette	10 ml	50
Pipette	25 ml	20
Pipette	50 ml	20
Pipette	100 ml	20
Erlenmeyer flask	100 ml	50
Erlenmeyer flask	300 ml	100
Erlenmeyer flask	500 ml	10
Erlenmeyer flask	1000 ml	10
Erlenmeyer flask	2000 ml	5
Erlenmeyer flask	3000 ml	5
Erlenmeyer flask	4000 ml	5
Erlenmeyer flask	5000 ml	5
Erlenmeyer flask	6000 ml	5
Erlenmeyer flask	10000 ml	3
Graduated glass cylinder	100 ml	4
Graduated glass cylinder	250 ml	4
Graduated glass cylinder	500 ml	4
Graduated glass cylinder	1000 ml	4

Annex XVIII (continued)

BCG Lab. NIH. Budapest. Materials for BCG vacc. production. p.2.

Description	Defin.	Quant.
Funnel	200-300 mm	6
Glass-stick	40 cm	10
Legroux flask	standard	30
Medium container	250 ml	30
Beaker	250 ml	30
Beaker	1000 ml	5
Filtering flask	1000 ml	5
Filtering flask	2000 ml	5
Glass or stainless-steel sterilizing cylinder for pipettes	4-6x40-50 cm	10
Pipette, curved	100 ml	5
Büchner funnel	8-10 cm	5
Seitz filter	5-8 cm	5
Stainless steel spatula for BCG culture	22x1 cm	5
Glass tube for BCG culture	12x3 cm	5
Homogeneizing vessel	50x240 mm	5
Stainless steel balls	4 mm	2500 g
Vaccine filling flask	190x130 mm	5
Pasteur pipette	standard	100
BCG dropping pipette for VU inoc.	200x8 mm	100
Injection needle for BCG dropping pipette	0.02 ml/drop	100
Cornwall pipette	1 ml	5
Cornwall pipette	2 ml	5
Petri dish	10 cm	500
Test tube	160x16 mm	500
Record syringe	2 ml	5
Sodium-L-glutaminat	g	3000
Dextran 40000	g	3000
Glucose	g	4500
L-Asparagin	g	500

Annex XVIII (continued)

BCG Lab. NIH. Budapest. Materials for BCG vacc. production. p. 3.

Description	Defin.	Quant.
Acid. citricum	g	200
K_2HPO_4	g	200
$MgSO_4.7H_2O$	g	200
Fe. amm. citricum	g	10
KH_2PO_4	g	50
Na_2HPO_4	g	80
Agar No. 3.	g	500
Human transfusion blood for BOAA medium	ml	2000
$CaCl_2$	g	10
$ZnSO_4$	g	10
Triton WR 1339	g	10
Bovine albumin fraction V	g	500
NaOH	g	10
NaCl	g	500
Oleic acid	g	10
Glycerin	ml	7000
Denaturated alcohol	ml	100,000
Chloroform	ml	2000
HCl pro analyses	ml	1000
Ethylic alcohol	ml	20000
Benzin	ml	2000
Aceton	ml	1000
Malachit-green	g	10
$CuSO_4$	g	5
Blood-agar plate medium	15.0 ml	450
Sodium-thiosulphate medium	15.0 ml	750
Slope-agar medium	5.0 ml	750
Dextr. bouillon medium	5.0 ml	750

Annex XVIII (continued)

BCG Lab. NIH. Budapest. Materials for BCG vacc. production. p. 4.

Description	Defin.	Quant.
pH meter		1
Rotating mill for BCG suspension		1
Ampoule filling and seeling machine (ROTA)		1
Freeze-drying machine (USIFROID)		1
Machine for vial stoppering under vacuum (USIFROID)		1
Machine to seal vial with Alu. cap (Capsolut Spinner)		1
Machine for labelling vials (ROTA)		1
Incubators 37°C having separated electric circuit for BCG strain maintaining and for experimental investigations	90x60x70 cm	3
Incubator room 37°C	250x160x260 cm	1
Refrigerators 4°-10°C	1100 liter	5
Cold room 4°-10°C	260x180x260 cm	1
Binocular stereo-microscope		1
Bacteriological microscope		1
Balances		1
Washing-machine (MIELE G19)		1
Pure water apparatus 10 l/h		1
UV lamp		2
Autoclave	200 l	2
Hot air electric sterilizer	150x85x80 cm	2
Illuminator for microscope		2
Type writer		2
Timer		2
Vial label	10 or 20 doses	150,000
Packing boxe 65x63x23 mm	10 or 20 doses	30,000
Aqua dest. pro inj.	1.0 or 2.0 ml	150,000
Instructions for use		30,000

Annex XVIII (continued)

BCG Lab. NIH. Budapest. Materials for BCG vacc. production. p.5.

