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Summary protocol VERO Working cellbank

Working Cellbank: VERO-MWCB-7

Test in adult mice

Method	: According to Ph. Eur.; 5.2.3
Date of inoculation	: 29-09-2009
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 adult mice for LCM

Method	: According to Ph. Eur.; 5.2.3
Date of inoculation	: 29-09-2009
Volume inoculated	: 0.01 ml ic
Period of observation	: 28 days
No°. of mice inoculated	: 10
No°. of mice died > 24 h	: 1
Result	: no evidence of infection with LCM

Test in SPF eggs

Method	: According to Ph. Eur.; 5.2.3
Number of eggs in each test	: 10
Date of inoculation allantoic cavity	: 07-05-2009
Period of observation	: 7 days
Date of inoculation yolk sac	: 01-05-2009
Period of observation	: 7 days
Number of embryos surviving the test	: 10
Result	: no evidence of infection

Test in Cercopithecus cell cultures

Method	: According to Ph. Eur.; 5.2.3
Observation period	: 14 days
Result	: No extraneous agents found
Date	: 27-04-2009

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

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Datum:	03-MRT-2010
Paraaf:	AK
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CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15448
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29536925

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Summary protocol VERO Working cellbank

Working Cellbank: VERO-MWCB-7

Remarks:

Reference is made to relevant parts of the current Ph. Eur.

TSE compliance

Materials derived from ruminants used in the manufacture of this batch:

	n° certificate	date of submission dossier to competent authority
Donor bovine serum	CEP 2000-341-Rev 00	-
Fetal bovine serum	CEP-2000-093-Rev 01	-

Certification

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

I herewith certify that lot number Vero-MWCB-7 of this working cell bank was manufactured and tested according to the procedures approved by the competent authorities and complies with the quality requirements. This includes that, for any materials derived from ruminants (bovine, ovine, caprine) used in the manufacture and/or formulation of the batch of product specified above, all measures have been taken to demonstrate compliance with Commission Directive 2001/83/EC and amending Directives 2003/63/EC and 2004/27/EC.

Reviewed by:

R.F. Lijffijt

Initials : RFL

Date : 03-MRT-2010

Qualified Person:

Drs. L.C.Sundermann

Signature :

Date : 03 mr 2010

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Datum: 03-MRT-2010
Paraaf: RFL
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CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.248
Administradora de Investigaciones Farmaceuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 13478925

Table 1. Batch number, serotype and passage level of the batches tested.

Batch	Fermentation volume (L)	PolioType	Passage level
PVU 92-01	150	1	5
PVU 92-06	150	1	5
PVU 92-09	150	1	5

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.145
CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29378935

Batch	Fermentation volume (L)	PolioType	Passage level
PVU 92-10	150	1	5
PVU 92-02	150	2	5
PVU 92-04	150	2	5
PVU 92-07	150	2	5
PVU 92-03	150	3	5
PVU 92-08	150	3	5
PVU 96-01	150	1	5
PVU 96-02	150	2	5
PVU 96-03	150	3	5
PU 95-3400	350	3	5
PVU 97-101	350	1	5
PVU 97-201	350	2	5
PVU 97-301	350	3	5
PV 03-109	350	1	6
PV 03-110	350	1	6
PV 03-111	350	1	6
PV 03-112	350	1	6
PV 03-113	350	1	6
PV 03-114	350	1	6
PV 03-203	350	2	6
PV 03-204	350	2	6
PV 03-205	350	2	6
PV 03-304	350	3	6
PV 03-304	350	3	6
PV 03-304	350	3	6

Methods of analysis

The D-antigen content was determined by an ELISA method unless described otherwise. Other methods used were SDS-PAGE, protein determination (colorimetric assay according to Bradford) and OD by measuring absorbance at 260 and 280 nm.

Results

D-antigen recovery after inactivation.

The effect of the formaldehyde inactivation on the D-antigen content in the monovalent pool was determined in order to determine whether the inactivation process yields a similar product (Table 2). The D-antigen content of the fraction prior to inactivation was set to 100%.

Table 2. D-antigen recovery after the inactivation step.

Type	Recovery (% D-antigen)	
	monovalent pool at passage level 5	monovalent pool at passage level 6
1	67 ± 10 (n=4)	84.5 ± 2.5 (n=6)
2	89 ± 4 (n=3)	82 ± 5 (n=3)
3	79 ± 5 (n=2)	87 ± 5 (n=3)

Conclusion 1: Monovalent pools at both passage levels show a similar decrease in D-antigen content after inactivation. Hence, the critical inactivation step is not influenced by the change in passage level.

