10.2.4 Materiales de Referencia

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 20378825

CAIF
COMPAÑIA ARGENTINA DE INVESTIGACIONES FARMACEUTICAS S.A.

3.2.S.5. Materiales o estándares de referencia

Índice

3.2.S.5. Materiales o estándares de referencia

1 **Determinación del contenido de antígeno D (prueba del antígeno D) e identidad**

Material/estándar de referencia	Documentación
Estándar de referencia de poliovirus	Apéndice 1
Anticuerpos monoclonales del antígeno D	Apéndice 2

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.146

CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29379925

Standard Polio Reference

The standard polio reference for D-antigen content determination is polio reference trivalent bulk, code IPV08-143. The standard preparation IPV08-143 is prepared by the Netherlands Vaccine Institute.

IPV08-143 trivalent bulk is a concentrated bulk, which has been produced in the normal polio production process according to NVI standards, and has been filled in ampoules and stored at ≤-70°C. In short, IPV08-143 is a trivalent blend of formaldehyde inactivated monovalent pools produced on Vero cells with Poliovirus type 1 (Mahoney), type 2 (MEF), and type 3 (Saukett). The assigned titre of the reference vaccine is 425-75-262 Ph.Eur. D-antigen units per ml for type 1, 2 and 3, respectively. IPV08-143 has been calibrated against international standard polio reference PU91-01.

The summary protocol of batch IPV08-143 is presented below:

Standard Polio Reference

Summary protocol Reference preparation Poliomyelitis Vaccine IPV08-143

Manufacturer	
Name manufacturer	NVI
Address	P.O. Box 457 3720 AL Bilthoven The Netherlands
Proprietary name	Reference preparation Poliomyelitis Vaccine

Note: all dates are represented as DD-MM-YYYY

Final lot(s)					
Volume of one Ampoule			0.6 ml		
Batch number of final bulk			IPV08-143		
Date of inactivation of bulk			28-07-2008		
Lot no.	No. of ampoules filled	No. of ampoules packed	Volume filled	Date of manufacture	Expiry date storage at 2 - 8 °C
IPV08-143	76816	76805	0.6 ml	JUL2009	AA

Not for human use.

Labels according on the proposal

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.146

CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 20.775.615

Standard Polio Reference

Summary protocol Reference preparation Poliomyelitis Vaccine IPV08-142

TSE compliance
Materials derived from ruminants used in the manufacture of this batch

	certificate	date of submission dossier to competent authority
Calf bovine serum	CEP 2001-154 and/or CEP 2003-341	
Foetal bovine serum	CEP 2001-053	
Trypsine	Not applicable (contains only lactose)	

Certification
Certification by qualified person taking the overall responsibility for production and control of the Reference preparation Poliomyelitis Vaccine.

I hereby certify that IPV batch N° IPV08-143 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

Qualified Person: Drs. L.G. Sondermann

Signature: .....

Date of issue: .....

CAIF SA
Dra. Bernarda Beley
Co-Directora Tecnica
M.N. 95.140

CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Beley
Apoderada
DNI 29.139.925

FOLIO 450

Standard Polio Reference

Summary protocol Reference preparation Poliomyelitis IPV08-143

Final lot	Result or data	Specification
Corresponding final bulk	IPV08-143	
Filling site	G1	
Dates of filling	24-07-2009	
	27-07-2009	
	28-07-2009	
	29-07-2009	
Batch number of final lot	IPV08-143	
Volume of final lot	0.6 ml	

	Result or data	Specification
D-antigen content [illegible]		
Method	Ph. Eur. 2.7.1	
Date	04-08-2009	
Result (mean of [illegible] ampoules) type 1	447 DU/ml	[illegible]-501 DU/ml
type 2	91 DU/ml	[illegible]-92 DU/ml
type 3	291 DU/ml	220-328 DU/ml
appearance: orange-red solution	conforms	
Test for sterility		
Method	Ph. Eur. 2.6.1	
Media	thioglycollate / TSB	
Number of ampoules tested	4 X 20	
Date test on	28-07-2009, 31-07-2009 and 25-08-2009	
Date test off	11-08-2009, 14-08-2009 and 03-09-2009	
Result	no growth observed	no growth of bacteria or fungi
Potency test poliomyelitis		
Method: Ph. Eur. 2.7.20		
Number of ampoules tested	4 X 10	
Number of rats	10 per group	
Volume injected	0.5 ml, im	
Date of immunization	29-07-2009 and 05-08-2009	
Date of blood sampling	19-08-2009 and 26-08-2009	
Trivalent reference vaccine	PU91-01	
Potency reference vaccine [illegible] compared to [illegible]		
type 1	430.0	
type 2	95.0	
type 3	285.0	
Poliomyelitis vaccine [illegible] relative potency		
type 1	1.08	
95% confidence limits	(0.83 – 1.41)	
type 2	1.25	
95% confidence limits	(1.07 – 1.46)	
type 3	1.17	
95% confidence limits	(0.96 – 1.33)	

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

CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.

FOLIO 143

Standard Polio Reference

Production Flow Sheet IPV08-143

Monovalent pool type 1
PV06-127

Monovalent pool type 2
PV07-322

Monovalent pool type 1
PV06-129

Monovalent pool type 2
PV07-216

Monovalent pool type 3
PV07-323

Trivalent bulk
IPV08-143

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 293/8925

FOLIO 452

10.2.5 Certificados Analiticos

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 20.739.143

CAIF
COMPAÑIA ARGENTINA DE INVESTIGACIONES FARMACEUTICAS S.A.

Bilthoven Biologicals B.V.