Description	Defin.	Quant.
Files for ampoule		30,000
Plastic grid for ampoule separation in packing box		30,000
Packing box label		60,000
Date stamp inking pad	set	1
Guinea pig, same sex	200-250.0 g	320
Mice	16-18.0 g	50
Tray	70x44x4 cm	10
Basket for test tube	20x20x15 cm	30
Rack for 40x16 test tube	24x14x8 cm	30
Clamp tubing shut-off Mohr		5
Vacuum rubber tube	m	2
Filter paper	sheets	100
Paper "wool"	kg	150
Cotton - wool	kg	50
Wrapping paper	sheets	500
Silk paper	sheets	500
Aluminium foil	sheets	100
Preprinted record sheet for media		100
Preprinted record sheets vaccine production		100
Preprinted vaccine for strain transfer		100
Inoculating clasper		5
Bell-shaped glass		4
Chemical spoon		4
String		20
Boxe for vials of final product	42x30x12.5 cm	50
Sterilizer instr. boiling type	30x13x6 cm	3
Metal basket for vials (ampoules)	240x16x7 cm	25
Stainless steel cylinder for discarding pipettes	48x8 cm	6

Annex XVIII (continued)

BCG Lab. NIH. Budapest. Materials for BCG vacc. production. p.6.

Description	Defin.	Quant.
Bucket to discard glassware	44x30 or 30x25 cm	5
Support stand for burette		4
Screw clamp		10
Support stand for tubes		10
Support stand for flasks		10
Gauze	m	200
Sterilizer instr. boiling type	25x17x11 cm	12
Rubber stopper for Legroux flask		400
Knife		4
Scissor		10
Forceps		10
Thermometer		10
Cylinder (Alu) of sterile Petri dishes	25x12 cm	50
Polyethylen bag	40x60 cm	50
Glass jar for mice	18x15 cm	10
Metal cage for mice	37x26x14 cm	20
Metal cage for guinea pig	42x32x12 cm	30
Chromium-sulfuric acid	ml	25,000
Na-Hypochlorite	ml	160,000
Chemicals for washing	kg	100
Safety match		
Neomagnol (Chlorogenium Chloraminium-B)	tbl	500
Dermatograph (water proof pen)		
Ultrasol (Chloraminium-T)	ml	125,000
Soap		

Remark: Manufacturers in parenthesis not express any preference for the companies mentioned.

Annex XIX

BCG Laboratory. National Institute of Hygiene.
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Cost of production and quality control of one batch freeze-dried BCG vaccine containing 10 000 vials a 10 or 20 doses.

Description	Defin.	Price
Sauton medium	5000.00 ml	
K_2HPO_4	2.50 g	
L-Asparagin	20.00 "	
Acid. citr.	10.00 "	
$MgSO_4$	2.50 "	
Fe. amm. citr.	0.25 "	
Glycerin	300.00 ml	
Bovin albumin fraction V.	3.00 g	
NH_4OH 10%	10.00 ml	
D-G-S excipient	3600.00 ml	
Dextran	180.00 g	
Na-L-Glutaminat	180.00 "	
Glucose	270.00 "	
BOAA medium	3000.00 ml	
KH_2PO_4	3.00 g	
Na_2HPO_4	6.00 "	
L-Asparagin	7.50 "	
Agar	30.00 "	
Oleic-acid	15.00 ml	
Bovin albumin fraction V.	14.00 g	
Human transfusion blood	285.00 ml	
Saboureaud medium	500.00 ml	
Tripcasin	8.00 g	
Yeast extract	25.00 ml	
NaCl	1.25 g	
Dextrose	10.00 "	
Agar	0.38 "	
HCl 25%	2.00 ml	
Slope agar medium	500.00 ml	
Beef extract	7.25 g	
Agar	8.50 "	
Peptone	5.00 "	
NaCl	1.50 "	
$Na_2HPO_4.12H_2O$	2.00"	
	Total	

Annex XIX (continued)

BCG Lab. NIH. Budapest. Cost of BCG vacc. production. p.2.

