

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° S4312 (clinical batch) 166/229


HAEMOPHILUS INFLUENZAE
Working seed lot n° FA214905


Master seed Lot n° : 141098
Preparation date : 28.07.05
Release date : 25.10.05

CONTROLS

- Purity
Identification
- Morphology
 - Growth characteristics
 - Biochemical characteristics
 - Antigenic characteristics

The tests results show that working seed lot has the same characteristics as the master seed lot.


ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.


CHRISTIAN DOMÍNGUEZ
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° S4312 (clinical batch)

167/229

TETANUS TOXOID
 Strain : Clostridium tetani 1472-C

STRAIN



Name	: Harvard 49205 strain identified by the n°Y-IV-5A
Origin	: Rijks Instituut (RIV), Bilthoven Netherlands
Received	: 17.11.82
Institut Mérieux number	: IM 1472-C

TETANUS TOXOID
 Master seed lot n° 221091

Strain	: IM 1472-C
Preparation date	: 22.10.91
Release date	: 14.11.91

CONTROLS

- Purity
- Identification
- Morphology
 - Growth characteristics
 - Biochemical characteristics

 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° S4312 (clinical batch) 168/229



TETANUS TOXOID
Working seed lot n°FA123048

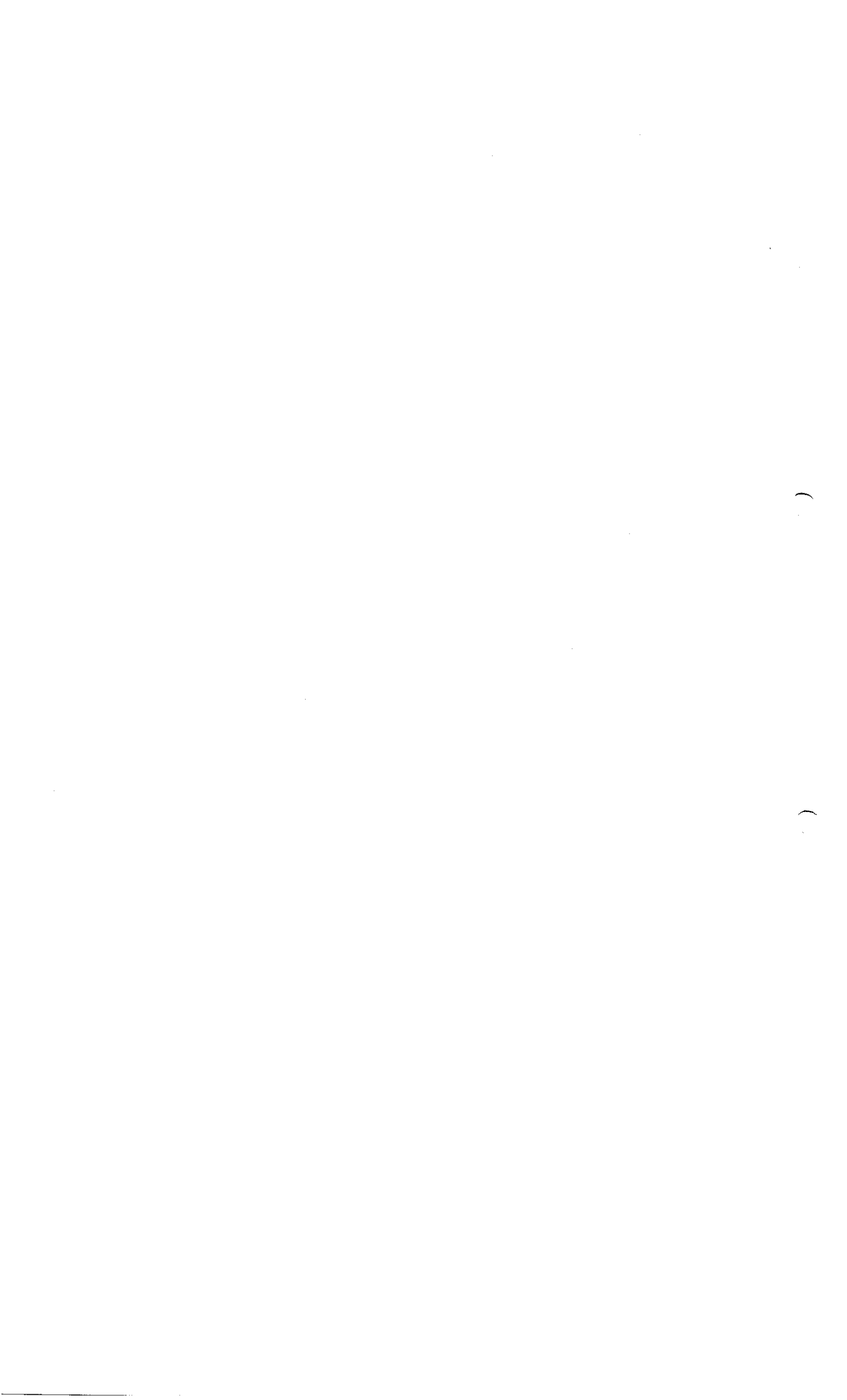
Master seed lot n°	: 221091
Preparation date	: 07.08.02
Release date	: 26.02.03

