



Product Quality and Disposition Department

The vaccines division of sanofi-aventis Group


Diphtheria, tetanus, pertussis (acellular, component),
 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
 influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)

197/211

 **

Bacterial and fungal sterility

Method	:	Membrane filtration	
Media	:	Thioglycollate medium	Soybean Casein Digest medium
Temperatures	:	30-35° C	20-25° C
Volume tested	:	20 ml	
Inoculum per Steritest	:	10 ml	10 ml
Volume of medium per Steritest	:	100 ml	100 ml
Test on	:	24.10.08	
Test off	:	07.11.08	
Result	:	No microbial growth - Conforms	


 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.


 CHRISTIAN DOMÍNGUEZ
 SUPERVISOR
 SANOFI PASTEUR S.A.

Product Quality and Disposition Department

Diphtheria, tetanus, pertussis (acellular, component),
hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)

198/211

**

PART 2
FINAL BULK PRODUCT


ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.


CHRISTIAN DUMITRESCU
GERENTE
SANOFI PASTEUR S.A.



Product Quality and Disposition Department


Diphtheria, tetanus, pertussis (acellular, component),
 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
 influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)


199/211

**

FINAL BULK

TESTS	SPECIFICATIONS
OSMOLALITY	300 - 400 mosmol/kg
FREE FORMALDEHYDE CONTENT	≤30 µg/mL
BACTERIAL AND FUNGAL STERILITY	No microbial growth
HISTAMINE-SENSITIZING ACTIVITY	≥95% survival
NON ADSORBED POLYRIBOSYL RIBITOL PHOSPHATE (PRP)	≥8.0 µg/dose
DEPOLYMERIZED PRP	<20.0%
PERCENT ADSORPTION – DIPHTHERIA TOXOID	≥23% At time of release, this acceptance criterion was not defined
PERCENT ADSORPTION – HEPATITIS B	≥64% At time of release, this acceptance criterion was not defined
DIPHTHERIA POTENCY	Activity ≥30 IU/dose Lower confidence limit (P = 0.95) of the estimated potency ≥ 20 IU/dose
TETANUS POTENCY	Lower confidence limit (P = 0.95) of the estimated potency ≥ 40 IU/dose
IMMUNOGENICITY PERTUSSIS IN MICE	Anti-PT and anti-FHA antibody GMTs for the vaccine are not significantly lower than those of the reference vaccine (analysis of variance) at the level 5%.
D-ANTIGEN CONTENT	TYPE 1 : 20.0 – 43.0 DU/DOSE TYPE 2 : 5.0 – 9.0 DU/DOSE TYPE 3 : 17.0 – 36.0 DU/DOSE
HEPATITIS B IN VITRO RELATIVE POTENCY (IVRP)	≥0.70 At time of release, this acceptance criterion was not defined


 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.


 CHRISTIAN DUMITRESCU
 SUPERVISOR
 SANOFI PASTEUR S.A.

11

12



sanofi pasteur

Product Quality and Disposition Department

The vaccines division of sanofi-aventis Group

Diphtheria, tetanus, pertussis (acellular, component),
 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
 influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)

200/211

**

FINAL BULK
 Lot number : FDV01420

Date of manufacture : 02.12.2009
 Storage temperature : + 5°C ± 3°C
 Expiry date : 02 September 2010

Composition

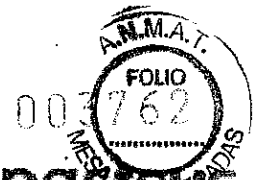
INGREDIENT	Lot number	Volume (ml)
Purified Diphtheria Toxoid	FA320479	2 728
Purified Tetanus Toxoid	FA309471	1 112
Adsorbed Pertussis Toxoid	FA326714	18 554
Adsorbed FHA Pertussis	FA360425	17 278
Concentrated Polio Trivalent (5C)	FA331636	50 000
Hepatitis B Surface Antigene C	AC005	1 724
	AC013	1 656
Haemophilus Polysaccharide Conjugate	FA327148	16 155
	FA328210	15 000
Aluminium hydroxide	FA367904	35 500
Buffer solution (Disodium hydrogen phosphate and potassium dihydrogen phosphate)	FA367891	20 000
Essential Amino acids solution	FA367893	25 000
Buffer solution (trometamol and saccharose)	FA368886	6 269
Water for injections	ABS1E335A	35 543
	ABS1E336A	
APPROXIMATE TOTAL VOLUME		242 800

352_CC5_SPI_AE.dot /19.09.11

ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.

CHRISTIAN DUMINIL
 APODERADO
 SANOFI PASTEUR S.A.





sanofi pasteur

The vaccines division of sanofi-aventis Group

Product Quality and Disposition Department

Diphtheria, tetanus, pertussis (acellular, component),
hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
Influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)

201/211

**

CONTROLS :

Osmolality

Method : Measure of the freezing point reduction
Date : 15.12.2009
Result : 336 mosmol/kg - Conforms

Free formaldehyde content

Method : Spectrophotometry / colorimetric assay
Date : 30.12.2009
Result : 1.36 µg/ml - Conforms

Bacterial and fungal sterility

Method	:	Membrane filtration
Media	:	Thioglycollate medium Soybean Casein Digest medium
Temperatures	:	30-35° C 20-25° C
Volume tested	:	20 ml
Inoculum per Steritest	:	10 ml 10 ml
Volume of medium per Steritest	:	100 ml 100 ml
Test on	:	11.12.2009
Test off	:	28.12.2009
Result	:	No microbial growth - Conforms

Histamine-sensitizing activity

Injection (Day 0)

Animals and sex : mice CD1 - Female
Number of animals : 20
Route : Intraperitoneal
Dose tested per mouse : 2 human doses (1ml)
Date of injection : 22.01.2010


Histamine challenge (Day 5)


Challenge dose per mouse : 2 mg histamine base
Route : Intraperitoneal
Date of injection : 27.01.2010