The relation between D-antigen content and virus content.

The antigenicity of both products was also determined by calculating the D-antigen as a fucntion of the virus concentration. The virus concentration of purified virus was therefore determined by measuring the absorbance at 260 nm (OD_{260}) (tabel 3) (Van Steenis et al, 1981).

Table 3. D-antigen content per absorbance unit at 260 nm of purified virus.

Type	D-antigen units/absorbance unit at 260 nm	
	monovalent pool at passage level 5	monovalent pool at passage level 6
1	7873 ± 1330 (n=6)	8482 ± 962 (n=6)
2	2717 ± 510 (n=5)	2097 ± 509 (n=3)
3	5681 ± 1105 (n=4)	5077 ± 992 (n=3)

Conclusion 2 The antigenicity for virus manufactured at passage level 5 is similar to virus manufactured at passage level 6 as determined from the ratio between D-antigen content en virus concentration.

Purity; the relation between D-antigen content and protein content.

The purity of purified virus and monovalent pool, as expressed in D-antigen units per mg protein, has been determined for both passage levels.

Table 4. Purity of purified virus and monovalent pool preparations.

Type	Production stage	D-antigen units/µg protein	
		monovalent pool at passage level 5	monovalent pool at passage level 6
1	virus	72 ± 20 (n=4)	79.7 ± 15 (n=6)
	monovalent pool	72 ± 9 (n=4)	75.1 ± 15 (n=6)
2	virus	30 ± 4 (n=3)	22 ± 3.5 (n=3)
	monovalent pool	29 ± 1 (n=3)	20 ± 4 (n=3)
3	virus	63 ± 1 (n=2)	51.8 ± 9 (n=3)
	monovalent pool	68 ± 4 (n=2)	47.9 ± 7 (n=3)

Conclusion 3 The purity of products at both passage levels is similar.

Purity; the relation between virus content and protein content.

Conclusion 2 and 3 imply that the ratio between virus concentration and protein concentration is similar as well. This is confirmed by the data in table 5.

Table 5. Virus concentration (OD_{260}) per mg protein

Type	OD_{260} per mg protein	
	monovalent pool at passage level 5	monovalent pool at passage level 6

1	9.5 ± 1.0 (n=4)	9.4 ± 0.3 (n=6)
2	12.6 ± 1.9 (n=3)	10.6 ± 1.0 (n=3)
3	12.1 ± 1.1 (n=2)	10.2 ± 0.7 (n=3)

Conclusion 4 The ratio between virus concentration and protein concentration for both passage levels is comparable, indicating a comparable purity.

Purity; the OD_{60}/OD_{280} ratio.

The absorbtion at 260 nm, which has been defined earlier as a measure for virus concentration, reflects the total amount of nucleic acids, i.e. viral RNA and cellular DNA. Absorbtion at 280 nm reflects the amount of protein. The ratio OD_{260}/OD_{280} for purified virus is 1.71 (Ferguson et al., 1993). Major deviations from this ratio and hence in the purity of the virus are known to occur in the following situations:

1. If the virus is present as procapsid, the OD_{260}/OD_{280} ratio is 0.75 (Van Steenis et al., 1981). Procapsids are an immature form of poliovirus in which the capsid proteins have not been processed completely.
2. If the virus is denatured, theOD_{260}/OD_{280} is 0.94. This is due to the formation of empty capsids, resulting in the conversion of the native D-antigen into C-antigen which is not immunogenic.
3. If there is a substantial amount of protein impurities
4. If there is an increase in the amount of nucleic acids

The measured OD_{260}/OD_{280} ratios for purified virus at both passage levels are very close to 1.71, indicating a similar high purity. The ratios are presented in table 6.

Table 6. The OD_{260}/OD_{280} ratio of purfied virus

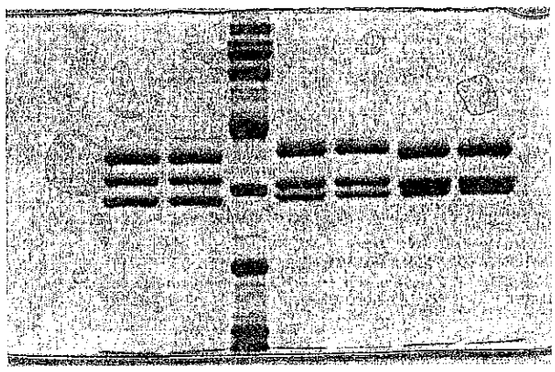
type	OD_{260}nm/OD_{280}nm	
	monovalent pool at passage level 5	monovalent pool at passage level 6
1	1.72 ± 0.13 (n=6)	1.75 ± 0.02 (n=6)
2	1.76 ± 0.03 (n=5)	1.69 ± 0.08 (n=3)
3	1.74 ± 0.09 (n=4)	1.69 ± 0.1 (n=3)

Conclusion 5 The OD_{260}/OD_{280} ratio of purified virus, and therefore the purity, are comparable for passage level 5 and 6.