CONTROLS

- Purity
 Identification
- Morphology
 - Growth characteristics
 - Biochemical characteristics

The tests results show that working seed lot has the same characteristics as the Master seed lot.

 ROXANA MONTEMILONE DIRECTORA TÉCNICA SANOFI PASTEUR S.A.	 CHRISTIAN DOMÍNGUEZ SUPERVISOR 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° S4312 (clinical batch)

169/229


TETANUS TOXOID
Working seed lot n°FA229010


Master seed lot n° : 221091
Preparation date : 20.12.05
Release date : 19.01.09

CONTROLS

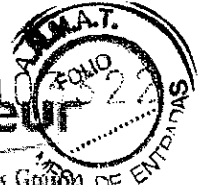
- Purity
- Identification
 - Morphology
 - Growth characteristics
 - Biochemical characteristics

The tests results show that working seed lot has the same characteristics as the Master seed lot.


 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.


 CHRISTIAN DOMÍNGUEZ
 COORDINADOR
 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° S4312 (clinical batch)

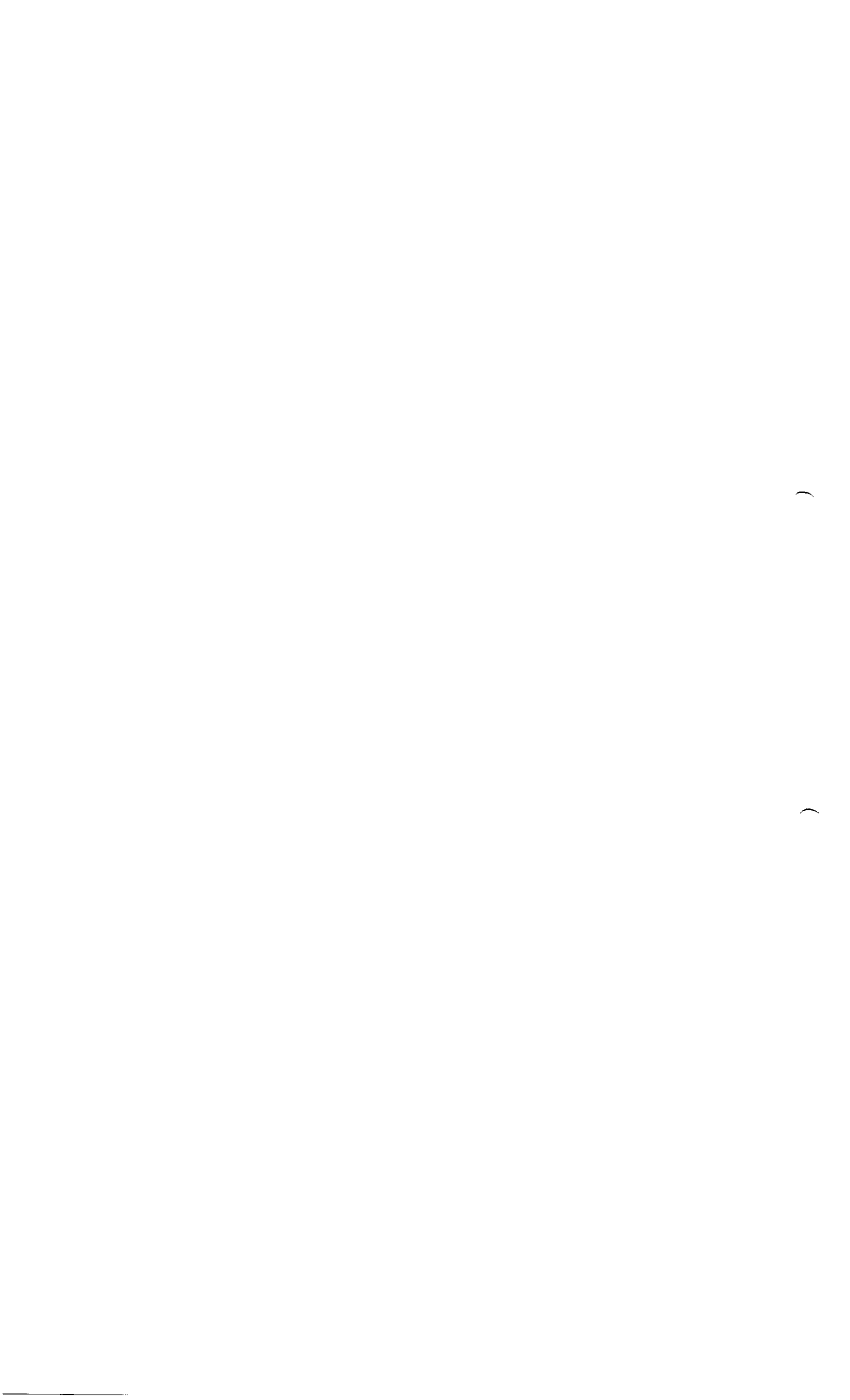
170/229

CONCENTRATED TETANUS PROTEIN

TESTS	SPECIFICATIONS
TETANUS PROTEIN	
PROTEIN NITROGEN CONTENT (PN)	For calculation
TOTAL NITROGEN CONTENT (TN)	For calculation
PN/TN RATIO	≥ 0.8
OD280/OD260 RATIO	≥ 1.3
FLOCCULATING TITRE	For calculation
ANTIGENIC PURITY	> 1500 Lf/mg of protein nitrogen
MOLECULAR SIZE DISTRIBUTION	≥ 50 % of monomers
BACTERIAL AND FUNGAL STERILITY	No microbial growth
CONCENTRATED TETANUS PROTEIN	
PROTEIN NITROGEN CONTENT	For calculation
OD280/OD260 RATIO	≥ 1.3
PHOSPHORUS CONTENT	< 1.5 µg/mg of protein
RESIDUAL FORMALDEHYDE CONTENT	< 10 µg/mg of protein
FLOCCULATING TITRE	For calculation