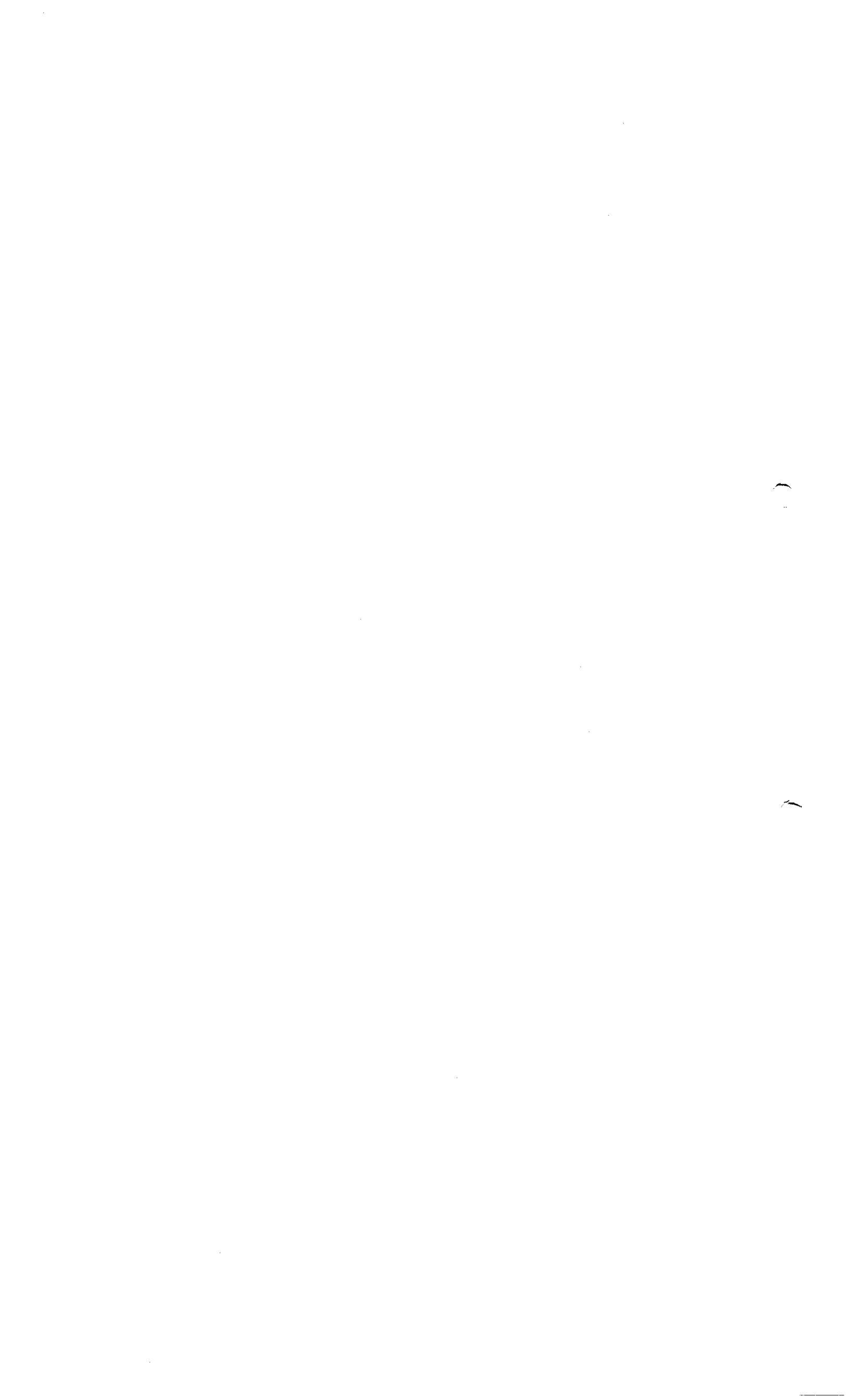
Reading date (Day 6)

Result : 28.01.2010
: 100 % of survival - Conforms

352_CC5_SPL_AE.dot /19.09.11


 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.


 CHRISTIAN DOMINGUEZ
 GERENTE
 SANOFI PASTEUR S.A.





sanofi pasteur

The vaccines division of sanofi-aventis Group

Product Quality and Disposition Department

Diphtheria, tetanus, pertussis (acellular, component),
hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)

202/211

Polysaccharide content :



Method	: High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection method (HPAEC-PAD)
Standard	: CP09E055
Date	: 10.12.2009
Non adsorbed PRP	: 10.5 µg/dose
Depolymerized PRP	: 7.3 % - Conforms

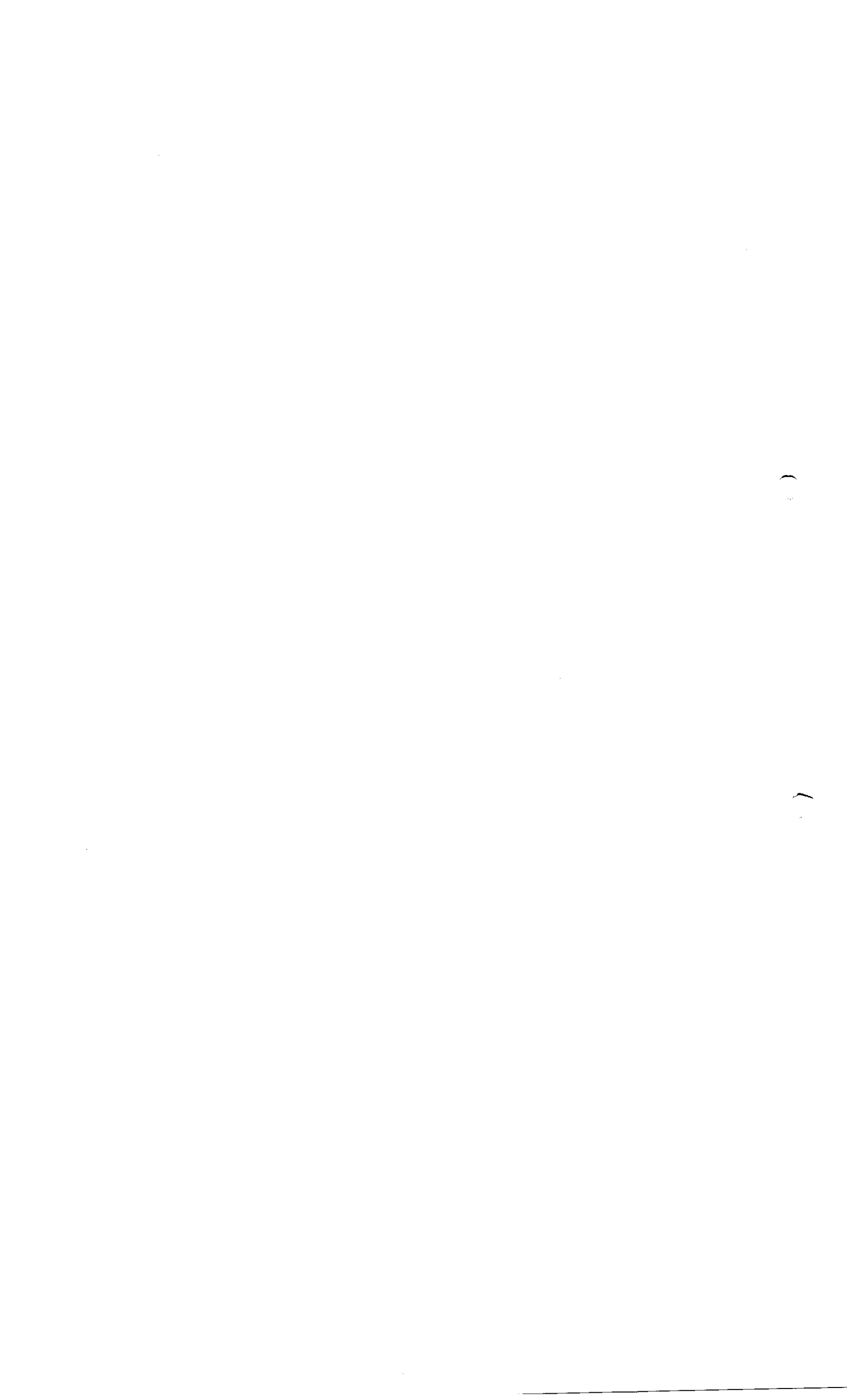
Percent adsorption – Diphtheria toxoid:

Method	: Rocket Immunoelectrophoresis method
Date	: 17.12.2009
Result	: 49 %

Percent adsorption – Hepatitis B:

Method	: ELISA
Date	: 10.12.2009
Result	: 98 %

	
ROXANA MONTEMILONE DIRECTORA TÉCNICA SANOFI PASTEUR S.A.	CHRISTIAN DUNNE APODERADO SANOFI PASTEUR S.A.





