Appendix 4: Batch analysis of Vero master cell banks

rivm research for man and environment | RIJKSINSTITUUT VOOR VOLKSGEZONDHEID EN MILIEUHYGIENE
NATIONAL INSTITUTE OF PUBLIC HEALTH AND ENVIRONMENTAL PROTECTION

SUMMARY PROTOCOL OF VERO CELLS

Summary of preparation of Cell Seed and Manufacturers Working Cell Bank (MWCB) and tests performed on the MWCB

MASTER CELL SEED	
Code	Vero cells, WHO seed 134th passage, Oct. 1987
Obtained from	ECCAC
Date	August 1990
CELL SEED	
Code	Vero 134th-A2
Date	051290
Number of subcultivations	2 (monolayer)
Passage number	136
Cell doublings	5.0
Number of ampoules	30
Cells/ampoule	10×10^6
MWCB	
Code	Vero-MWCB UCC 91-02, passage 141, 200391
Nr subcultivations in monolayer cultures	3
Cell doublings in monolayer cultures	6.2
Nr subcultivations in microcarrier cultures	2
Cell doublings in microcarrier cultures	7.3
Number of frozen ampoules	171
Volume/ampoule	15 ml
Cells/ampoule	$\leq 300 \times 10^6$
Viability	$\pm$ 90%

TESTS PERFORMED ON MWCB (according to WHO TRS 745, 1987, Annex 3, p.93-107)

Test	Result
Test for sterility	satisfactory
Test for mycoplasma	satisfactory
Test for absence of adventitious agents	satisfactory
Test for identity	Cercopithecus cells

CONCLUSION: Meets relevant requirements

DATE: 920214

Dr E.C.Beuvery
Head Laboratory for
Inactivated Virus Vaccines

Dr G.van Steenis
Head Laboratory for
Control of Biological Products

Antonie van Leeuwenhoeklaan 9, P.O. Box 1, 3720 BA BILTHOVEN, The Netherlands
Telephone: ..(31)30 - 749111, Telex: 47215 rivm nl, Telefax: ..(31)30 - 742971

NVI
nederlands vaccin instituut

Summary protocol VERO Master cellbank

Master Cellbank: VERO-MWCB-2 = UCC 91-04

Manufacturer

Name manufacturer	: NVI (Netherlands Vaccine Institute)
Address of manufacturer	: P.O. Box 457
	: 3720 AL Bilthoven,
	The Netherlands

Note

This cellbank has been produced in the early 90's by the RIVM as a working cellbank and was originally reviewed and released in October 1993 (release protocol added to this summary batch protocol). In order to ascertain a sufficient supply of VERO cells for IPV production it was decided that this working cellbank should be upgraded to a master cellbank. Partly based on this new status several tests on the cellbank have been repeated or initiated.
This document summarizes the most recent data, generated in 1991-1992 and in 2001-2003.

Cell source

Master Cell Bank no.	: WHO VERO seed, 134th passage, October 1987
Obtained from	: ECACC
Obtained in	: August 1990

Cultivation to obtain an intermediate cell seed

Batch number	: Vero; 134th-A2
Date	: 03-12-1990
Cells are subcultivated two times in monolayer cultures.	
Number of cell doublings	: 5.0
Number of ampoules frozen	: 30
Cells per ampoule	: 10^7
Passage number	: 136

Cultivation to obtain a working cell bank

Batch number	: Vero-MWCB-2, UCC 91-04
Date	: 23-12-1991
Cells are subcultivated three times in monolayer cultures.	
Number of cell doublings	: 6.4
Cells are subcultivated two times in microcarrier cultures.	
Number of cell doublings	: 7.0
Number of ampoules frozen	: 193
Volume per ampoule	: 15 ml
Cells per ampoule	: $\leq 3 \times 10^6$
Viability	: ≥90%
Passage number	: 141

Serum for cell cultures

Origin of serum used	: foetal calf	donor bovine
Batch no°	: SF 90517	
	SF 80428	SD 901004

CAIF SA
Dra. Bernarda Belay
Co-Directora Técnica
M.N. 15.141
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29378925

NVI
nederlands vaccin instituut

Summary protocol VERO Master cellbank

Master Cellbank: VERO-MWCB-2 = UCC 91-04

Tests performed on cell bank

Sterility test

Method	: (45C-St-1)
Media	: Thioglycollate; Trypton Soya Broth
Temperature	: 30-32 °C 20-25 °C
Inoculum	: 10 ml 10 ml
Date of test	: 26-02-1993
Result	: Passed

Test for mycoplasmas

Method	: (17C-St-MP)
Media	: PPLO agar and broth,
	: Chanock agar and broth
Date	: 05-03-1993
Result	: Passed

Test for absence of cytopathogenic and haemadsorbing viruses

Method	: (46C-IPV-1)
Date	: 18-02-1993
Result	: Passed

Test in rabbit kidney cell cultures

Method	: (17C-IPV-03)
Date	: 19-11-2001
Result	: Passed

Test in VERO cell cultures

Method	: (46C-IPV-01)
Date	: 08-03-1993
Result	: Passed

Test in cercopithecus kidney cell cultures

Method	: (46C-IPV-01)
Date	: 08-03-1993
Result	: Passed

Test in MRC5 cell cultures

Method	: (46C-IPV-01)
Date	: 08-03-1993
Result	: Passed

NVI
nederlands vaccin institut

Summary protocol VERO Master cellbank

Master Cellbank: VERO-MWCB-2 = UCC 91-04

Test for identity

Method	: Authenikit "Corning"
Date	: 05-03-1993
Result	: Monkey cercopitheeus species
Method	: DNA fingerprint
Date	: 03-03-2002
Result	: identical to standard vero DNA
Method	: Morphology
Date	: 23-05-2002
Result	: uniform cell type; identical to 86UC558 VERO cells.
Over all conclusion	: VERO cells