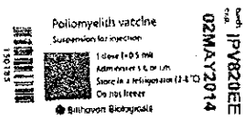
Summary protocol IPV vaccine.....IPV820E

Manufacturer	
Name manufacturer Address	**Bilthoven Biologicals B.V.** P.O. Box 457 3720 AL **Bilthoven** **The Netherlands**
Proprietary name	**Inactivated poliomyelitis vaccine (IPV)**
Trade name	**Poliomyelitis vaccin**
Marketing authorization no°	17642

Note: all dates are represented as dd-mm-yyyy

Final lot(s)					
Volume of 1 human dose				0.5 ml	
Batch number of final bulk				IPV820	
Date of manufacture of final bulk				04-04-2012	
Lot no.	**No. of vials filled**	**No. of vials Labeled**	**Volume filled**	**Date of manufacture**	**Expiry date** *storage at 5±3 °C*
IPV820E	29397		0.6 ml	26-04-2012	02-05-2014
IPV820EE		43	0.6 ml	26-04-2012	02-05-2014

Labels appearing on the vials:



CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.145

Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 20276025

FOLIO 40

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 and/or CEP 2001-184	-
Foetal bovine serum	CEP 2000-093 and/or CEP 2001-083	-
Trypsine	Not applicable (contains only lactose)	-

Certification
Certification by qualified person taking the overall responsibility for production and control of the inactivated poliomyelitis vaccine.

I herewith certify that IPV batch N° **IPV820E** 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.

For registration purpose only

Qualified Person: Drs. L.C.Sundermann

Signature: _____

Date of issue: 23 Oct 2012

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 23273925

Bilthoven Biologicals B.V.

FOLIO 455

IPV vaccine, final lot.....IPV820E

Final lot		Result or data	Specification
Corresponding final bulk		IPV820	
Filling-site		G7	
Date of filling		26-04-2012	
Batch number of final lot		IPV820E	
Volume of final lot		0.5 ml	
Expiry date, storage at 5 ± 3°C		02-05-2014	*max. 24 months after date of potency test*
		Result or data	***Specification***
D-antigen content, confirmation of identity			
method		Ph. Eur. 2.7.1	
date		18-06-2012	
result	type 1	82 DU/ml	*≥ 60*
	type 2	19 DU/ml	*≥ 12*
	type 3	56 DU/ml	*≥ 48*
appearance: orange/red solution		conforms	
Test for sterility			
method		Ph. Eur. 2.6.1	
media		thioglycollate / TSB	
number of vials tested		40	
date test on		05-06-2012	
date test off		19-06-2012	
result		no growth observed	*no growth of bacteria or funghi*
Test for protein content			
method		Calculated from trivalent bulk value	
result		4.1 µg/ml	*≤ 20 µg/ml*
Test for endotoxins			
method		Ph. Eur. 2.6.14	
date of test		06-06-2012	
result		< 0.50 IU/ml	*≤ 10 IU/ml*
Test for pH			
method		electrometric with glass electrode	
date		05-06-2012	
result		7.1	*6.8 – 7.4*
Test for extractable volume			
method		ANA-20073	
number of vials tested		5	
date test on		05-06-2012	
Result, mean of 5 vials		0.6 ml	

Remarks:

DATE 23-10-2012

CAIF SA
Dra. Maria Berarda Belay
Gerente Directora Tecnica
M.N. 15.146

CAIF
Compañía Argentina de Industrias Farmacéuticas S.A.
Dra. María Berarda Belay
Apoderada
DNI 20379825

10.2.6 Validacion de Metodos Analiticos

3.2.S.4.3 Validación de procedimientos analíticos

Informe de validación de la prueba del límite de ADN residual de células Vero

Apéndices de IPVV.3.2.S.4.3
IPVV.3.2.S.4.3.Validation rest Vero cell DNA limit test.01.pdf

Informe de validación de la determinación del antígeno D

Apéndices de IPVV.3.2.S.4.3
IPVV 3.2.S.4.3 Validation of D-antigen.01.pdf

Informe de validación de la prueba de esterilidad

Apéndices de IPVV.3.2.S.4.3
IPVV.3.2.S.5.3 validation sterility test.01.pdf

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

CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Barbara

NVI
nederlands vaccin instituut

Report RAP-10013
Version: 2

Validation report
Limiting test for Vero cell residual DNA

General information

Old code	RAP-10013
Expiry period	25 years
Management	Management
Key words	Poliomyelitis; IPV-Vero; residual-DNA

Authorisation
This document has been authorised by the document management system Quality On-line. The authorisation process was carried out according to procedure SOP-20091.

This document was compiled by Arja Olivier, laboratory technician.

	Function	Name	Signature	Date
For consent	Document manager			
Approved by				

Distribution
Hard copy to VVC

Amendment history

Version	Date	Amended
1	10-Jun-2003	New document
2		See 3.3

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.149

CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29378125

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

CAIF
Laboratorios Argentinos de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada

1 Introduction

Vero cells, a continuous cell line, are used for the production of inactivated polio-myelitis vaccine. The European Pharmacopoeia recommends that if continuous cell-lines are used for production, it must be demonstrated that during the purification process the content of substrate-cell DNA is reduced to not more than 100 pg per single human dose (0.5 or 1 ml). The undiluted monovalent harvest is tested. This monovalent harvest is diluted at least 5x (final volume 0.5 ml) or 10x (final volume 1 ml), depending on the required volume of the final lot. If this monovalent harvest contains less than 1 ng host-derived DNA per ml, then a single human dose contains not more than 100 pg DNA.
The procedure is validated (VLP2000/002).

2 Identification

2.1 Reference(s) to Validation Plan

Validation of Limiting test for Vero cell residual DNA
LCB-VLP nr.: 2000/002
Date: 26-03-2002

Request for amendment submitted on 09-04-2004 relating to a number of changes in the Validation plan. These were necessary for the optimisation of the test.
The changes were discussed and approved in the V.A.T. consultation on 27-03-2003. Marloes de Bruijn, Wim van den Ham, Lonneke Levels and Arja Olivier were present at this meeting. The changes are listed below (see § 3.4).