Purity; polyacrylamide gelelectrophoresis.

Purified monovalent pools cultured on Vero cells at virus passage levels 5 and 6 as well as purified monovalent pools at virus passage level 6 cultured on monkey kidney cells were subjected to SDS-PAGE stained with Coomassie (figure 1a and b). The dense bands in the gels reflect the three capside proteins. Due to its small size (7 kD), VP4 is not always visible as it runs off the gel.

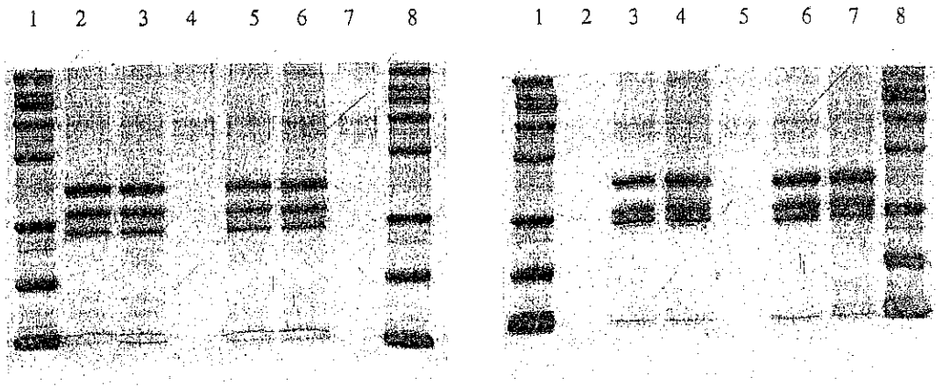
Fig. 1a Monovalente pools on Vero cells and level 6 on monkey kidney cells at virus passage level 5



1 2 3 4 5 6 7

1. Type 1 MKC PU 00-1314-4.1 passage 6
2. Type 1 VERO PVU98-102-4.1 passage 5
3. Broad Range Reference proteins
4. Type 2 MKC PU 00- 288-4.1 passage 6
5. Type 2 VERO PVU01-202-4.1 passage 5
6. Type 3 MKC PU 01-3433-4.1 passage 6
7. Type 3 VERO PVU01-303-4.1 passage 5

Fig. 1b. Monovalent pools on Vero cells at passage level 5 and 6



gel 1 gel 2

1 = marker	1 = marker
2 = type 1 passage 5 (PVU 98-102-4.1)	2 = monsterbuffer
3 = type 1 passage 5 (PVU 01-103-4.1)	3 = type 2 passage 5 (PVU 01-202-4.1)
4 = monsterbuffer	4 = type 2 passage 6 (PV 03-203-4.1)
5 = type 1 passage 6 (PV 03-110-4.1)	5 = monsterbuffer
6 = type 1 passage 6 (PV 03-112-4.1)	6 = type 3 passage 5 (PVU 01-303-4.1)
7 = monsterbuffer	7 = type 3 passage 6 (PV 03-306-4.1)
8 = marker	8 = marker

Conclusion 7

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 5.1 CAIF
Consejo Argentino de Investigaciones Farmaceuticas S.A.
Dra. Maria Bernarda Belay
Apoderada
DNI 20379923

Monovalent pools of poliomyelitis vaccin of passage levels 5 and 6 on Vero cells show the same pattern on SDS-PAGE.

General conclusion

No differences between the active substance of the poliomyelitis vaccin, i.e. the monovalent pool or its preceeding products, manufactured at passage level 5 or 6 have been detected with the performed characterisations. It can be concluded that the (intermediate) products produced on the two different passage levels demonstrate a high degree of similarity.

References

1. Ferguson, M., Wood, D.J., Minor, P.D. (1993) Antigenic structure of poliovirus in inactivated vaccines, Journal of General Virology, 74, 685-690
2. Van Steenis, van Wezel and Sekhuis (1981) Potency testing of killed polio vaccine in rats. Dev. Biol. Stand. 47, 119-128.