Tests performed on high passage

The following tests are performed on passage 151

Co-cultivation on Vero cell cultures

Method	: According to Ph. Eur.: 5.2.3 (17C-CC-01)
Observation period	: 21 days
Result	: No extraneous agents found
Date	: 26-06-2001

Co-cultivation on MRC5 cell cultures

Method	: According to Ph. Eur.: 5.2.3 (17C-CC-01)
Observation period	: 21 days
Result	: No extraneous agents found
Date	: 26-06-2001

Co-cultivation on Cercopitheeus cell cultures

Method	: According to Ph. Eur.: 5.2.3 (17C-CC-01)
Observation period	: 21 days
Result	: No extraneous agents found
Date	: 26-06-2001

Test in suckling mice

Method	: According to Ph. Eur.: 5.2.3 (17C-CeL-02)
Date of inoculation	: 11-09-2001
Volume inoculated	: 0.1 ml sc
Period of observation	: 28 days
No°. of mice inoculated	: 10
No°. of mice died < 24 h	: 2
No°. of mice died > 24 h	: 0
Result	: no evidence of infection

NVI
nederlands vaccin instituut

Summary protocol VERO Master cellbank

Master Cellbank: VERO-MWCB-2 = UCC 91-04

Test in adult mice

Method	: According to Ph. Eur.; 5.2.3 (17C-CeL-03)
Date of inoculation	: 26-06-2001
Volume inoculated	: 0.1 ml im
Period of observation	: 28 days
No°. of mice inoculated	: 10
No°. of mice died > 24 h	: 0
Result	: no evidence of infection

Test in SPF eggs

Method	: According to Ph. Eur.; 5.2.3 (17C-CeL-07)
Number of eggs in each test	: 10
Date of inoculation allantoic cavity	: 10-09-2001
Period of observation	: 9 days
Date of inoculation yolk sac	: 04-09-2001
Period of observation	: 9 days
Number of embryos surviving the test	: 9
Result	: no evidence of infection

Test in Cercopithecus cell cultures

Method	: According to Ph. Eur.; 5.2.3 (17C-IPV-01)
Observation period	: 14 days
Result	: No extraneous agents found
Date	: 03-07-2001

The following tests are performed on passage 156

Absence of retroviruses

Method	: According to Ph. Eur.; 5.2.3 Electron Microscopy
Result	: No endogenous virus particles found
Date	: March 2003

Absence of retroviral reverse transcriptase activity

Method	: According to Ph. Eur.; 5.2.3 (PERT assay)
Date test on	: 06-02-2003
Date test off	: 14-02-2003
Result	: no activity detected

Absence of Simian Imunodeficiency Virus

Method	: According to Ph. Eur.; 5.2.3 (PCR)
Date test on	: 13-02-2003
Date test off	: 27-02-2003
Result	: free of detectable SIV

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148

CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 23378925

NVI
nederlands vaccin instituut

Summary protocol VERO Master cellbank

Master Cellbank: VERO-MWCB-2 = UCC 91-04

Absence of Simian T-cell Leukemia Virus

Method	: According to Ph. Eur.; 5.2.3 (PCR)
Date test on	: 13-02-2003
Date test off	: 25-02-2003
Result	: free of detectable STLV

Test for tumorigenicity

Method	: According to Ph. Eur.; 5.2.3 (17C-IPV-02)
Date of inoculation	: 02-12-2002
Volume inoculated	: 0.1 ml sc
Period of observation	: 21 / 84 days
No°. of rats inoculated	: 10
No°. of rats died due to aspecific reasons	: 1
Result	: no evidence of tumorigenicity

Remarks:

Reference is made to relevant parts of the current Ph. Eur., references between brackets are to current internal SOP's of NVI.
Current means the version that was valid at the date the tests have been performed.

18-11-2004: addition of two serum batches used during production, as a correction of an omission from the past.

Conclusion

Certification by person taking overall responsibility for production and control of the master cell bank VERO.

I certify that lot number Vero-MWCB-2, UCC 91-04 of this master cell bank meets the requirements of the European Pharmacopoeia (monograph 5.2.3) and/or WHO monographs where applicable.

Reviewed by:		Qualified Person:	
L.C. Sundermann		Drs. L.C.Sundermann	
Initials	: [signature]	Signature	: [signature]
Date	: 18-11-04	Date	: 18-11-04

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI: 25378925

NVI
nederlands vaccin instituut

Summary protocol VERO Master cellbank

Master Cellbank: VERO-MWCB-2 = UCC 91-04

Original RIVM protocol on which MWCB-2 was released as working cellbank

RIVM research for man and environment

RIJKSINSTITUUT VOOR VOLKSGEZONDHEID EN MILIEUHYGIENE
NATIONAL INSTITUTE OF PUBLIC HEALTH AND ENVIRONMENTAL PROTECTION

SUMMARY PROTOCOL OF VERO CELLS

Summary of preparation of Cell Seed and Manufacturers Working Cell Bank (MWCB-2) and tests performed on MWCB-2.