ANTIGENIC PURITY	> 1500 Lf/mg of protein nitrogen
MOLECULAR SIZE DISTRIBUTION	≥ 50 % of monomers
BACTERIAL AND FUNGAL STERILITY	No microbial growth
SPECIFIC TOXICITY	Conforms
IRREVERSIBILITY OF TETANUS TOXOID	Conforms
PYROGENS	Conforms

ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.

CHRISTIAN DOMÍNGUEZ
COORDINADOR
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° S4312 (clinical batch)

171/229

CONCENTRATED TETANUS PROTEIN
lot number : FA280431

Date of manufacture : 26.07.07
Storage temperature : + 5 °C ± 3°C
Expiry date : 26 JULY 2008
Volume : 1.910 liters

TETANUS PROTEIN CONTROLS :

Protein nitrogen content (PN)

Method : Kjeldahl after trichloroacetic acid precipitation
Date : 26.04.07
Result : 0.112 mg/ml

Total nitrogen content (TN)

Method : Kjeldahl
Date : 26.04.07
Result : 0.127 mg/ml

PN/TN ratio : 0.88 - Conforms

OD280/OD260 ratio : 1.83 - Conforms

Flocculating titre

Method : Flocculation determined from in-house reference
Date : 07.05.07
Result : 270 Lf/ml

Antigenic purity

Result : 2411 Lf/mg of protein nitrogen - **Conforms**

ROXANA MONTEMILONE 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° S4312 (clinical batch) 172/229

Molecular size distribution

Method : size exclusion Chromatography
Date : 22.05.07
Result : 69.5 % of monomers - 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 : 26.04.07
Test off : 10.05.07
Result : No microbial growth - Conforms

CONCENTRATED TETANUS PROTEIN CONTROLS :

Protein nitrogen content

Method : Kjeldahl after trichloroacetic acid precipitation
Date : 18.09.07
Result : 5.15 mg/ml