Description	Defin.	Price
Na hydrosulfit medium	500.00 ml	
Tripcasine	8.00 g	
Yeast extract	25.00 "	
NaCl	1.25 "	
Dextrose	2.50 "	
Na2SO4	0.25 "	
Agar	2.00 "	
Methylenblue	0.60 "	
N2 gaz		
Vial 2.5 g	10,000 No	
Vacuum rubber stopper 188 typ.no.	10,000 "	
Al. capsule 188 typ.no.	10,000 "	
Vial label	10,000 "	
Instruction for use	2,000 "	
Packing box	2,000 "	
Aqua dest.pro inj. 1.0 or 2.0 ml	10,000 "	
Files for ampule	2,000 "	
Packing box label	4,000 "	
Additional costs		
Other materials		
Electric energy		
Water		
Machine amortizations		
Salaries		
3 microbiologists		
5 senior technicians		
5 technicians		
2 other personnel		
	G. Total	

Annex XX

BCG Laboratory. National Institute of Hygiene.
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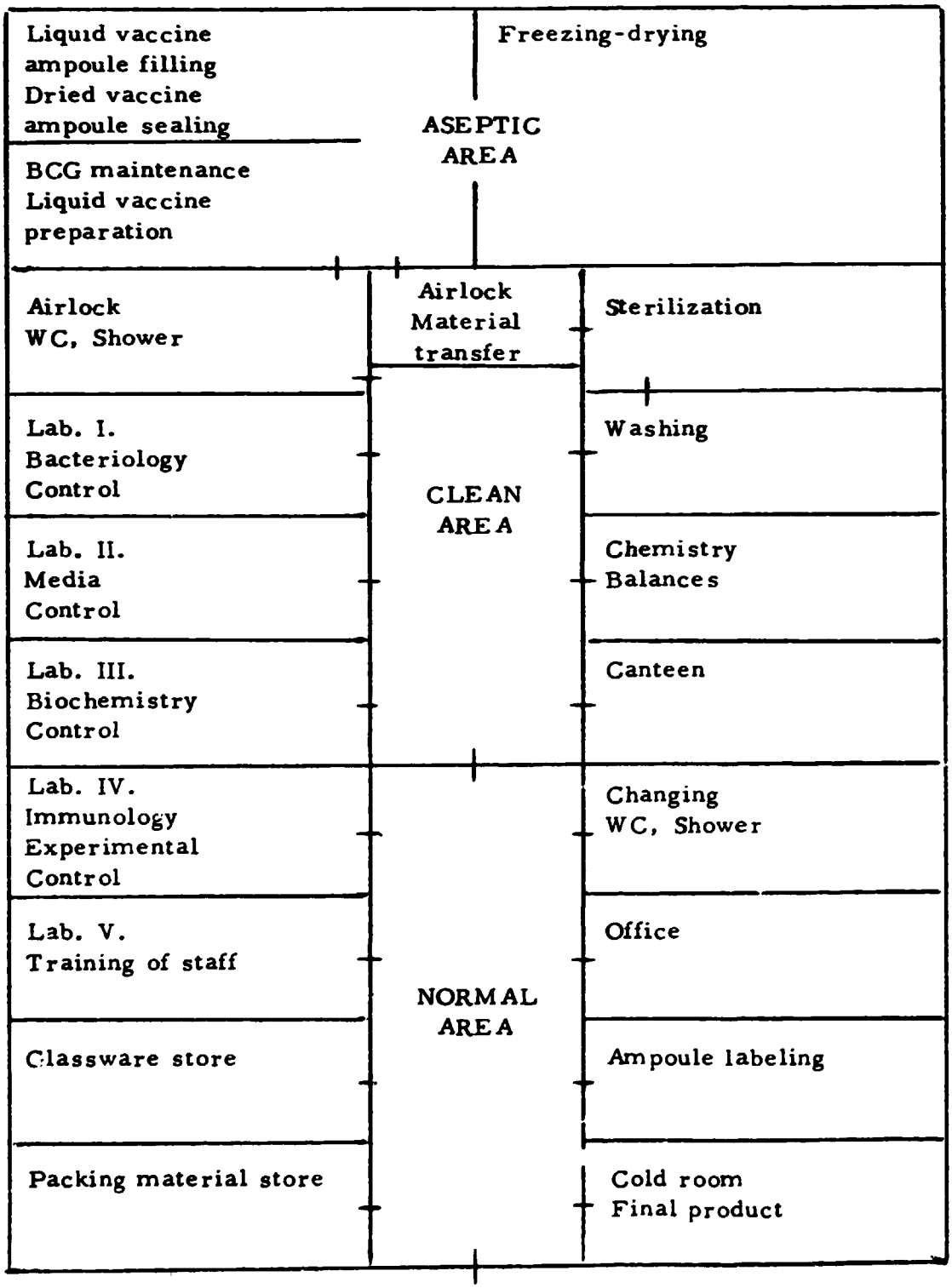
Schematic design of BCG laboratory building. 25 m x 12 m = 300 m^2 (37).