MASTER CELL SEED	
code	Vero cells, WHO seed 134th passage, Oct. 1987
obtained from	ECCAC
date	august 1990
CELL SEED	
code	Vero 134th-A2
date	031290
number of subcultivations	2 (monolayer)
Passage number	136
Cell doublings	5.0
Number of ampoules frozen	30
Cells/ampoule	10 x10E6
MWCB-2	
code	Vero-MWCB-2, UCC 91-04, passage 141, 23-12-91 (ampoules labeled passage 138)
nr. subcult.in monolayercultures	3
Cell doublings in monolayercultures	6.4
nr. subcult.in microcarriercultures	2
Cell doublings in microcarriercultures	7.0
Number of ampoules frozen	193
Volume/ampoule	15 ml
Cells/ampoule	≤ 300 x 10E6
Viability	≥ 90 %

TESTS PERFORMED ON MWCB-2 (acc.to WHO TRS 745,1987,Annex 3,p.93-107)

Test	Result
Test for sterility	satisfactory
Test for Mycoplasma	satisfactory
Test for absence of adventitious agents	satisfactory
Test for identity	Cercopithecus species
CONCLUSION	Meets relevant requirements.
DATE 04-10-93	04-10-93

Dr.E.C.Beuvery
Head Laboratory for
Inactivated Viral Vaccines

Dr.G.van Steenis
Head Quality and
Regulatory Affairs

ERIAUX ORGANIQUES
Bocknek
ORGANIC MATERIALS

Bocknek Ltd.
165 Bethridge Road
Rexdale, (Toronto) Ontario
Canada, M9W 1N4
Tel: 416-745-0796
Telex: 06-989319
Fax: 416-745-8843

DONOR BOVINE SERUM
CERTIFICATE OF ANALYSIS

LOT NO: AD 901004

TESTS	RESULTS	TESTS	RESULTS
STERILITY TESTS (1)	PASSED	PHOSPHORUS	2.02 mmol/L
MYCO/UREAPLASMA	NEGATIVE	POTASSIUM	6.5 mmol/L
VIRUSES: BVD	NEGATIVE	SGOT	67 U/L
IBR	NEGATIVE	SGPT	21 U/L
PI-3	NEGATIVE	SODIUM	137 mmol/L
ENDOTOXIN	0.4 ng/ml	UREA	3.3 mmol/L
BILIRUBIN	1 mmol/L	TOTAL PROTEIN	69 g/L
CALCIUM	2.36 mmol/L	ALBUMIN	35 g/L
CHOLESTEROL	2.67 mmol/L	GLOBULINS	34 g/L
CREATININE	121 umol/L	pH	7.5
GLUCOSE	0.2 mmol/L	HEMOGLOBIN	120 mg/L
LDH	1191 U/L	SPECIFIC GRAVITY 1.039	g/ml

Notes: (1) BACTERIA AND FUNGI, (2) LACTATE DEHYDROGENASE.
(3) ALPHA, BETA, AND GAMMA

CELL GROWTH PROMOTION (DIPLOID AND HETERPLOID CELLS)

PLATING EFFICIENCY.....PASSED.

9 - 10 POPULATION DOUBLINGS IN THREE SUBCULTIVATIONS.....PASSED.

CELL TOXICITY.....NONE.

GROWTH PROMOTING QUALITY RATING.....EXCELLENT.

ORIGIN
BLOOD FOR DONOR BOVINE SERUM PRODUCTION HAS BEEN ASEPTICALLY COLLECTED FROM YOUNG, HEALTHY STEERS KEPT IN ISOLATION. THE STEERS ARE KNOWN TO BE FREE OF BLUE-TONGUE, Q-FEVER, BOVINE-SPONGIFORM ENCEPHALOPATHY, LEPTOSPIROSIS, LEUKOSIS, BRUCELLOSIS, TUBERCULOSIS, OR ANY OTHER PATHOGENIC AGENTS. THE SERUM HAS BEEN FILTER STERILIZED. PROCESSING AND TESTING HAS BEEN CARRIED OUT IN FACILITIES AND BY METHODS APPROVED BY F.D.A. (US) AND H.P.B. (CANADA).

DATE: December 5/90

Barbara Foster
TRAFFIC MANAGER

Adolf von Seefried, Ph.D.
QUALITY CONTROL MANAGER

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148
CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 2932[illegible]925

Agriculture Canada

Food Production and Inspection Branch / Direction générale, Production et inspection des aliments

AGRICULTURE CANADA
FOOD PROD. & INSP. BRANCH
10077G YONGE ST.
RICHMOND HILL, ONT. L4C 1T7

Your file / Votre référence

Our file / Notre référence

This is to certify that from documentary evidence received, the following described shipment of donor bovine serum was produced from animals originating and maintained in the province of Ontario, Canada. The said bovines were kept within an isolation unit, and did not come in contact with any other bovines. The animals were health monitored by a qualified veterinarian. Blue tongue, Foot and Mouth Disease, Rinderpest, Bovine Spongiform Encephalopathy, Bovine Pleuropneumonia, and Rift-Valley-Fever have not been diagnosed in the Province of Ontario.