2.2 Reference(s) to SOP / Method

Limiting test for Vero cell residual DNA
SOP 17C-BIC-06.doc (concept)
Date: 21-03-2002 (Original SOP, not in use)

Residual DNA determination
SOP 17C-BIC-02
Date: 08-12-1997 (Radioactive method, not in use)

Limiting test for Vero cell residual DNA
ANA-21606
Date: 22-03-2005 (Current method)

Limiting test for Vero cell residual DNA. Detection using staining
RAP-20522
Date: 3-8-2004

Isolation of Vero cell DNA
BRV-21858
Date: 11-6-2004

2.3 Changes to report

1. Introduction added. This stresses the importance of the volume of the end product.The volume of a human dose of DTP is 1 ml, the volume of a human dose of IPV-Vero plain or DaKTP is 0.5 ml. The volume has no influence on the amount of DNA per dose of vaccine, should any be present.
2. Documents that are not in QoI are attached as appendices. These are SOP 17C-BIC-02 and SOP 17C-BIC-06. These precede ANA-21606 and reflect the history of the procedure.3.6

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

2.4 Requirements and criteria

A requirement of the European Pharmacopoeia (01/2002:0214) is that when using a continuous cell line for the production of the poliomyelitis vaccine, a human vaccine dose of 1 ml (DTP) or 0.5 ml (IPV-Vero plain and DaKTP) must be shown not to contain more than 100 picograms (pg) of cellular DNA.
This test is used to show whether or not the 6.1-fraction of each of the three types of inactivated, monovalent poliomyelitis vaccine (IPV-Vero) contains more that 1 nanogram (ng) of cellular DNA per ml. After dilution, a final human dose must not contain more than 100 pg DNA.

Specificity The values of the test controls should be low or undetectable, while the values of the lowest assay limit should be at least 2x as high.
The different vaccines, namely the trivalent vaccine and the 3 monovalent vaccines, should have no significant difference in the amount of DNA detected.

Linearity There should be a linear relationship between the concentration of DNA and the intensity of the spot on the film, within the limits of 0.25 ng DNA / ml to 1.5 ng DNA / ml buffer. The correlation coefficient of the graph should be >0.9.

Assay limits DNA [1)] is added to the reference vaccine [2)] and to the test samples.
The chosen test control is a control without DNA, the chosen lower assay limit is 0.25 ng DNA / ml vaccine and the maximum permitted value chosen is 1.0 ng DNA / ml vaccine.
The average measured value of the reference vaccine + 0.25 ng DNA / ml should be at least 2x the value of the control. The average measured value of the reference vaccine + 1.0 ng DNA should be at least 2x that of 0.25 ng DNA / ml.
1) Isolation of Vero-DNA and measurement of its purity and concentration according to SOP 17C-BIC-05.
2) The reference vaccine is shown not to contain any host-derived DNA.
This has already been determined for the trivalent vaccine (see logbook for composition) in tests according to SOP 17C-BIC-02 (the radioactive test).
For the monovalent vaccines used in this validation, this has been determined according to the above-mentioned trivalent vaccine. These monovalent vaccines can serve as references during testing of production samples. They are:
PVU98-102-6.1, PVU97-201-6.1 and PVU98-302-6.1.

Precision-Repeatability Losing one of the bands from the blot is an inherent part of this test. To overcome this the test must be carried out at least 4 times. Only 1 of the measured values may clearly deviate from the others. Following visual inspection of the X-ray film it may be necessary to use Grubb's test in excel to test for outliers.

2.5 Amendments

Concerning Validation plan **1.3 Quality requirements** :

Specificity. It was previously stipulated that there should be no more than 20% difference between the spot intensities for vaccine + DNA and buffer + DNA and between vaccine – DNA and buffer – DNA. This is no longer required. During *optimisation* of the test it seems that there is always a difference between these values. This is not important since every test incorporates control samples of reference vaccine: the reference for each vaccine sample is a vaccine control.
Linearity. To be determined.
Assay limits. Have been determined.
Detection limit. The lowest demonstrability limit has not been determined. It is not relevant to test values that fall below the lowest assay limit of 0.25 ng DNA / ml.
Concerning Validation plan **2.1 Initiator** :
The test is carried out on an RVP product, namely a component of DTP and DKTP.
Concerning Validation plan **2.3 Time planning** :
The validation and the report were completed after the given end dates.

End date Validation:	01-03-2003
End date Report:	01-06-2003

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

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

Concerning Validation plan **4 Method** :

The concept-SOP 17C-BIC-06 was adapted in a few places during *optimisation* of the test. These changes concern the following points:
The effect of the vaccine itself upon the test. This matrix effect was reduced as much as possible by diluting the vaccine and by incorporating an extra Proteinase K step.
An improved implementation of the test. This refers to the ethanol precipitation being replaced by isopropanol; this gives a better result and also allows precipitation to be carried out in eppendorf tubes instead of the glass Corex tubes required previously. A larger number of samples can now be processed. This also refers to the fact that all procedures involving denatured DNA are done on ice to prevent rehybridisation.
The details will be incorporated in the final SOP.

3 Results

Tests I to VIII were used to optimise the assay. The details are recorded in lab notebooks AVC 0026 and AVC 0027.
Tests IX to XV were used for validation. The details and raw data are recorded in lab notebook AVC 0027.
The processed data is shown in the table in Appendix 1.
A short overview of these tests is listed below.

IX First test according to the optimised method.
Trivalent vaccine. Standard curve in buffer.

X Trivalent vaccine. Standard curve in buffer. Concentrations of DNA changed to 0, 0.5, 1.0 and 1.5 ng / ml.

XI Trivalent vaccine and the 3 monovalent vaccines. Standard curve.