Annex XXI

BCG Laboratory. National Institute of Hygiene.
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Flow chart of the production and quality control of the freeze-dried i.d. BCG vaccine from surface culture on Sauton liquid medium.

PRODUCTION		CONTROL
Ampoule of Seed-Lot		Identity
↓		
Sauton 1. (1st passage)		Sterility
↓		
Sauton 2. (2nd passage) →	Harvest	Sterility
	Stock suspension	Safety
	Liquid bulk	Viability
	Liquid filling lot	Stability
	Freeze-drying	Potency
	Final product (Dried lot)	Field trial
↓		
Sauton 3. (3rd passage) →	Harvest	Sterility
	Stock suspension	Safety
	Liquid bulk	Viability
	Liquid filling lot	Stability
	Freeze-drying	Potency
	Final product (Dried lot)	Field trial
↓		
.		
.		
.		
↓		
Sauton 9. (9th passage) →	Harvest	Sterility
	Stock suspension	Safety
	Liquid bulk	Viability
	Liquid filling lot	Stability
	Freeze-drying	Potency
	Final product (Dried lot)	Field trial

Annex XXII

BCG Laboratory. National Institute of Hygiene.
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Personnel required for manufacturing and quality control of freeze-dried i.d. BCG vaccine (37).

1. A director of the laboratory.

   Qualifications: Medical microbiology and postgraduate experience with scientific degree in biomedical sciences (biochemistry, immunology, epidemiology, medical biometry).

2. Two scientists.

   Qualifications: Science graduates with certificated experience in microbiological and biochemical techniques.

3. Two senior technicians.

   Qualifications: Certificated education and at least 15 years experience in medical laboratory and microbiological techniques.

4. Four techniciens.

   Qualifications: Certificated education and at least 2 years experience in microbiological techniques.

5. Six other personnel.

   Qualifications: Local education and training for washing, sterilizing of glassware and equipments for vaccin production.

6. Two secretary.

   Responsibilities: One secretary for the day-to-day running of the laboratory and one for the production and control protocols.

Annex XXIII

BCG Laboratory. National Institute of Hygiene.
H-1097 Budapest, Gyáli ut 2.

Summary of the training in production and control of BCG vaccine.

The basic training of the scientific personnel for manufacturing and quality control of freeze-dried i.d. BCG vaccine in the Hungarian BCG Laboratory takes 6-12 months to obtain theoretical and practical knowledges and skills in the following subjects:

1. Production:
   - Planning of budget, supply and storage of materials as well as machines and instruments.
   - Cleaning, washing and sterilization of glassware.
   - Preparation of media to maintain BCG strain, for vaccine productions and for the sterility tests (Annexes III, IV, V, VI).
   - Maintenance of the BCG strain, Seed-Lot System, passage of cultures on Sauton medium for vaccine production (Annex II).
   - Harvesting of culture, Preparation of stock suspension, liquid bulk, filling lot, sterility tests (Annex VIII).
   - Excipient preparation, pH control, filling lot adjustment, filling of ampoules, freezing, drying, sealing of ampoules (vials) labeling, packing (Annexes VII, VIII, IX).

2. Quality control:
   - Monitoring the production laboratory, microbiological control of the steril area.
   - In process control during the manufacture.
   - Control of the final products (dried lot);
     -- In vitro tests; sterility, viable units determination on solid medium, counting of colonies, computation of viable units, statistical analysis of the viability after production and during storage, residual moisture.

Annex XXIII (continued)

BCG Laboratory. National Institute of Hygiene.
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-- In vivo test on animals; specific and aspecific safety tests on guinea-pigs and mice, residual virulence, tuberculin sensitivity, protective effect.

3. Field-trial. Organization of BCG vaccination.
   - Dispatching of BCG vaccine for institutes; obstetric departments, children's polyclinics, tuberculosis dispensaries, school-medical services.
   - Primovaccination of new-borns.
   - Revaccination of tuberculin negative children.
   - Pre and postvaccination tuberculin test training.
   - Epidemiological evaluation of the effectiveness of the BCG vaccination.

Remarks:

- After the basic training the 3-5 years postgraduate educations are organized in the Institutes of the Medical University and in the Postgraduate Medical School.
- Complete list of references for basic training and postgraduate education is available in the Hungarian BCG Laboratory.