Description: One Hundred & Twenty-eight (128) only boxes containing:
765 x 800 mls, Lot SD 901004 of
Sterile Donor Bovine Serum

Consignor: Bocknek Ltd.,
165 Bethridge Road,
Toronto, Ontario. Canada
M9W 1N4

Consignee: R.I.V.M.,
Le Brandenburgerweg 78b,
3721 Mk Bilthoven,
Amsterdam, Holland

FOOD PRODUCTION AND INSPECTION BRANCH OFFICIAL EXPORT CERTIFICATE
AGRICULTURE CANADA
CERTIFICAT OFFICIEL D'EXPORTATION DIRECTION GÉNÉRALE PRODUCTION ET INSPECTION DES ALIMENTS

Official Veterinarian TOM POPPER DVM
Food Production and Inspection Branch

March 6, 1991.
Date

Canada

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148

CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29378925

AFZENDER:BODINCO BV; 24- 4- 1 15:38; +31 72 5670068 => +0302250610; #2/2

CANSERA INTERNATIONAL INC.
165A BETHRIDGE ROAD, REXDALE, ONTARIO, CANADA, M9W 1N4

CERTIFICATE OF ORIGIN

LOT # SF80428

STERILE FETAL BOVINE SERUM

ORIGIN: CANADA

THE UNDERSIGNED HAS EXAMINED THE MANUFACTURER'S INVOICE OR SHIPPER'S AFFIDAVIT CONCERNING THE ORIGIN OF THE MERCHANDISE AND DEEMS IT TO BE TRUE AND CORRECT TO THE BEST OF HIS/HER KNOWLEDGE AND BELIEF.

JANE ELIZABETH HAFFENDEN, a Commissioner, etc.,
Municipality of Metropolitan Toronto, For Cansera International Inc.
Expires December 15, 2001.

04/11/01 08:16 FAX 416 744 8401 CANSERA 002

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148
CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 25378525

ANMAT FOLIO 00283 MESA DE ENTRADAS

MATÉRIAUX ORGANIQUES
Bocknek
ORGANIC MATERIALS

Bocknek Ltd.
165 Bethridge Road
Rexdale, (Toronto) Ontario
Canada, M9W 1N1
Tel: 416-745-0796
Telex: 06-989339
Fax: 416-745-8811

CERTIFICATE OF ANALYSIS

PRODUCT: STERILE FETAL BOVINE SERUM

LOT #: SF-8-04-28

THE FOLLOWING TESTS WERE PERFORMED ON THE ABOVE LOT.

TEST	RESULT
STERILITY	PASSED
ENDOTOXIN	0.025 ng/mL
CELL CULTURE SUITABILITY	PASSED
MYCOPLASMA & UREAPLASMA	NEGATIVE
BVD	FREE OF CYTOPATHIC BVD
IBR	NEGATIVE
PI^3	NEGATIVE
TOTAL PROTEIN	38 g/L

Barbara Bartlett

PAUL HAFFENDEN
LABORATORY MANAGER

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 16.148
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 23378825

MATÉRIAUX ORGANIQUES
Bocknek
ORGANIC MATERIALS

Bocknek Ltd.
165 Bethridge Road
Rexdale, (Toronto) Ontario
Canada, M9W 1N4
Tel: 416-745-0796
Telex: 06-989319
Fax: 416-745-8843

FETAL BOVINE SERUM
CERTIFICATE OF ANALYSIS

LOT NO: SF 9-05-17

TESTS	RESULTS
STERILITY TESTS(1)	PASSED
MYCO/ UREOPLASMA	NEGATIVE
VIRUSES: BVD	NEGATIVE
IBR	NEGATIVE
P1-3	NEGATIVE
ENDOTOXIN	0.2 ng/ml
BILIRUBIN	4 umol/L
CALCIUM	3.41 mmol/L
CHOLESTEROL	0.86 mmol/L
CREATININE	264 umol/L
GLUCOSE	6.2 mmol/L
LDH(2)	444 U/L

TESTS	RESULTS
PHOSPHORUS	3.31 mmol/L
POTASSIUM	13.4 mmol/L
SGOT	32 U/L
SGPT	6 U/L
SODIUM	132 mmol/L
UREA	6.5 mmol/L
TOTAL PROTEIN	37 g/L
ALBUMIN	23 g/L
GLOBULINS(3)	14 g/L
pH	7.4
HEMOGLOBIN	250 mg/L
SPECIFIC GRAVITY	1.03 g/ml

NOTES: (1) BACTERIA AND FUNGI, (2) LACTATE DEHYDROGENASE,
(3) ALPHA, BETA, AND GAMMA.

CELL GROWTH PROMOTION (DIPLOID AND HETEROPLOID CELLS)

PLATING EFFICIENCY.....PASSED
J - 10 POPULATION DOUBLINGS IN THREE SUBCULTIVATIONS.....PASSED
CELL TOXICITY.....NONE
GROWTH PROMOTING QUALITY RATING.....EXCELLENT

ORIGIN

THIS SERUM HAS BEEN DERIVED FROM BLOOD ASEPTICALLY COLLECTED IN CANADIAN ABATTOIRS, AND PROCESSED IN FACILITIES WITH METHODS APPROVED BY F.D.A. (US) AND H.P.B. (CANADA).

DATE: June 26/89

Barbara Foster
Barbara Foster,
TRAFFIC MANAGER.

A. v. Seefried
Adolf von Seefried, Ph.D.,
Q.C. MANAGER.

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148

CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29378925

Appendix 7: Batch analysis of poliomyelitis working seed lots

NVI
nederlands vaccin instituut

Summary protocol Poliomyelitis Working Seedlot

Working Seedlot: 03/1/B5
Type: 1

Manufacturer

Name manufacturer	: NVI (Netherlands Vaccine Institute)
Address of manufacturer	: P.O. Box 11
	: 3721 MA Bilthoven,
	The Netherlands

Cell substrate

Type of cell culture used	: Vero
Manufacturers Working Cell Bank no.	: MWCB-5 (02UVC-029)
Cell culture code number	: 03VC030

Cultivation

Cells are subcultivated three times in microcarrier cultures.