XII Trivalent vaccine and the 3 monovalent vaccines. Standard curve. Concentrations of DNA: 0, 0.25, 0.5, 1.0 and 1.5 ng / ml. The lower assay limit of 0.25 ng / ml was added.

XIII Repeat of test XII.

XIV Only the monovalent vaccines. These were only tested at concentrations of 0, 0.25 and 1.0 ng / ml. Standard curve as before.

XV Monovalent vaccines and standard tested at assay concentrations.

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 23.318.825

The results of the tests X to XV attached in Appendix 1 are summarized below.

Added DNA in ng/ml vaccine/buffer	*Average spot intensity*					
	X	XI	XII	XIII	XIV	XV
Trivalent 0	0	11	0	12	-	-
Trivalent 0.25	-	-	30	30	-	-
Trivalent 0.5	71.5	50	70.5	53.5	-	-
Trivalent 1.0	120	98	110	50	-	-
Trivalent 1.5	138	117.5	129	71	-	-
Type 1 0	-	10	0	13.5	-	-
Type 1 0.25	-	-	34.5	2.5	28.6	24.8
Type 1 0.5	-	51	68.5	51.5	-	-
Type 1 1.0	-	88.5	92.5	68	91	70
Type 1 1.5	-	110	115.5	88.5	-	-
Type 2 0	-	15.5	0	12	-	-
Type 2 0.25	-	-	33.5	36	38.6	42
Type 2 0.5	-	60	70	54.5	-	-
Type 2 1.0	-	71.5	95	76	99.5	78.6
Type 2 1.5	-	108	122.5	104.5	-	-
Type 3 0	-	9.5	0	14	-	-
Type 3 0.25	-	-	33.5	37	44.6	40.78
Type 3 0.5	-	57.5	64.5	46.5	-	-
Type 3 1.0	-	106.5	92	65.5	108	84
Type 3 1.5	-	115.5	124.5	86	-	-
Buffer 0	14.75	9	0	13	12	-
Buffer 0.25		-	49	30	37.4	26.5
Buffer 0.5	69.75	57	81	50.5	63	-
Buffer 1.0	103.3	83	97	64.5	85.4	59.2
Buffer 1.5	123.3	112	128.5	67	112.4	-

CAIF SA
Dra. Bernarda Belay
Co-Directors Tecnica
M.N. 15.146

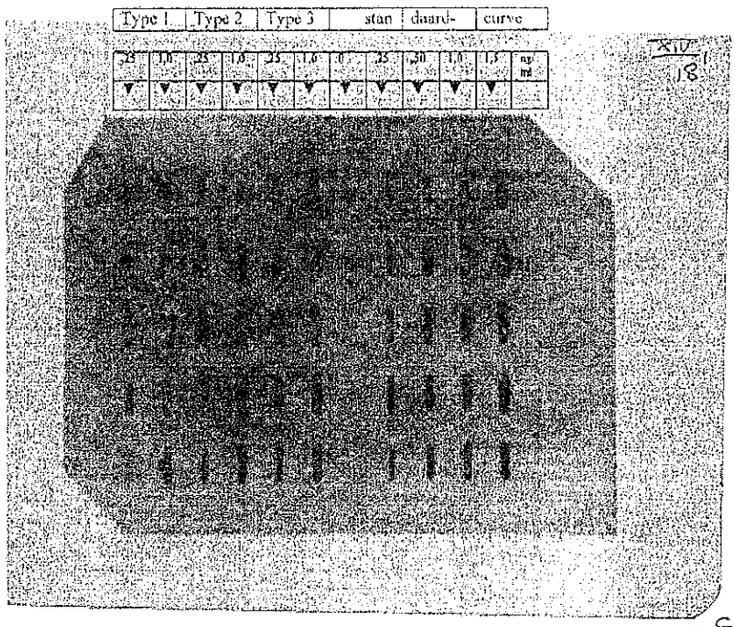
CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
DNI 20.376.425

3.1 Specificity

The specificity is proven in the table above: if the spot intensity of DNA in buffer is compared with that of DNA in vaccine, it is clear that the DNA can be detected independently of the matrix. The test controls containing buffer or vaccine without DNA give a result that is either not detectable or very low. In addition, there is no significant difference between the different types of vaccine. This is clearly visible on the X-ray films from the blots. The blot from test XIV is shown below as an example.

[Blot from test XIV]

Blot van test XIV



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

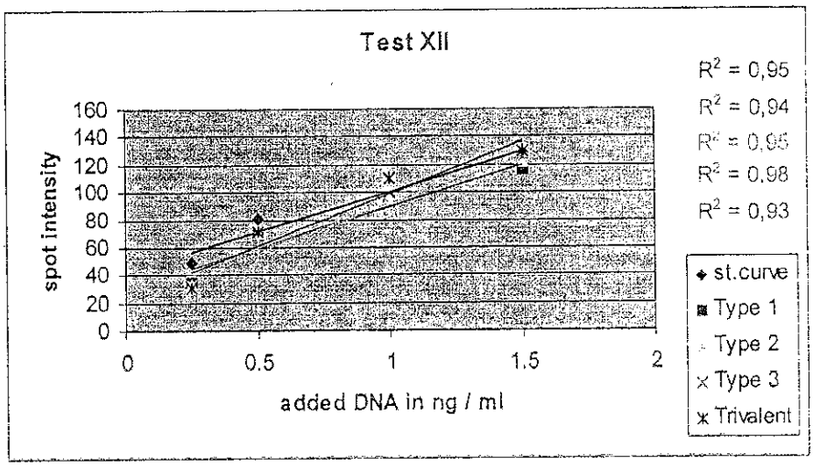
CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 23.376.735