OD280/OD260 ratio : 1.91 - Conforms

Phosphorus content

Method : Chen
Date : 30.08.07
Result : <0.04 µg/mg of protein - Conforms

Residual formaldehyde content

Method : Nash
Date : 14.08.07
Result : 0.91 µg/mg of protein - Conforms

ROXANA MONTEMILONE DIRECTORA TÉCNICA SANOFI PASTEUR S.A.
CHRISTIAN DOMÍNGUEZ ANDERADO 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° S4312 (clinical batch)
173/229

Flocculating titre

Method : Flocculation determined from in-house reference
 Date : 01.08.07
 Result : 12000 Lf/ml

Antigenic purity

Result : 2330 Lf/mg of protein nitrogen - **Conforms**

Molecular size distribution

Method : size exclusion Chromatography
 Date : 06.09.07
 Result : 64.0 % of monomers -- **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	:	31.07.07	
Test off	:	14.08.07	
Result	:	No microbial growth - Conforms	

Specific toxicity test

Animals : Guinea-pigs
 Number of animals : 5
 Lf of toxoid injected per animal : 2000
 Route : Subcutaneous
 Test on : 09.08.07
 Test off : 30.08.07
 Result : **Conforms**



ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.



CHRISTIAN DOMÍNGUEZ
 MODERADO
 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° S4312 (clinical batch)** 174/229

Irreversibility of tetanus toxoid


Method	:	WHO method		Technical report N° 800 and 814
Concentration of the toxoid solution	:	15 Lf/ml		15 Lf/ml
Incubation period	:			
Temperatures	:	5°C ± 3°C		37°C ± 1°C
Test on	:		01.08.07	
Test off	:		12.09.07	
 <i>Test in animals</i>				
Number of guinea pigs injected	:	5		5
Route	:		Subcutaneous	
Volume injected	:	5 ml		5 ml
Date of inoculation	:		12.09.07	
Date of end of observation	:		03.10.07	
Result	:		Conforms	


Pyrogens

Method : by intravenous injection of 2 µg/ml/kg of the rabbit body weight
 Date : 31.10.07
 Number of rabbits : 3

Weight of each rabbit (kg)	Initial temperature in °C (A)	Intravenous dose (ml)	Maximum temperature in °C (B)	Response (B) - (A)
2.7	38.81	2.70	38.91	0.10
2.9	38.63	2.90	39.05	0.43
2.6	38.85	2.60	38.93	0.08

The sum of the 3 individual maximum temperature rises : 0.61 °C
 Result : Conforms


 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.


 CHRISTIAN DOMÍNGUEZ
 FEDERADO
 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° S4312 (clinical batch)

175/229

CONCENTRATED TETANUS PROTEIN
 lot number : **FA289726**

Date of manufacture : **31.10.07**
 Storage temperature : **+ 5 °C ± 3°C**
 Expiry date : **31 OCTOBER 2008**
 Volume : **2.234 liters**

TETANUS PROTEIN CONTROLS :

Protein nitrogen content (PN)

Method : Kjeldahl after trichloroacetic acid precipitation
 Date : **03.01.07**
 Result : **0.091 mg/ml**

Total nitrogen content (TN)

Method : Kjeldahl
 Date : **03.01.07**
 Result : **0.096 mg/ml**

PN/TN ratio : 0.95 - Conforms

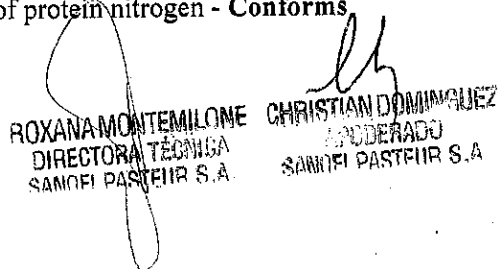
OD280/OD260 ratio : 1.76 - Conforms

Flocculating titre

Method : Flocculation determined from in-house reference
 Date : **24.11.06**
 Result : **200 Lf/ml**

Antigenic purity

Result : **2 198 Lf/mg of protein nitrogen - Conforms**



ROXANA MONTEMILONE **CHRISTIAN DOMINGUEZ**
 DIRECTORA TÉCNICA SUPERADJ
 SANOFI PASTEUR S.A. 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° S4312 (clinical batch)**

176/229

Molecular size distribution

Method : size exclusion Chromatography
Date : 27.11.06
Result : 66.8 % of monomers – **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	:	15.11.06	
Test off	:	29.11.06	
Result	:	No microbial growth - Conforms	

CONCENTRATED TETANUS PROTEIN CONTROLS :