Number of cell doublings	: 10.8
Amount of control cells set aside	: 1.1×10^8 cells

Serum for cell cultures

Origin of serum used	: donor bovine	foetal calf
Batch no°	: SD20312	SF91021

Tests performed on control cells

Test for absence of cytopathogenic and haemadsorbing viruses

Method	: According to Ph. Eur.; 2.6.16 (17C-IPV-V-01)
Date	: 17-02-2003
Result	: passed

Test in cell cultures

Method		: According to Ph. Eur.; M-0214 (17C-IPV-01)
Date of test	start	: 03-03-2003
	finish	: 25-03-2003
Result		: Passed

Test for identity

Method	: Authentikit "Corning"
Date	: 04-03-2003
Result	: VERO cells

Virus strain

Type	: 1
Strain used	: Mahoney
Origin and source strain	: Dr. J. Salk
Master seedlot	: PM 14
Date of preparation	: 58/02/06
Parent seedlot	: UPV 91-06
Date of preparation	: 12-08-1991

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148
CAIF
Compañía Argentina de
Investigaciones Farmaceuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29378925

NVI
nederlands vaccin instituut

Summary protocol Poliomyelitis Working Seedlot

Working Seedlot: 03/1/B5
Type: 1

Virus harvest

Date of inoculation of cells with virus	: 21-02-2003	
Date of virus harvest	: 23-02-2003	
Volume	: 60 l	
Special observations on virus harvests	: None	
Seedlot no°.	: 03/1/B5	

Filling and freezing of seedlot

Filling date	: 07-03-2003	
Volume filled	: 75 ml	750 ml
No°. of containers	: 400	38
Freezing dates	: 07-03-2003	
Storage at –70°C		

Tests on virus harvest / seedlot

Tests are performed either on the virus harvest (as bulk) or on the filled seedlot.

Identity test

Method	: 17C-IPV-06
Date	: 17-03-2003
Result	: type 1

Virus concentration

Method	: 17C-IPV-13
Date	: 11-03-2003
Result (log CCID50 per ml)	: 8.55

Sterility test

Method		: 17C-Ster	
Media		: Thioglycollate	Trypton Soya Broth
Temperature		: 30-35 °C	20-25 °C
Inoculum		: 10 ml	10 ml
Date of test	start	: 12-03-2003	
	finish	: 26-03-2003	
Result		: Passed	

Test for mycoplasmas

Method		: 17C-St-MP-01
Media		: PPLO agar and broth,
		: Chanock agar and broth
Date of test	start	: 18-03-2003
	finish	: 22-04-2003
Result		: Passed

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148

CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29378925

NVI
nederlands vaccin instituut

Summary protocol Poliomyelitis Working Seedlot

Working Seedlot: 03/1/B5
Type: 1

Test for mycobacteria

Method		: According to Ph. Eur.; 2.6.2 (17C-MB)[1]
Media		: Loewenstein; Middlebrook; Tween albumin
Period of observation		: 56 days
Date of test	start	: 18-03-2003
	finish	: 14-05-2003
Result		: Passed

Test in Vero cell cultures

Method		: According to Ph. Eur.; 2.6.16 (17C-Polio-ZV-02)
Total volume inoculated		: 50 ml
Observation period		: 14 days
Date of test	start	: 19-03-2003
	finish	: 02-04-2003
Result		: No extraneous agents found

Test in MRC5 cell cultures

Method		: According to Ph. Eur.; 2.6.16 (17C-Polio-ZV-02)
Total volume inoculated		: 50 ml
Observation period		: 14 days
Date of test	start	: 19-03-2003
	finish	: 02-04-2003
Result		: No extraneous agents found

Test in adult mice

Method	: According to Ph. Eur.; 2.6.16 (17C-PSV-01)
Date of inoculation	: 03-04-2003
Volume inoculated	: 0.03 ml ic / 0.5 ml ip
Period of observation	: 21 days
No°. of mice inoculated	: 10
No°. of mice died > 24 h	: 0
Result	: no evidence of infection

Test in suckling mice

Method	: According to Ph. Eur.; 2.6.16 (17C-PSV-02)
Date of inoculation	: 03-04-2003
Volume inoculated	: 0.01 ml ic / 0.1 ml ip
Period of observation	: 14 days
No°. of mice inoculated	: 21
No°. of mice died < 24 h	: 1
No°. of mice died > 24 h	: 0
Result	: no evidence of infection

[1] Sample has been treated by washing steps to remove antibiotics prior to the test for mycobacteria

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 16.148
CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29378925

NVI
nederlands vaccin instituut

Summary protocol Poliomyelitis Working Seedlot

Working Seedlot: 03/1/B5
Type: 1

Test in guinea-pigs

Method	: According to Ph. Eur.; 2.6.16 (17C-PSV-03)
Date of inoculation	: 29-04-2003
Volume inoculated	: 5 ml
Period of observation	: 42 days
No°. of guinea-pigs inoculated	: 5
No°. of guinea-pigs died > 24 h	: 0
Result	: no evidence of infection

Remarks:

Conclusion

Certification by person taking overall responsibility for production and control of the Poliomyelitis Working Seedlot.

I certify that lot number 03/1/B5 of this Poliomyelitis Working Seedlot meets the requirements of the European Pharmacopoeia (monograph 0214) and/or WHO monographs where applicable.