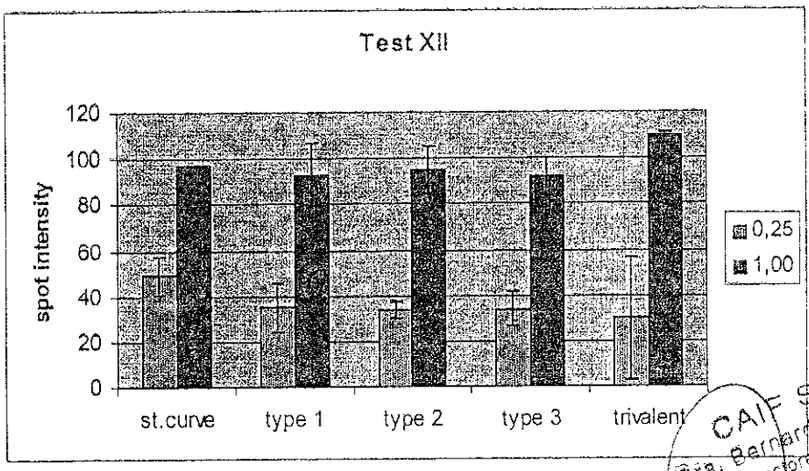
3.2 Linearity

For data see Appendix 1.
The relationship between the concentration of DNA and the intensity of the spot on the film is linear between 0.25 ng DNA / ml and 1.5 ng DNA / ml for both the standard curve and the reference vaccines.
The correlation coefficient (R^2-value) is >0.9 in all cases.
The graph of the results of test XII is shown below as an example; see Appendices 2 to 5 for the graphs and corresponding R^2-values of all tests.

3.3 Assay limits (range)

The lower and higher assay limits previously used for the validation were 0.25 ng DNA / ml and 1.5 ng DNA / ml respectively. In this range there is a linear relationship between the DNA concentration and the spot intensity on the film. Because of this linearity, only the values 0 ng / ml, 0.25 ng / ml and 1.0 ng / ml (i.e. the maximum permitted value) are required when testing production samples.
The bar charts demonstrating a significant difference between the spot intensities of these results are shown in Appendices 3 to 5.
The graph from test XII is shown below as an example.

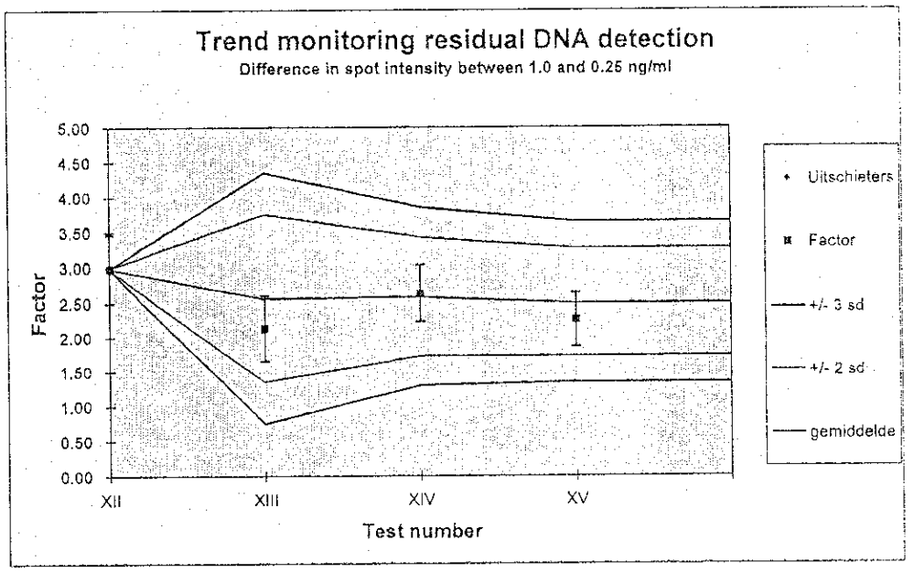




CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148

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

The ratio between the results for 1.0 ng DNA / ml and 0.25 ng DNA / ml is an indication of the reliability of the test implementation and of the linearity; it is also an indication monitor of the assay limits. This ratio shall continue to be determined in all tests to monitor the trend of the test. The results until now are shown below.



3.4 Detection limit

N/A

3.5 Quantification limit

N/A

3.6 Accuracy

N/A

3.7 Precision

3.7.1 Repeatability

In Appendix 1 the results of the test are found in the third column 'Spot intensity in 10^6 pixels / band'. Outliers are given in brackets. Outliers are found no more than once per fourfold sample, on average just once per blot.

The software connected to the scanner calculates a standard curve from the spot intensities of the standard DNA samples in buffer. This can be used to calculate the concentration of DNA in the band for each sample. In practice however, the spot intensities of the samples will simply be compared to those of the reference samples.

3.7.2 Intermediate precision

N/A

3.7.3 Reproducibility

N/A

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

CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 23378025

3.8 Robustness
N/A

3.9 Suitability of the test system
N/A

3.10 Suitable feasibility in the lab
N/A

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 151148
CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 25374925

4 Discussion

* The repeatability of the test is problematic, a consequence of the test complexity and interference due to other factors, namely the presence of proteins, large amounts of salts and possibly the formaldehyde. Further problems can be caused when every now and then a band on the blot deviates considerably from the rest. This occurs on average roughly once per blot. To compensate for this problem each test must be carried out at least 4 times. When the test is applied to production samples it may be necessary to use Grubb's test in excel to test for outliers after inspection of the X-ray film.
* Should 2 out of 4 samples have extreme values, then the sample should be tested again.
* The test is suitable for use on the 3 monovalent vaccines as well as the trivalent vaccine.
* The test is sensitive enough to detect the lower assay limit of 0.25 ng DNA / ml. The spot intensity is > 2x that of the test controls containing either buffer or vaccine without added DNA.
* The spot intensities of the samples + 1.0 ng DNA / ml is > 2x that of the samples + 0,25 ng DNA / ml. This is apparent from the trend monitoring, shown above in 4.3. The bar charts included in Appendices 3 to 5 also show that there is a significant difference between these values.
* Production samples can be analysed using the test in this form.