Appendix 6: Comparison of drug substances produced with WSLs at passage levels 4 and 5

Introduction

The active substances of Inactivated Poliomyelitis Vaccine (IPV) are three different monovalent pools poliomyelitis virus. Each pool contains a virus serotype, i.e. type 1 (Mahoney), type 2 (MEF-1) or type 3 (Saukett). These viruses are grown, purified and inactivated in separate production runs resulting in monovalent pools.

The viruses were grown on a Vero cell line produced with a WSL at passage level 4. Due to a limited number of ampoules of the WSL at passage level 4 a new WSL is prepared at passage level 5. As a consequence the viruses cultured in production are at passage level 6. In order to determine whether an additional passage changes the characteristics of the virus, monovalent pools obtained from both passage levels are compared with respect to some relevant biochemical and immunological characteristics. This appendix describes the results of these studies and shows that the monovalent pools from both passage levels have a high degree of similarity.

Batches tested

Table 1 gives an overview of the batches of monovalent pools that were used for the these comparability studies. Most of the batches produced on Vero cells at WSL passage level 4 were produced for experimental purposes only with the exception of PVU 92-10 (type 1), PVU 92-07 (type 2), and PVU 92-08 (type 3) which were used for the production of vaccine used in clinical trial studies. All batches produced with WSL passage level 5 are full scale production batches.

3.2.S.2.3 – Control of Materials

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Certificate of Analysis

Product: DEAE Sepharose™ Fast Flow

Code Numbers: 17-0709-01, 17-0709-05, 17-0709-10, 17-0709-60, 17-0709-99

Lot No: 289339

Test/Characteristic:		Limits:	Results:
1	**Function** Retention volume; mL - GammaBind™ G type 2 - β-Lactoglobulin B - β-Lactoglobulin A	 37 – 47 56 – 70 67 – 83	 44 65 77
2	**Total capacity** mmol Cl^- / mL packed gel	0.11 - 0.16	0.14
3	**Flow rate at 0.1 MPa; cm / hour** Bed height: 14 - 16 cm	300 - 600	466
4	**Particle size distribution** Volume share within 45 and 165 µm; %	min. 95	98
5	**Microbial contamination** microorganisms / mL suspension	max. 100	0

Approval date: 2001-09-27 **Expiry date:** 2006-07

Tests and limits according to AS 45-6001-46 Ed. AM

Quality Assurance
Issued by Britta Medin

Amersham Pharmacia Biotech
Björkgatan 30
SE-751 84 Uppsala
Sweden
tel +46 18 612 00 00
fax +46 18 612 12 00
web-site
http://www.apbiotech.com
Reg No SE 556106 1019 01

50-3037-63, ed 08

3.2.S.2.3 – Control of Materials

No-mammalian certificate for DEAE Sepharose Fast Flow

amersham pharmacia biotech

To whom it may concern

ON MAMMALIAN DERIVED MATERIALS IN DEAE SEPHAROSE ™ FAST FLOW CHROMATOGRAPHY MEDIA

It is hereby stated that no mammalian derived substances are used either in the raw materials or the manufacture of the above product, and thus that the product does not contain, and is not derived from, specified risk material as defined in Commission Decision 97/534/EC (as amended). In addition, Certificate of Suitability are not relevant for this product.

Uppsala, Sweden, February 27, 2001

Amersham Pharmacia Biotech AB
Regulatory Support

Lena Hellqvist

Amersham Pharmacia Biotech AB
Björkgatan 30
SE-751 84 Uppsala
Sweden
Reg no. SE 55 61 06 1915 01

tel +46 18 612 00 00
fax +46 18 612 12 00
web site
http://www.apbiotech.com

Trypsin 1: 250

This specification is based on internal specification: SPC-20158 version 3

Product origin

Origin: Animal

Animal species: Porcine (pancreas) and Bovine (milk)

Tissue: pancreas and milk (lactose)

Country of Origin: Canada, USA

TSE certificate: not applicable

Steps in Production process that reduce possible microbial contaminations:

- Pancreas is extracted after precipitation for 24 – 26 hours at 55 - 60°C and gamma irradiated at 25 kGy

Trypsin 1: 250

Quality

Trypsin is a white or almost white amorphous powder. Sparingly soluble in water. The amorphous is hygroscopic.