Product Quality and Disposition Department

Diphtheria, tetanus, pertussis (acellular, component), hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch) 203/211

**

Diphtheria potency

Method : European Pharmacopoeia
Strain and sex of animals : Guinea pigs - Dunkin-Hartley - female
Weight : 250 - 350 g
Number of animals per dose : 8

Immunization



Date : 08.12.2009
Volume tested : 1 ml
Route : Subcutaneous

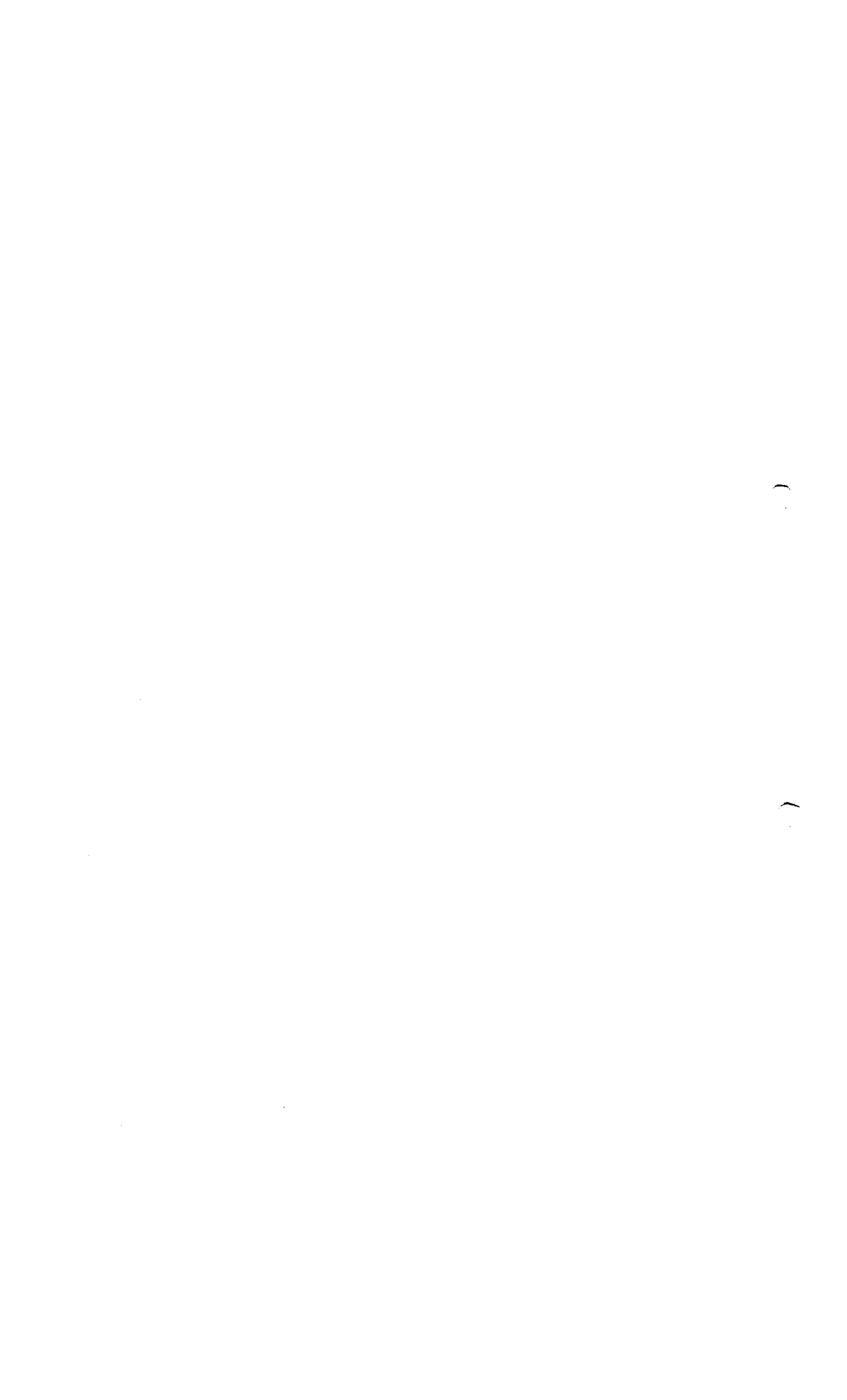
Challenge

Date : 05.01.2010
Challenge dose : 0.0512 - 0.0128 - 0.0032 - 0.0008
0.0002 and 0.00005 Lf/0.2 ml
Route : Intradermal
Reading date : 07.01.2010

Results	Dilutions	Score	Log ED ₅₀
Reference toxoid BRP - D4 97 IU/ml	1/16	31/48	- 1.514
	1/32	29/48	
	1/64	18/48	
	1/128	5/48	
Product tested FDV01420	1/17.5	34/48	- 1.711
	1/35	27/48	
	1/70	24/48	
	1/140	12/42	

Potency : 76 IU/dose
Confidence limits (P=0.95) : 57 - 113 IU/dose


 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.

 CHRISTIAN DUMINEZ
 SUPERADO
 SANOFI PASTEUR S.A.