5 Revalidation

N/A

6 Conclusion

This validation demonstrates that the adapted version of the method Limiting test for Vero cell residual DNA, as described in SOP 17C-BIC-6, complies with the given criteria and can be released for use.

7 Appendices

APPENDIX 1	Data from tests IX to XV	Page 12
APPENDICES 2 to 5	Linear graphs and Bar charts	Pages 18-21

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

CAIF
Compañía Argentina de
Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 20238025

APPENDIX 1 DATA FROM TESTS IX TO XV

TEST IX 13-11-'02					
First test according to the optimised method. Trivalent vaccine only. Standard curve.					
Added DNA in ng/ml vaccine	*Added DNA in ng/ml buffer*	*Spot intensity in 10^6 pixels / band*	*Average spot intensity in 10^6 pixels / band*	*Calculated DNA in pg / 200 µl vaccine*	*Average DNA in ng/ml vaccine*
1.25	-	( ) ; 97	97	; 218	1.09
1.00	-	92 ; 86	89 +/- 4.2	209 ; 197	1.02
0.75	-	50 ; 51	50.5 +/- 0.7	126 ; 127	0.64
0.50	-	40 ; 41	40.5 +/- 0.7	106 ; 107	0.54
-	1.25	100 ; 109	104.5 +/- 6.4		
-	1.00	86 ; 100	93 +/- 9.9		
-	0.75	42 ; 63	52.5 +/- 14.8		
-	0.50	58 ; 42	50 +/- 11.3		

TEST X 27-11-'02					
Trivalent vaccine only. Standard curve.					
Added DNA in ng/ml vaccine	*Added DNA in ng/ml buffer*	*Spot intensity in 10^6 pixels / band*	*Average spot intensity in 10^6 pixels / band*	*Calculated DNA in pg / 200 µl vaccine*	*Average DNA in ng/ml vaccine*
0.5	-	80 ; 70 76 ; 60	71.5 +/- 8.7	153 ; 130 143 ; 108	0.67
1.0	-	126 ; 127 118 ; 110	120.25 +/- 7.9	270 ; 271 248 ; 228	1.27
1.5	-	123 ; 141 145 ; 140	138 +/- 8.3	261 ; 307 315 ; 303	1.49
-	0	17 ; 15 13 ; 14	14.75 +/- 1.7		
-	0.5	70 ; 74 72 ; 63	69.75 +/- 4.8		
-	1.0	- ; 90 104 ; 116	103.3 +/- 13.0		
-	1.5	141 ; 109 120 ; 141	123.3 +/- 13.3		

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 20329625

TEST XI 08-01-'03					
Trivalent and the 3 monovalent vaccines. Standard curve.					
Added DNA in ng/ml vaccine	*Added DNA in ng/ml buffer*	*Spot intensity in 10^6 pixels / band*	*Average spot intensity in 10^6 pixels / band*	*Calculated DNA in pg / 200 µl vaccine*	*Average DNA in ng/ml vaccine*
0 (mixture)	-	11 ; 11	11	0 ; 0	0
0.5 "	-	38 ; 62	50 +/- 17.0	73 ; 142	0.54
1.0 "	-	95 ; 101	98 +/- 4.2	237 ; 253	1.23
1.5 "	-	126 ; 109	117.5 +/- 12.0	326 ; 277	1.51
0 (type 1)	-	10 ; 10	10	0 ; 0	0
0.5 "	-	58 ; 44	51 +/- 9.9	130 ; 90	0.55
1.0 "	-	84 ; 93	88.5 +/- 6.4	204 ; 231	1.09
1.5 "	-	110 ; 110	110	279 ; 278	1.40
0 (type 2)	-	19 ; 12	15.5 +/- 4.9	18 ; 0	0.05
0.5 "	-	57 ; 63	60 +/- 4.2	128 ; 144	0.68
1.0 "	-	52 ; 91	71.5 +/- 27.6	111 ; 224	0.84
1.5 "	-	111 ; 105	108 +/- 4.2	281 ; 265	1.37
0 (type 3)	-	11 ; 8	9.5 +/- 2.1	0 ; 0	0
0.5 "	-	52 ; 63	57.5 +/- 7.8	112 ; 143	0.64
1.0 "	-	99 ; 114	106.5 +/- 10.6	247 ; 291	1.35
1.5 "	-	115 ; 116	115.5 +/- 0.7	292 ; 297	1.58
-	0	9 ; 9	9		
-	0.5	65 ; 49	57 +/- 11.3		
-	1.0	83 ; 83	83		
-	1.5	109 ; 115	112 +/- 4.2		