Reviewed by:

J. Westendorp

Initials :

Date : 22-09-03

Qualified Person:

Drs. L.C.Sundermann

Signature :

Date : 22-09-03

NVI
Nederlands vaccin institute

Summary protocol Poliomyelitis Working Seedlot

Working Seedlot: 01/2/B5
Type: 2

Manufacturer

Name manufacturer	: NVI (Netherlands Vaccine Institute)
Address of manufacturer	: P.O. Box 11
	: 3721 MA Bilthoven,
	The Netherlands

Cell substrate

Type of cell culture used	: Vero
Manufacturers Working Cell Bank no.	: MWCB-2 (UCC91-04)
Cell culture code number	: 01UVC024

Cultivation

Cells are subcultivated three times in microcarrier cultures.

Number of cell doublings	: 10.2
Amount of control cells set aside	: 1.5 x 10^9 cells

Serum for cell cultures

Origin of serum used	: donor bovine	foetal calf
Batch no[s]	: SD00404	SF91021

Tests performed on control cells

Test for absence of cytopathogenic and haemadsorbing viruses

Method	: According to Ph. Eur.; 2.6.16 (17C-IPV-V-01)
Date	: 03-09-2001
Result	: passed

Test in cell cultures

Method		: According to Ph. Eur.; M-0214 (17C-IPV-01)
Date of test	start	: 24-09-2001
	finish	: 29-10-2001
Result		: Passed

Test for identity

Method	: Authentikit "Corning"
Date	: 02-11-2001
Result	: VERO cells

Virus strain

Type	: 2
Strain used	: MEF1
Origin and source strain	: SSI Kopenhagen
Master seedlot	: F451
Date of preparation	: 56/03/16
Parent seedlot	: UPV 91-07
Date of preparation	: 26-08-1991

NVI
nederlands vaccin instituut

Summary protocol Poliomyelitis Working Seedlot

Working Seedlot: 01/2/B5
Type: 2

Virus harvest

Date of inoculation of cells with virus	: 07-09-2001
Date of virus harvest	: 09-09-2001
Volume	: 60 l
Special observations on virus harvests	: None
Seedlot no°.	: 01/2/B5

Filling and freezing of seedlot

Filling date	: 25-10-2001	
Volume filled	: 50 ml	500 ml
No°. of containers	: 400	40
Freezing dates	: 25-10-2001	
Storage at –70°C		

Tests on virus harvest / seedlot

Tests are performed either on the virus harvest (as bulk) or on the filled seedlot.

Identity test

Method	: 17C-IPV-06
Date	: 29-11-2001
Result	: type 2

Virus concentration

Method	: 17C-IPV-13
Date	: 05-02-2002
Result (log CCID50 per ml)	: 8.33

Sterility test

Method		: 17C-Ster	
Media		: Thioglycollate	Trypton Soya Broth
Temperature		: 30-35 °C	20-25 °C
Inoculum		: 10 ml	10 ml
Date of test	start	: 30-10-2001	
	finish	: 13-11-2001	
Result		: Passed	

Test for mycoplasmas

Method		: 17C-St-MP-01
Media		: PPLO agar and broth, : Chanock agar and broth
Date of test	start	: 15-01-2002
	finish	: 18-02-2002
Result		: Passed

NVI
nederlands vaccin instituut

Summary protocol Poliomyelitis Working Seedlot

Working Seedlot: 01/2/B5
Type: 2

Test for mycobacteria

Method		: According to Ph. Eur.; 2.6.2 (17C-MB)[1]
Media		: Loewenstein; Middlebrook; Tween albumin
Period of observation		: 56 days
Date of test	start	: 26-10-2001
	finish	: 21-12-2001
Result		: Passed

Test in Vero cell cultures

Method		: According to Ph. Eur.; 2.6.16 (17C-Polio-ZV-02)
Total volume inoculated		: 50 ml
Observation period		: 14 days
Date of test	start	: 20-06-2002
	finish	: 04-07-2002
Result		: No extraneous agents found

Test in MRC5 cell cultures

Method		: According to Ph. Eur.; 2.6.16 (17C-Polio-ZV-02)
Total volume inoculated		: 50 ml
Observation period		: 14 days
Date of test	start	: 20-06-2002
	finish	: 04-07-2002
Result		: No extraneous agents found

Test in adult mice

Method	: According to Ph. Eur.; 2.6.16 (17C-PSV-01)
Date of inoculation	: 29-11-2001
Volume inoculated	: 0.03 ml ic / 0.5 ml ip
Period of observation	: 21 days
No°. of mice inoculated	: 10
No°. of mice died > 24 h	: 0
Result	: no evidence of infection

Test in suckling mice

Method	: According to Ph. Eur.; 2.6.16 (17C-PSV-02)
Date of inoculation	: 29-11-2001
Volume inoculated	: 0.01 ml ic / 0.1 ml ip
Period of observation	: 14 days
No°. of mice inoculated	: 21
No°. of mice died < 24 h	: 1
No°. of mice died > 24 h	: 0
Result	: no evidence of infection

[1] Sample has been treated by washing steps to remove antibiotics prior to the test for mycobacteria

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148

CAIF
Compañia Argentina de
Investigaciones Farmaceuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29378925

NVI
nederlands vaccin instituut

Summary protocol Poliomyelitis Working Seedlot

Working Seedlot: 01/2/B5
Type: 2

Test in guinea-pigs

Method	: According to Ph. Eur.; 2.6.16 (17C-PSV-03)
Date of inoculation	: 27-11-2001
Volume inoculated	: 5 ml
Period of observation	: 42 days
No°. of guinea-pigs inoculated	: 5
No°. of guinea-pigs died > 24 h	: 0
Result	: no evidence of infection

Remarks:

Conclusion

Certification by person taking overall responsibility for production and control of the Poliomyelitis Working Seedlot.