Product Quality and Disposition Department

**Diphtheria, tetanus, pertussis (acellular, component),
 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
 influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)**

204/211

Tetanus potency

Method : European Pharmacopoeia
 Strain and sex of animals : Mice Swiss OF1 - Female
 Age : 5 weeks
 Number of animals per dose : 16

Immunization

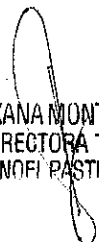
Date : 07.12.2009
 Volume : 0.5 ml
 Route : Subcutaneous


Challenge

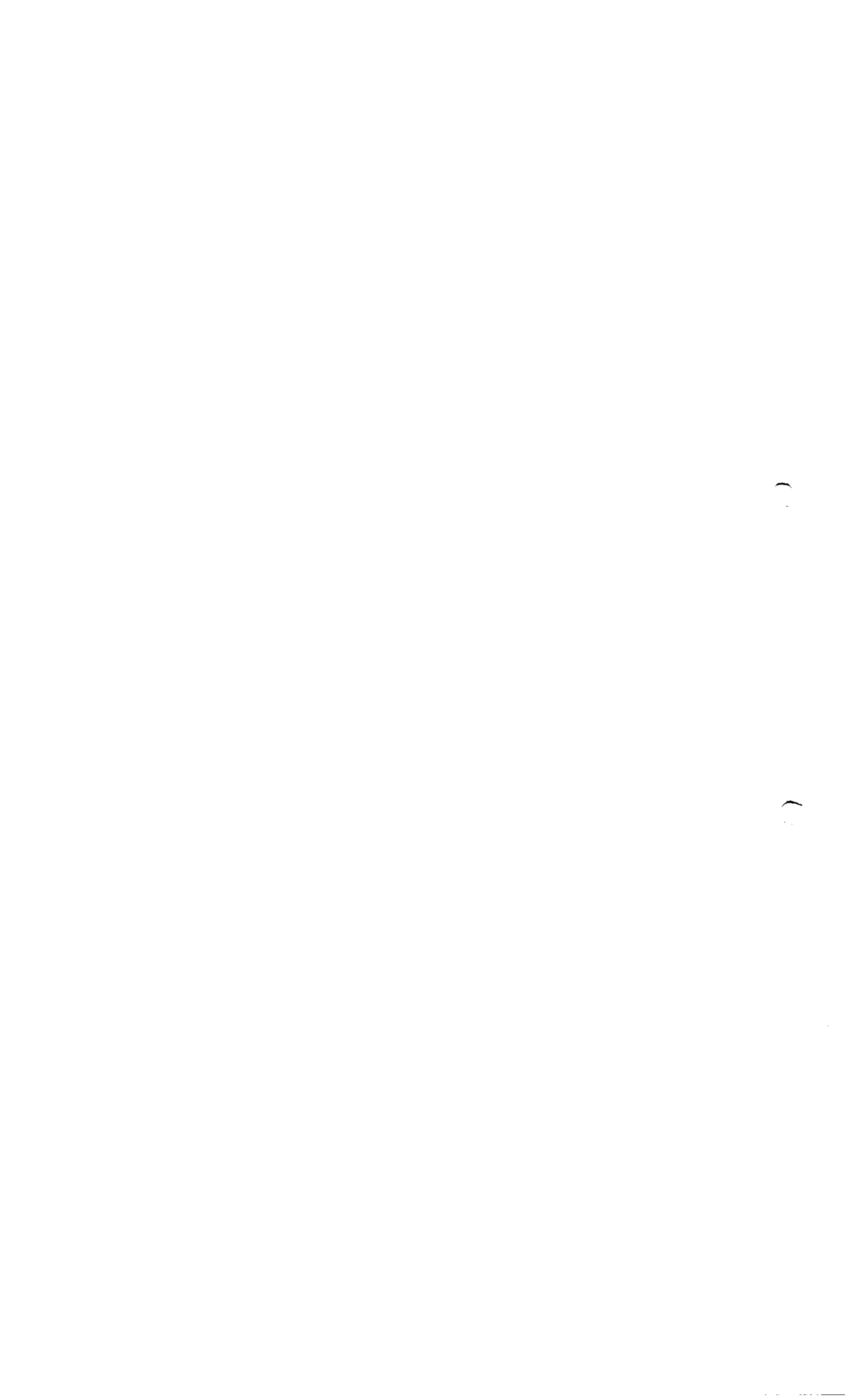
Date : 04.01.2010
 Challenge dose : 1/320
 Volume : 0.5 ml
 Route : Subcutaneous
 Reading date : 08.01.2010

Results	Dilutions	Score/Total	Log ED ₆₀
Reference toxoid NIBSC-T 98/552 250 IU/ml	1/25	16/16	- 2.009
	1/50	15/16	
	1/100	7/16	
	1/200	2/16	
Product tested FDV01420	1/150	16/16	- 2.760
	1/300	10/16	
	1/600	9/16	
	1/1200	4/16	

Potency : 705 IU/dose
 Confidence limits (P=0.95) : 485 – 1 017 IU/dose


 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.


 CHRISTIAN DUMINGUÉZ
 SUPERADU
 SANOFI PASTEUR S.A.



Product Quality and Disposition Department

Diphtheria, tetanus, pertussis (acellular, component),
 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
 influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)

205/211

**

Immunogenicity Pertussis in mice

Animals and sex : Mice Swiss OF1- Female
 Weight : 20 - 22 g
 Number of animals per group : 10

Immunization

Date : 11.12.2009
 Volume : 0.5 ml
 Route : Intraperitoneal
 Reference vaccine : PFAGI003-03
 Dose per mouse : 1/5 human dose

Bleeding date : 08.01.2010

Titration

1. Anti-PT titers

Method : ELISA
 Pertussis toxin for coating : FA287985
 Standard anti-PT serum : BRP BATCH1
 Date : 26.01.2010
 Results
 Reference vaccine : 269 EU/ml (196 - 367)
 Product tested : 219 EU/ml (133 - 361)
 Probability : 0.452 (p >0.05)