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 29276915

TEST XII 15-01-'03					
Trivalent and monovalent vaccines. Minimum detection limit of 0.25 ng / ml. Standard curve.					
Added DNA in ng/ml vaccine	*Added DNA in ng/ml buffer*	*Spot intensity in 10^6 pixels / band*	*Average spot intensity in 10^6 pixels / band*	*Calculated DNA in pg / 200 µl vaccine*	*Average DNA in ng/ml vaccine*
0 (mixture)	-	-	-	-	-
0.25 "	-	49 ; 11	30 +/- 26.9	51 ; 0	0.13
0.5 "	-	62 ; 79	70.5 +/- 12.0	89 ; 134	0.56
1.0 "	-	111 ; 109	110 +/- 1.4	224 ; 219	1.11
1.5 "	-	122 ; 136	129 +/- 9.9	253 ; 293	1.37
0 (type 1)	-	-	-	-	-
0.25 "	-	42 ; 27	34.5 +/- 10.6	32 ; 0	0.08
0.5 "	-	63 ; 74	68.5 +/- 7.8	91 ; 120	0.53
1.0 "	-	102 ; 83	92.5 +/- 13.4	200 ; 147	0.87
1.5 "	-	111 ; 120	115.5 +/- 6.4	223 ; 201	1.06
0 (type 2)	-	-	-	-	-
0.25 "	-	36 ; 31	33.5 +/- 3.5	16 ; 3	0.05
0.5 "	-	68 ; 72	70 +/- 2.8	103 ; 115	0.55
1.0 "	-	88 ; 102	95 +/- 9.9	161 ; 199	0.90
1.5 "	-	120 ; 125	122.5 +/- 3.5	248 ; 262	1.28
0 (type 3)	-	-	-	-	-
0.25 "	-	39 ; 28	33.5 +/- 7.8	24 ; 0	0.06
0.5 "	-	69 ; 60	64.5 +/- 6.4	108 ; 82	0.48
1.0 "	-	87 ; 97	92 +/- 7.1	158 ; 184	0.86
1.5 "	-	119 ; 130	124.5 +/- 7.8	245 ; 277	1.30
-	0	-	-		
-	0.25	43 ; 55	49 +/- 8.5		
-	0.5	103 ; 59	81 +/- 31.1		
-	1.0	97 ; 97	97		
-	1.5	123 ; 134	128.5 +/- 7.8		

Note: The test controls with 0 ng DNA / ml are visible to the naked eye but are too weak to be detected.

CAIF SA
Dra. Bernarda Beley
Co-Directora Tecnica
M.N. 15.146

CAIF
Compañía Argentina de
Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Beley
Apoderada
DNI 20370024

TEST XIII 22-01-'03					
Repeat of test XII					
Added DNA in ng/ml vaccine	*Added DNA in ng / ml* TEST *buffer*	*Spot intensity in 10^6 pixels / band*	*Average spot intensity in 10^6 pixels / band*	*Calculated DNA in pg / 200 µl vaccine*	*Average DNA in ng/ml vaccine*
0 (mixture)	-	(37) ; 12	12	; 0	0
0.25 "	-	33 ; 27	30 +/- 4.2	74 ; 46	0.30
0.5 "	-	52 ; 55	53.5 +/- 2.1	163 ; 176	0.85
1.0 "	-	37 ; 63	50 +/- 18.4	95 ; 214	0.77
1.5 "	-	57 ; 85	71 +/- 19.8	189 ; 316	1.26
0 (type 1)	-	14 ; 13	13.5 +/- 0.7	0 ; 0	0
0.25 "	-	26 ; 21	23.5 +/- 3.5	41 ; 21	0.16
0.5 "	-	53 ; 50	51.5 +/- 2.1	166 ; 154	0.80
1.0 "	-	64 ; 72	68 +/- 5.7	219 ; 255	1.19
1.5 "	-	85 ; 92	88.5 +/- 4.9	318 ; 349	1.67
0 (type 2)	-	12 ; 12	12	0 ; 0	0
0.25 "	-	32 ; 40	36 +/- 5.7	70 ; 107	0.44
0.5 "	-	57 ; 52	54.5 +/- 3.5	186 ; 160	0.87
1.0 "	-	77 ; 75	76 +/- 1.4	279 ; 273	1.38
1.5 "	-	118 ; 91	104.5 +/- 19.1	473 ; 344	2.04
0 (type 3)	-	13 ; 15	14 +/- 1.4	0 ; 0	0
0.25 "	-	35 ; 39	37 +/- 2.8	82 ; 103	0.47
0.5 "	-	44 ; 49	46.5 +/- 3.5	127 ; 150	0.69
1.0 "	-	63 ; 68	65.5 +/- 3.5	213 ; 237	1.13
1.5 "	-	92 ; 80	86 +/- 8.5	352 ; 294	1.62
-	0	13 ; 13	13		
-	0.25	30 ; 30	30		
-	0.5	49 ; 52	50.5 +/- 2.1		
-	1.0	65 ; 64	64.5 +/- 0.7		
-	1.5	59 ; 75	67 +/- 11.3		

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 20.139.945

TEST XIV 05-02-'03					
Monovalent vaccines only, tested at assay concentrations. Standard curve.					
Added DNA in ng/ml vaccine	*Added DNA in ng/ml buffer*	*Spot intensity in 10^6 pixels / band*	*Average spot intensity in 10^6 pixels / band*	*Calculated DNA in pg / 200 µl vaccine*	*Average DNA in ng/ml vaccine*
0.25 (type 1)	-	31; 33; 36; 26; 17	28.6 +/- 7.4	39; 45; 54; 25; (0)	0.21
1.0 "	-	105; 94; 91; 79; 86	91 +/- 9.7	255; 221; 213; 178; 199	1.07
0.25 (type 2)	-	46; 44; 43; 26; 34	38.6 +/- 8.4	84; 76; 76; 27; 48	0.31
1.0 "	-	(19); 107; 99; 101; 91	99.5 +/- 6.0	(4); 259; 238; 243; 215	1.20
0.25 (type 3)	-	50; 45; 46; 46; 36	44.6 +/- 5.2	95; 80; 83; 84; 53	0.40
1.0 "	-	97; 125; 116; 108; 94	108 +/- 12.9	230; 310; 287; 263; 221	1.31
-	0	15; 13; 12; 11; 9	12 +/- 2.2		
-	0.25	37; 40; 35; 39; 36	37.4 +/- 2.1		
-	0.5	59; 81; 57; 61; 57	63 +/- 10.2		
	1.0	81; 85; 99; 85; 77	85.4 +/- 8.3		
	1.5	110; 102; 121; 126; 103	112.4 +/- 10.7		

CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.146

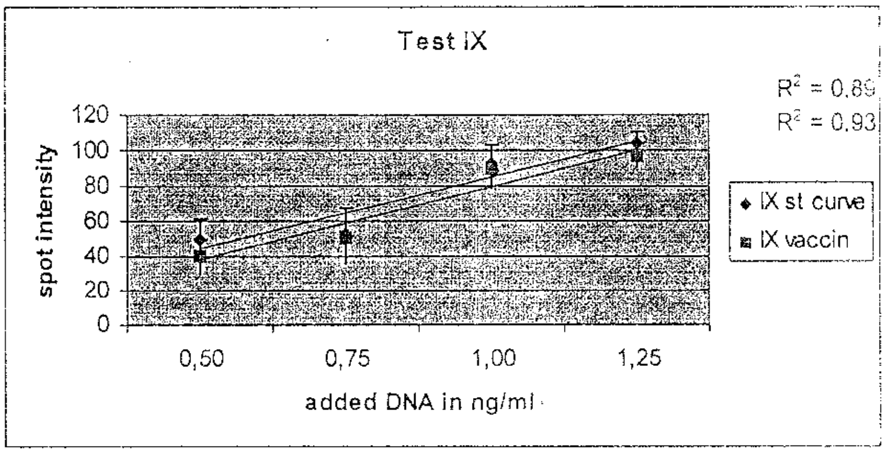
CAIF
Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 29178925

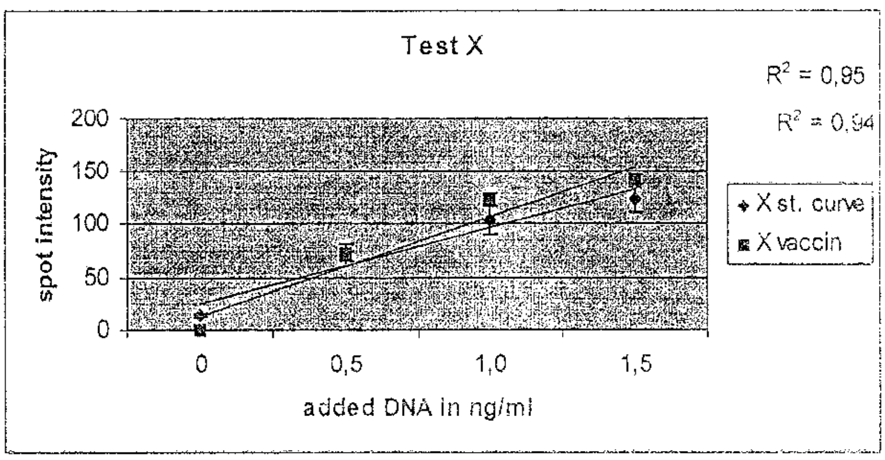
TEST XV 19-02-'03					
Assay concentrations only.					
Added DNA in ng/ml vaccine	*Added DNA in ng/ml buffer*	*Spot intensity in 10^6 pixels / band*	*Average spot intensity in 10^6 pixels / band*	*Calculated DNA in pg / 200 µl vaccine*	*Average DNA in ng/ml vaccine*
0.25 (type 1)	-	38; 20; 27; 14; 25	24.8 +/- 8.9	114; 63; 85; 47; 79	0.39
1.0 "	-	89; 69; 52; 68; 72	70 +/- 13.2	262; 204; 155; 201; 211	1.04
0.25 (type 2)	-	46; 42; 33; 38; 51	42 +/- 7.0	137; 125; 99; 115; 150	0.63
1.0 "	-	103; 85; 64; 75; 66	78.6 +/- 16.0	301; 250; 189; 221; 196	1.16
0.25 (type 3)	-	44; (15); 35; 45; 39	40.75 +/- 4.6	131;(39); 106; 134; 117	0.61
1.0 "	-	80; 105; 74; 78; 83	84 +/- 12.2	235; 306; 218; 230; 245	1.24
-	0	-	-		
-	0.25	44; 28; 15; 19; (12)	26.5 +/- 12.9		
-	1.0	76; 75; 42; 53; 50	59.2 +/- 15.4		

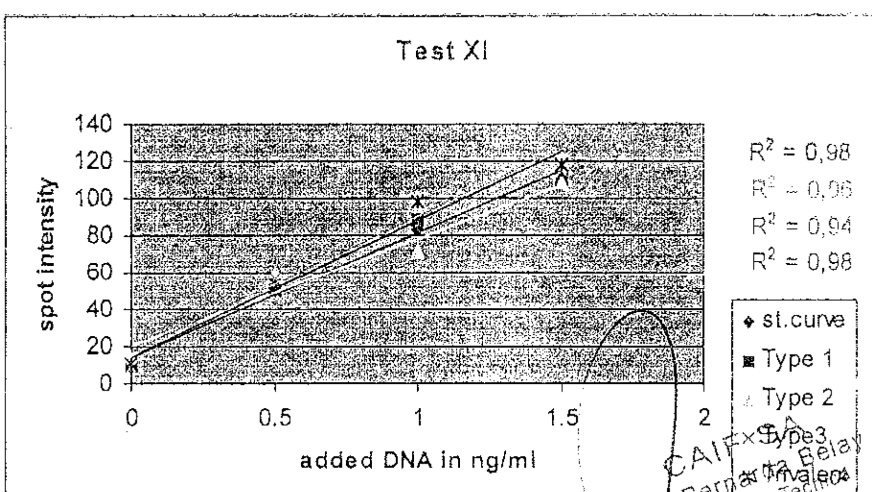
CAIF SA
Dra. Bernarda Belay
Co-Directora Tecnica
M.N. 15.148

Compañía Argentina de Investigaciones Farmacéuticas S.A.
Dra. María Bernarda Belay
Apoderada
DNI 20378025

APPENDIX 2   GRAPHS OF TESTS IX, X AND XI







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