I certify that lot number 01/2/B5 of this Poliomyelitis Working Seedlot meets the requirements of the European Pharmacopoeia (monograph 0214) and/or WHO monographs where applicable.

Reviewed by:		Qualified Person:	
J. Westendorp		Drs. L.C.Sundermann	
Initials	: JW	Signature	:
Date	: 22.09.03	Date	: 22-09-03

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148

CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29378925

NVI
nederlands vaccin instituut

Summary protocol Poliomyelitis Working Seedlot

Working Seedlot: 01/3/B5
Type: 3

Manufacturer

Name manufacturer	: NVI (Netherlands Vaccine Institute)
Address of manufacturer	: P.O. Box 11
	: 3721 MA Bilthoven,
	The Netherlands

Cell substrate

Type of cell culture used	: Vero
Manufacturers Working Cell Bank no.	: MWCB-2 (UCC91-04)
Cell culture code number	: 01UVC027

Cultivation

Cells are subcultivated three times in microcarrier cultures.

Number of cell doublings	: 10.3
Amount of control cells set aside	: 2.6 x 10^9 cells

Serum for cell cultures

Origin of serum used	: donor bovine	foetal calf
Batch no°	: SD00404	SF91021

Tests performed on control cells

Test for absence of cytopathogenic and haemadsorbing viruses

Method	: According to Ph. Eur.; 2.6.16 (17C-IPV-V-01)
Date	: 17-09-2001
Result	: passed

Test in cell cultures

Method		: According to Ph. Eur.; M-0214 (17C-IPV-01)
Date of test	start	: 09-10-2001
	finish	: 05-11-2001
Result		: Passed

Test for identity

Method	: Authentikit "Corning"
Date	: 02-11-2001
Result	: VERO cells

Virus strain

Type	: 3
Strain used	: Saukett
Origin and source strain	: SSI Kopenhagen
Master seedlot	: 7TV261
Date of preparation	: 64/11/30
Parent seedlot	: UPV 91-08
Date of preparation	: 09-09-1991

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148

Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 20378925

NVI
nederlands vaccin instituut

Summary protocol Poliomyelitis Working Seedlot

Working Seedlot: 01/3/B5
Type: 3

Virus harvest

Date of inoculation of cells with virus	: 21-09-2001	
Date of virus harvest	: 23-09-2001	
Volume	: 60 l	
Special observations on virus harvests	: None	
Seedlot no°.	: 01/3/B5	

Filling and freezing of seedlot

Filling date	: 01-11-2001	
Volume filled	: 150 ml	300 ml
No°. of containers	: 200	91
Freezing dates	: 01-11-2001	
Storage at –70°C		

Tests on virus harvest / seedlot

Tests are performed either on the virus harvest (as bulk) or on the filled seedlot.

Identity test

Method	: 17C-IPV-06
Date	: 04-12-2001
Result	: type 3

Virus concentration

Method	: 17C-IPV-13
Date	: 05-02-2002
Result (log CCID50 per ml)	: 8.55

Sterility test

Method		: 17C-Ster	
Media		: Thioglycollate	Trypton Soya Broth
Temperature		: 30-35 °C	20-25 °C
Inoculum		: 10 ml	10 ml
Date of test	start	: 06-11-2001	
	finish	: 20-11-2001	
Result		: Passed	

Test for mycoplasmas

Method		: 17C-St-MP-01
Media		: PPLO agar and broth, : Chanock agar and broth
Date of test	start	: 15-01-2002
	finish	: 18-02-2002
Result		: Passed

NVI
nederlands vaccin instituut

Summary protocol Poliomyelitis Working Seedlot

Working Seedlot: 01/3/BS
Type: 3

Test for mycobacteria

Method		: According to Ph. Eur.; 2.6.2 (17C-MB)[1]
Media		: Loewenstein; Middlebrook; Tween albumin
Period of observation		: 56 days
Date of test	start	: 13-11-2001
	finish	: 10-01-2002
Result		: Passed

Test in Vero cell cultures

Method		: According to Ph. Eur.; 2.6.16 (17C-Polio-ZV-02)
Total volume inoculated		: 50 ml
Observation period		: 14 days
Date of test	start	: 20-06-2002
	finish	: 04-07-2002
Result		: No extraneous agents found

Test in MRC5 cell cultures

Method		: According to Ph. Eur.; 2.6.16 (17C-Polio-ZV-02)
Total volume inoculated		: 50 ml
Observation period		: 14 days
Date of test	start	: 20-06-2002
	finish	: 04-07-2002
Result		: No extraneous agents found

Test in adult mice

Method	: According to Ph. Eur.; 2.6.16 (17C-PSV-01)
Date of inoculation	: 05-02-2002
Volume inoculated	: 0.03 ml ic / 0.5 ml ip
Period of observation	: 21 days
No°. of mice inoculated	: 10
No°. of mice died > 24 h	: 0
Result	: no evidence of infection

Test in suckling mice

Method	: According to Ph. Eur.; 2.6.16 (17C-PSV-02)
Date of inoculation	: 04-02-2002
Volume inoculated	: 0.01 ml ic / 0.1 ml ip
Period of observation	: 14 days
No°. of mice inoculated	: 21
No°. of mice died < 24 h	: 0
No°. of mice died > 24 h	: 0
Result	: no evidence of infection

[1] Sample has been treated by washing steps to remove antibiotics prior to the test for mycobacteria

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148

CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29378925

NVI
nederlands vaccin institut

Summary protocol Poliomyelitis Working Seedlot

Working Seedlot: 01/3/B5
Type: 3

Test in guinea-pigs

Method	: According to Ph. Eur.; 2.6.16 (17C-PSV-03)
Date of inoculation	: 24-01-2002
Volume inoculated	: 5 ml
Period of observation	: 42 days
No°. of guinea-pigs inoculated	: 5
No°. of guinea-pigs died > 24 h	: 0
Result	: no evidence of infection

Remarks:

Conclusion

Certification by person taking overall responsibility for production and control of the Poliomyelitis Working Seedlot.