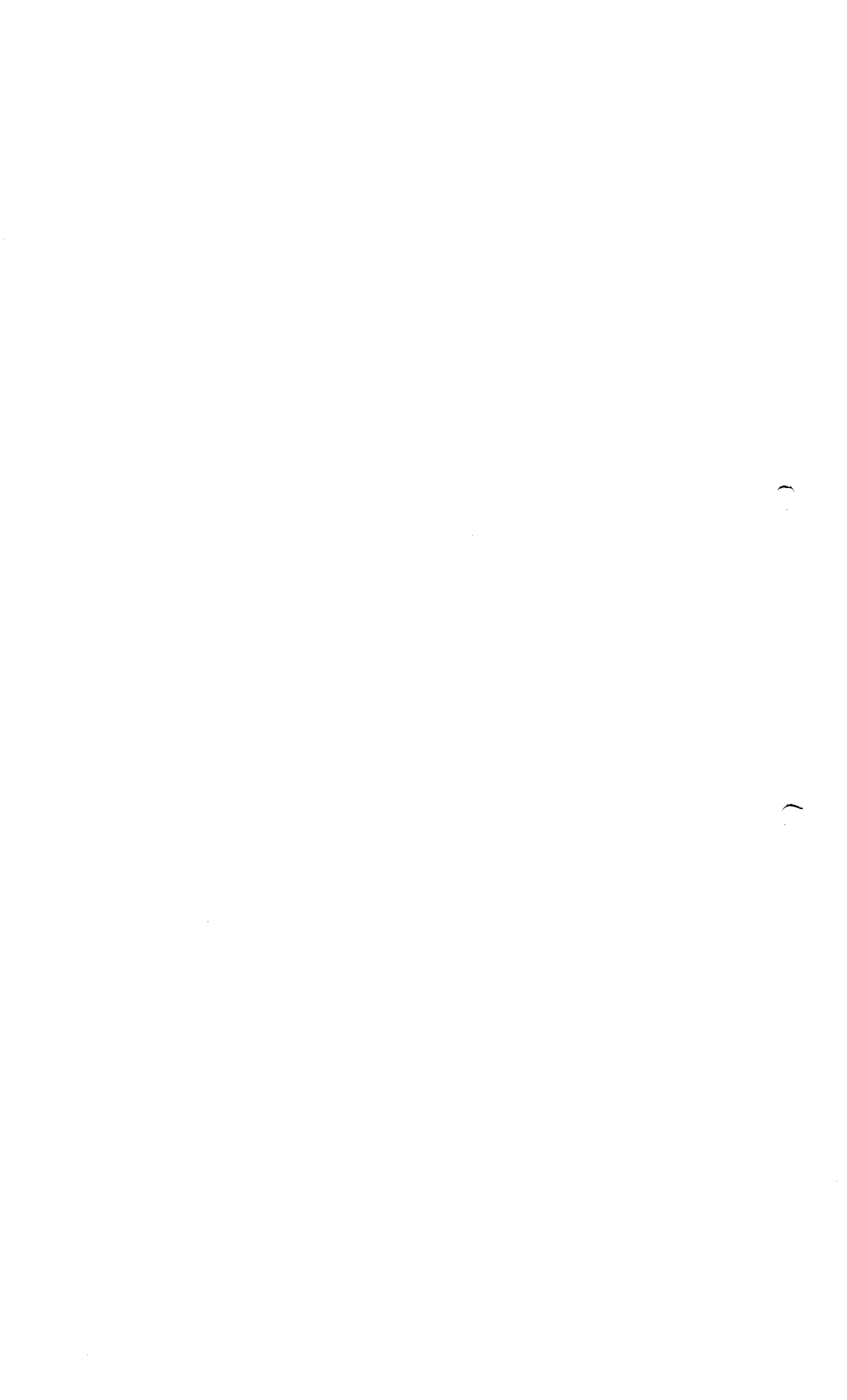
2. Anti-FHA titers

Method : ELISA
 FHA for coating : FA088651
 Standard anti-FHA serum : BRP BATCH1
 Date : 02.02.2010
 Results
 Reference vaccine : 206 EU/ml (130 - 328)
 Product tested : 257 EU/ml (134 - 496)
 Probability : >0.50 (p >0.05)

Conclusion : Conforms


 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.


 CHRISTIAN DOMINGUEZ
 SUPERVISOR
 SANOFI PASTEUR S.A.



Product Quality and Disposition Department

Diphtheria, tetanus, pertussis (acellular, component),
 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
 influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)

206/211

D Antigen content

Method : ELISA
 Standard : 11.07.07 versus EWS
 Date : 12.01.2010

	D Antigen unit/dose		
	Type 1	Type 2	Type 3
Product tested	28.1	5.8	24.6

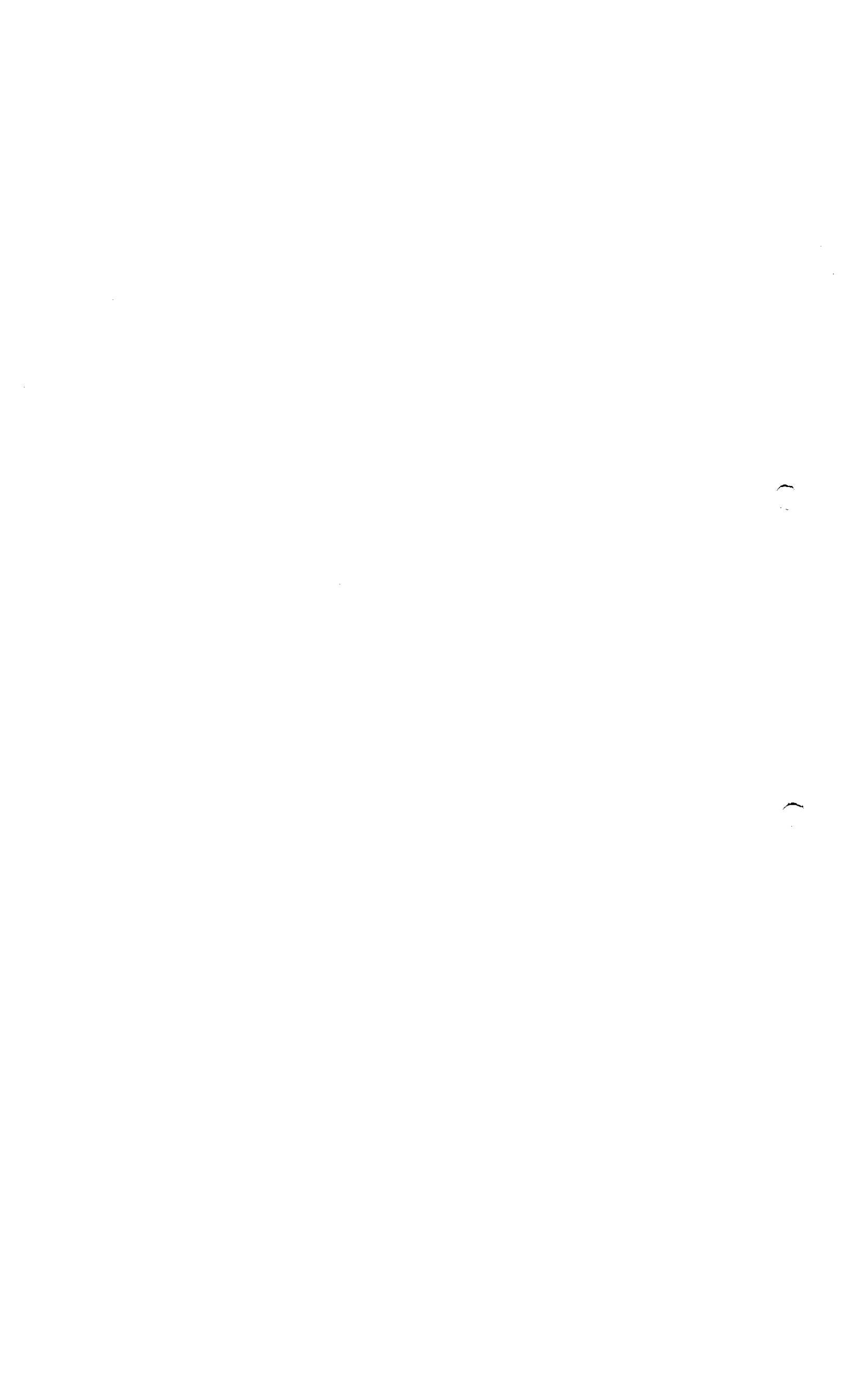
Result : Conforms

Hepatitis B in vitro Relative Potency

Method : ELISA
 Date : 10.12.2009
 Result : Conforms


 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.