Specification	Requirement
Identity	
Identity A (EP)	Conform (Within 3 minutes red-violet colour)
Identity B (EP)	Conform (Within 3 minutes no red-violet colour)
Chemical tests	
Appearance of solution (EP)	Conform (Not more opalescent as reference solution III)
pH	≥ 3 – ≤ 6
Absorption maximum at 280 nm	≥ 13.5 – ≤ 16.5
Absorption minimum at 250 nm	≤ 7.0
Chymotrypsin	Conform (pH of test solution is not higher as reference solution)
Loss on drying	≤ 5.0 %
Content	≥ 0.5 microkatal/mg
Microbiological properties	
Total aerobic viable count	≤ 10^4 micro org./gr
E.Coli	absent
Salmonella	absent
Mycoplasma	Absent
Virus (porcine parvo)	Absent

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

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CERTIFICATE OF ORIGIN STATEMENT

Product Description: Trypsin 1:250
Component No: 740-00328

This is to certify that the component mentioned above has the following source and origin:

Source:	Origin:
Porcine Pancreas	USA/Canada
Lactose	USA

We can confirm that USA and Canada are considered to be free of BSE/TSE.

Debbie Hamilton
Quality Systems

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.1

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

2-Deoxy-d-ribose

Specification	Requirement
Identity	
Specific optic rotation	≥ -57 ° and ≤ -55 °
Chemical tests	
Loss on drying	≤ 1.0 %
Total ash	≤ 0.5 %
Microbiological properties	
-	

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 28378525

Adenosine 5'-monophosphate monohydrate

Specification	Requirement
Identity	
Infrared	Match > 70
Specific optic rotation	≥ -49.0 ° and ≤ -47.0 °
Chemical tests	
Melting point	≥ 183 °C and ≤ 188 °C
Content (HPLC)	≥ 99.0 %
Microbiological properties	
-	

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

Adenosine-triphosphate

Specification	Requirement
Identity	
IR	IR complies
Chemical tests	
Melting point	185 -190°C
Solubility	Clear and colourless
Purity	99.0 %
Microbiological properties	
-	

Asparaginum anhydrous

Specification	Requirement
Identity	
Specific optic rotation	≥ +33.7 ° and ≤ +36.0 °
Infrared	Match > 70
Chemical tests	
Appearance of solution	Complies
pH	≥ 4.0 and ≤ 6.0
Ninhydrin-positive substances	≤ 0.5 %
Chlorides	≤ 200 ppm
Sulphates	≤ 200 ppm
Ammonia	≤ 0.1 %
Iron	≤ 10 ppm
Heavy metals	≤ 10 ppm
Loss on drying	≤ 0.1 %
Sulphated ash	≤ 0.1 %
Content	≥ 99.0 %
Microbiological properties	
-	

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

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

D-ribose

Specification	Requirement
Identity	
Infrared	Match > 70
Chemical tests	
Specific optic rotation	≥ -21.0 ° and ≤ -18.5 °
TLC (thin layer chromatography)	Complies
Water	≤ 0.5 %
Heavy metals	≤ 0.001 %
Microbiological properties	
-	

Phenol red soluble

Specification	Requirement
Identity	
Specific extinction	≥ 1400 and ≤ 1900
Chemical tests	
pH	≥ 5.1 and ≤ 5.5
Water solubility	Complies
Colour change	Complies (colour changes from red to orange by adding diluted acid)
Microbiological properties	
-	

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

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

Hypoxanthine

Specification	Requirement
Identity	
IR	IR complies (Correlation coefficient > 0.950)
Chemical tests	
Loss on drying	≤ 1.0 %
Assay	97-103 %
Absorption of solution	> 95 %
Heavy metals	< 10 ppm
Sulphated ash	< 0.2 %
Microbiological properties	
-	

Iron(III) nitrate nonahydrate

Specification	Requirement
Identity	
Iron	Complies
Nitrates	Complies
Chemical tests	
Content	> 98 % and ≤ 101 %
Chlorides	≤ 0.0005 %
Phosphates	≤ 0.005 %
Sulphates	≤ 0.005 %
Calcium	≤ 0.005 %
Copper	≤ 0.005 %
Potassium	≤ 0.005 %
Magnesium	≤ 0.001 %
Manganese	≤ 0.02 %
Sodium	≤ 0.005 %
Lead	≤ 0.001 %
Zinc	≤ 0.001 %
Microbiological properties	
-	

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

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

NVI nederlands vaccin instituut	**Module 3 – Quality** Inactivated poliomyelitis vaccine, suspension for injection	Doc.:ENG-50371-Hydroxyproline.01 Replaces: - Date: 25 mrt 2008 Drafted by: MS Page 1 of 1

Hydroxyproline

This specification is based on internal specification SPC-20112 version 6

Specification	Requirement
Identity	
Specific optic rotation	≥ -77.0 ° and ≤ -74.0 °
Infrared	Match > 70
Chemical tests	
assay	≥99.0 %
Microbiological properties	
-	

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

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