I certify that lot number 01/3/B5 of this Poliomyelitis Working Seedlot meets the requirements of the European Pharmacopoeia (monograph 0214) and/or WHO monographs where applicable.

Reviewed by:		**Qualified Person:**	
J. Westendorp		Drs. L.C.Sundermann	
Initials	:	Signature	:
Date	: 22-09-03	Date	: 22-09-03

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148
CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29378925

NVI
nederlands vaccin instituut

Summary protocol VERO Working cellbank

Working Cellbank: VERO-MWCB-7

Manufacturer

Name manufacturer	: NVI (Netherlands Vaccine Institute)
Address of manufacturer	: P.O. Box 457
	: 3720 AL Bilthoven,
	The Netherlands

Production of the working cellbank

Master Cellbank	: VERO-MWCB-2 = UCC 91-04
Date of production	: 23-12-1991
Passage number	: 141

Cultivation to obtain a working cell bank

Batch number	: VERO-MWCB-7
Date	: 27-11-2008
Cells are subcultivated two times in microcarrier cultures.	
Number of cell doublings	: 8.6
Number of ampoules frozen	: 477
Volume per ampoule	: 8 ml
Cells per ampoule	: appr. 160 x 10^6
Viability	: appr. 100 %
Passage number	: 143

Serum for cell cultures

Origin of serum used	: Foetal Calf	donor bovine
Batch no°	: A 70106 7019	DRK 0265

Tests performed on cell bank

Sterility test

Method	: According to Ph. Eur.; 2.6.1
Media	: Thioglycollate;Trypton Soya Broth
Temperature	: 30-32 °C 20-25 °C
Date test on	: 29-09-2009
Date test off	: 20-10-2009
Result	: Passed

NVI
nederlands vaccin instituut

Datum: 03-mrt-2010
Paraaf:
Certified Copy

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148
CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29320925

NVI
nederlands vaccin instituut

Summary protocol VERO Working cellbank

Working Cellbank: VERO-MWCB-7

Test for mycoplasmas

Method	: According to Ph. Eur.; 2.6.7
Media	: PPLO agar and broth,
	: Chanock agar and broth
Date test on	: 20-02-2009
Date test off	: 25-03-2009
Result	: Passed

Test for absence of cytopathogenic and haemadsorbing viruses

Method	: According to Ph. Eur.2.6.16;
Date test on	: 08-12-2008
Date test off	: 07-01-2009
Result	: Passed

Test in rabbit kidney cell cultures

Method	: According to Ph. Eur.2.6.16;
Date test on	: 22-04-2009
Date test off	: 06-05-2009
Result	: Passed

Test in cercopithecus kidney cell cultures

Method	: According to Ph. Eur.2.6.16;
Date test on	: 22-12-2008
Date test off	: 12-01-2009
Result	: Passed

Test in MRC5 cell cultures

Method	: According to Ph. Eur.2.6.16;
Date test on	: 22-12-2008
Date test off	: 21-01-2009
Result	: Passed

Test for identity

Method	: Authentikit "Corning"
Date	: 11-12-2008
Result	: Monkey cercopithecus species

NVI
nederlands vaccin instituut

Datum: 03-MRT-2010
Paraaf:
Certified Copy

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148
CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29379925

NVI
nederlands vaccin instituut

Summary protocol VERO Working cellbank

Working Cellbank: VERO-MWCB-7

Method	: DNA fingerprint
Date test on	: 27-05-2009
Date test off	: 05-06-2009
Result	: identical to standard vero DNA
Method	: Morphology
Date	: 22-05-2009
Result	: VERO Cells
Over all conclusion	: VERO cells

Tests performed on high passage

The following tests are performed on passage 156

Co-cultivation on Vero cell cultures

Method	: According to Ph. Eur.; 5.2.3
Observation period	: 28 days
Result	: No extraneous agents found
Date	:14-04-2009

Co-cultivation on MRC5 cell cultures

Method	: According to Ph. Eur.; 5.2.3
Observation period	: 28 days
Result	: No extraneous agents found
Date	: 14-04-2009

Co-cultivation on Cercopithecus cell cultures

Method	: According to Ph. Eur.; 5.2.3
Observation period	: 28 days
Result	: No extraneous agents found
Date	: 14-04-2009

Test in suckling mice

Method	: According to Ph. Eur.; 5.2.3
Date of inoculation	: 06-10-2009
Volume inoculated	: 0.1 ml sc
Period of observation	: 28 days
No°. of mice inoculated	: 10
No°. of mice died < 24 h	: 0
No°. of mice died > 24 h	: 0
Result	: no evidence of infection

NVI
nederlands vaccin instituut

Datum: 03-MRT-2010
Paraaf:
Certified Copy

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148
CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29378928