 CHRISTIAN DOMINGUEZ
 (APODERADO)
 SANOFI PASTEUR S.A.



Product Quality and Disposition Department

Diphtheria, tetanus, pertussis (acellular, component),
hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)

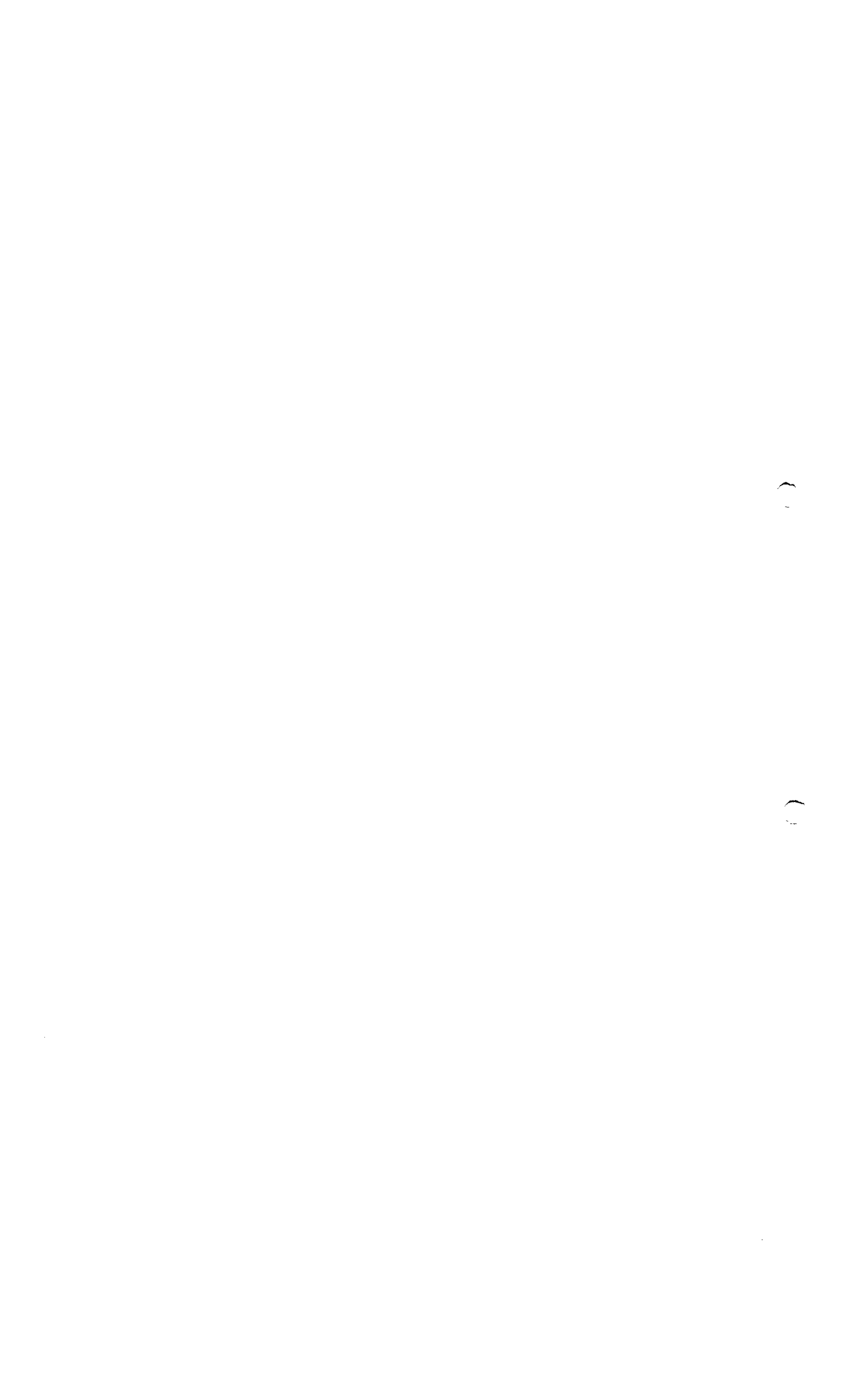
207/211

**

PART 3
FINAL PRODUCT


ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.


CHRISTIAN DOMÍNGUEZ
SUPERADO
SANOFI PASTEUR S.A.





Product Quality and Disposition Department

Diphtheria, tetanus, pertussis (acellular, component),
 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
 Influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)


208/211


**

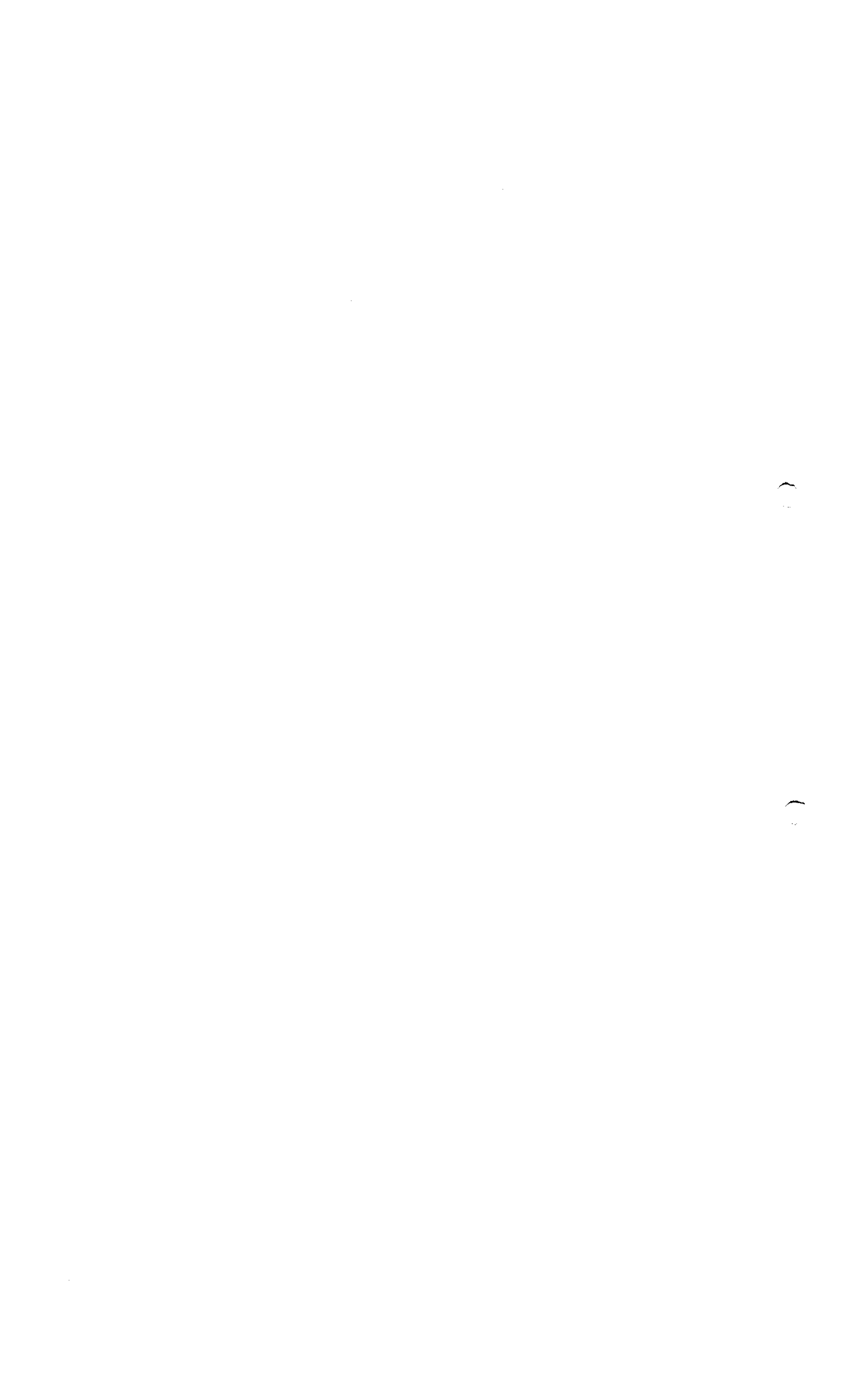
FINAL PRODUCT

TESTS	SPECIFICATIONS
APPEARANCE	Whitish and cloudy suspension
pH	6.5 - 7.5
EXTRACTABLE VOLUME	At least the nominal volume
ALUMINIUM CONTENT	0.40 - 0.80 mg/dose
BACTERIAL AND FUNGAL STERILITY	No microbial growth
PYROGEN TEST	Conforms to Ph. Eur. criterion
ABNORMAL TOXICITY TEST	No death nor sickness sign At time of release, the test was performed
IDENTITY OF EACH VALENCE : DIPHTHERIA, TETANUS, PERTUSSIS, POLIOMYELITIS, HEPATITIS B, HAEMOPHILUS	Positive

352_CC5_SPL_AE.dot /19.09.11


 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.


 CHRISTIAN DOMÍNGUEZ
 FEDERADO
 SANOFI PASTEUR S.A.





sanofi pasteur

The vaccines division of sanofi-aventis Group

Product Quality and Disposition Department

Diphtheria, tetanus, pertussis (acellular, component),
hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)

209/211

**

FINAL PRODUCT
Lot number : S4313

Date of manufacture: 10.02.2010

CONTROLS :

Appearance

Method : Visual inspection
Date : 11.03.2010
Result : Conforms

pH

Method : Glass electrode
Date : 17.03.2010
Result : 7.20 - Conforms

Extractable Volume

Method : European Pharmacopoeia
Date : 15.03.2010
Result : Conforms

Aluminium content

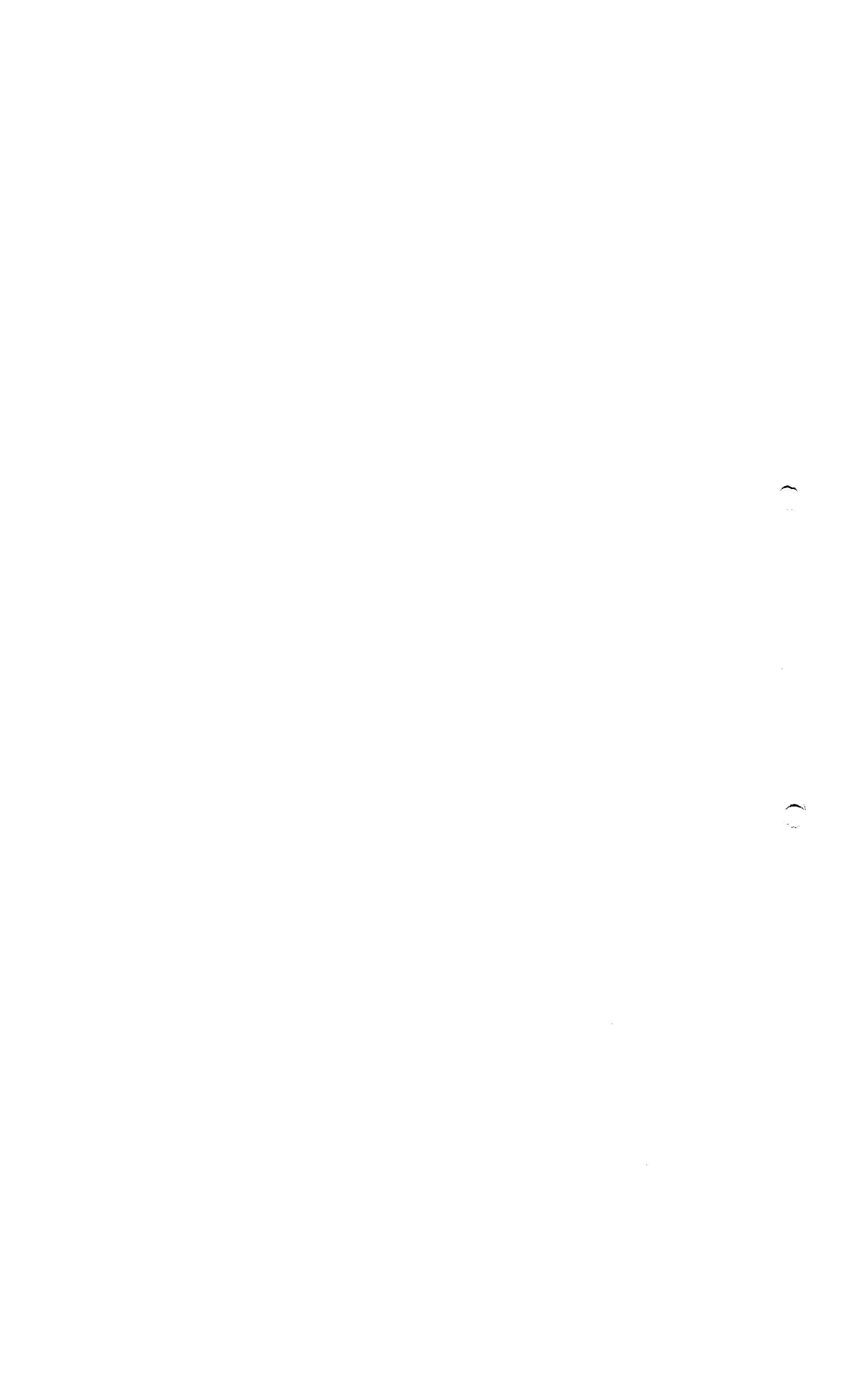
Method : Complexometry
Date : 18.03.2010
Result : 0.62 mg/dose - Conforms

Bacterial and fungal sterility

Method	:	Membrane filtration
Media	:	Thioglycollate medium Soybean-Casein Digest medium
Temperature	:	30-35° C 20-25° C
Number of units tested	:	40
Inoculum per Steritest	:	10 ml 10 ml
Volume of medium per Steritest	:	100 ml 100 ml
Test on	:	17.02.2010
Test off	:	03.03.2010
Result	:	No microbial growth - Conforms

HUXANA MONTEMLONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.

CHRISTIAN LUMINGUE
ENCUERADO
SANOFI PASTEUR S.A.



Product Quality and Disposition Department

Diphtheria, tetanus, pertussis (acellular, component),
 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
 influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch) 210/211

 **

Pyrogen test

Method : by intravenous injection 0.1 µg/ml/kg of the rabbit body weight
 Date : 11.03.2010
 Number of rabbits : 3

Weight of each rabbits (kg)	Initial temperature in °C (A)	Intravenous dose (ml)	Maximum temperature in °C (B)	Response (B) - (A)
2.5	39.24	0.5	39.45	0.22
2.5	39.13	0.5	39.22	0.10
2.7	39.13	0.5	39.26	0.13


The sum of the 3 individual maximum temperature rises : 0.44 °C
 Result : Conforms


Diphtheria, Tetanus, Pertussis (FHA and Pertussis toxoid), Poliomyelitis, Hepatitis B and Haemophilus identity

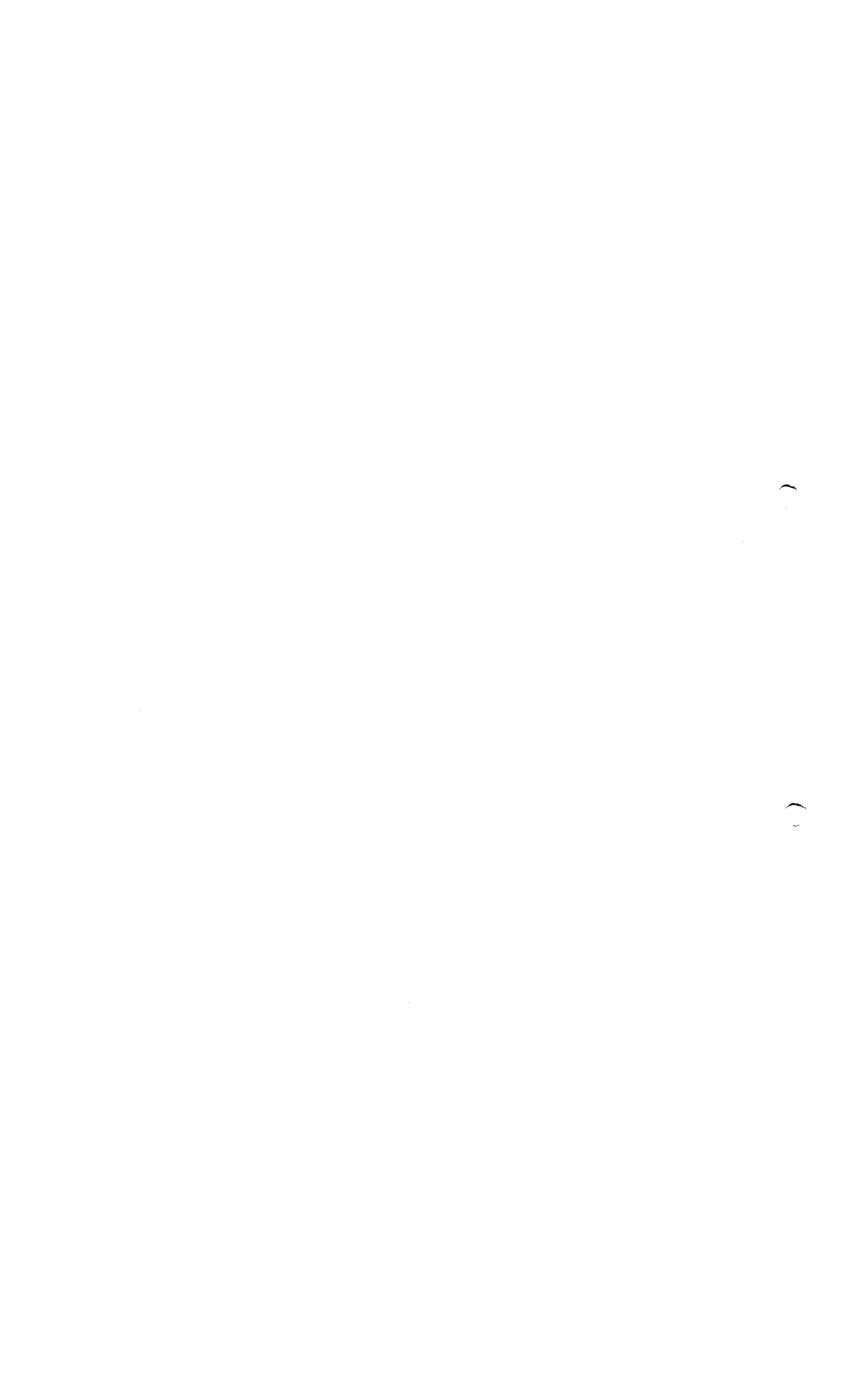
Method : Luminex
 Date : 08.04.2010 and 09.04.2010
 Result : Positive - Conforms

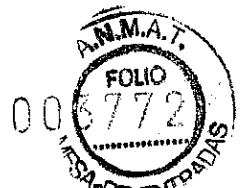
Abnormal toxicity

Animals	:	Mice	Guinea pigs
Number of animals	:	5	2
Volume injected	:	0.5 ml	5 ml
Route of injection	:	Intraperitoneal	
Test on	:	08.03.10	
Test off	:	15.03.10	
Result	:	No death nor sickness sign - Conforms	


 ROXANA MONTMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.


 CHRISTIAN DOMÍNGUEZ
 GERENTE
 SANOFI PASTEUR S.A.





sanofi pasteur

The vaccines division of sanofi-aventis Group

Product Quality and Disposition Department

Diphtheria, tetanus, pertussis (acellular, component),
hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus

influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)

210/210

MANUFACTURER' S CERTIFICATION

I herewith certify that **DIPHTHERIA, TETANUS, PERTUSSIS (ACELLULAR, COMPONENT), HEPATITIS B (RDNA), POLIOMYELITIS (INACTIVATED) AND HAEMOPHILUS INFLUENZAE TYPE B CONJUGATE VACCINE (ADSORBED)**, batch number **S4314**, was manufactured and tested according to cGMP and was submitted in the frame of the CTD submission via the Article 58 of regulation N°726/2004 in July 2011, procedure number : EMEA/H/W/002495.

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 Directive 2001/83/EC and amending directives 2003/63/EC and 2004/27/EC .

GENERAL CONCLUSION : Conforms

Date : 07 December 2011

Name :

A. PABIEU-SECURET

R&QA

Product Quality and Disposition Department

ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.

CHRISTIAN LUMINISQUE
GERENTE
SANOFI PASTEUR S.A.