Protein nitrogen content

Method : Kjeldahl after trichloroacetic acid precipitation
Date : 14.11.07
Result : 4.87 mg/ml

OD280/OD260 ratio : 2.01 - Conforms

Phosphorus content

Method : Chen
Date : 21.11.07
Result : < 0.04 µg/mg of protein - **Conforms**

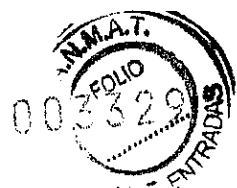
Residual formaldehyde content

Method : Nash
Date : 14.11.07
Result : 0.85 µg/mg of protein - **Conforms**

ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.

CHRISTIAN DOMINGUEZ
ENCARGADO
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° S4312 (clinical batch) 177/229

Flocculating titre

Method : Flocculation determined from in-house reference
Date : 06.11.07
Result : 12 000 Lf/ml

Antigenic purity

Result : 2 464 Lf/mg of protein nitrogen - **Conforms**

Molecular size distribution

Method : size exclusion Chromatography
Date : 12.11.07
Result : 65.8 % of monomers – **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	:	06.11.07
Test off	:	20.11.07
Result	:	No microbial growth - Conforms

Specific toxicity test

Animals : Guinea-pigs
Number of animals : 5
Lf of toxoid injected per animal : 2000
Route : Subcutaneous
Test on : 12.11.07
Test off : 03.12.07
Result : **Conforms**

ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.

CHRISTIAN DOMÍNGUEZ
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° S4312 (clinical batch)
178/229

Irreversibility of tetanus toxoid

Method	:	WHO method	Technical report N° 800 and 814
Concentration of the toxoid solution	:	15 Lf/ml	15 Lf/ml
Incubation period	:		
Temperatures	:	5°C ± 3°C	37°C ± 1°C
Test on	:		06.11.07
Test off	:		18.12.07

Test in animals


Number of guinea pigs injected	:	5	Subcutaneous	5
Route	:			
Volume injected	:	5 ml		5 ml
Date of inoculation	:		18.12.07	
Date of end of observation	:		08.01.08	
Result	:		Conforms	

Pyrogens


Method : by intravenous injection of 2 µg/ml/kg of the rabbit body weight
 Date : 06.12.07
 Number of rabbits : 3

Weight of each rabbit (kg)	Initial temperature in °C (A)	Intravenous dose (ml)	Maximum temperature in °C (B)	Response (B) - (A)
2.1	38.82	2.10	38.93	0.12
2.1	39.32	2.10	39.39	0.07
2.1	39.13	2.10	39.29	0.17

The sum of the 3 individual maximum temperature rises : 0.35 °C
 Result : Conforms



 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.



 CHRISTIAN DOMÍNGUEZ
 SUPERVISOR
 SANOFI PASTEUR S.A.



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° S4312 (clinical batch)

179/229

HAEMOPHILUS type b POLYSACCHARIDE

TESTS	SPECIFICATIONS
PHOSPHORUS CONTENT	7.5 – 9.4 %
RIBOSE CONTENT	≥ 35 %
PROTEIN CONTENT	≤ 1.0 %
NUCLEIC ACID CONTENT	≤ 1.0 %
BACTERIAL ENDOTOXINS	< 25 IU/μg of polysaccharide
MOLECULAR SIZE : polysaccharide eluted before K _d 0.30	> 50 %
HAEMOPHILUS B IDENTIFICATION	Positive
PYROGENS	Conforms

352_CC5_SPL_AE.dot /19.09.11

Signature of Roxana Montemilone
ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.

Signature of Christian Domínguez
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° S4312 (clinical batch)

180/229

HAEMOPHILUS type b POLYSACCHARIDE
lot number : FA240998

Date of manufacture : 26.04.06
Storage temperature : ≤ - 35°C
Expiry date : 26 APRIL 2009
Quantity : 94.591 g

CONTROLS :

Phosphorus content

Method : Chen
Date : 15.05.06
Result : 8.09 % - Conforms

Ribose content

Method : Mejbaum
Date : 10.05.06
Result : 39.7 % - Conforms

Protein content

Method : Lowry
Date : 11.05.06
Result : <0.13 % - Conforms

Nucleic acid content

Method : Absorbance at 260 nm
Date : 11.05.06
Result : 0.08 % - Conforms

ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.

CHRISTIAN DOMINGUEZ
GERENTE
SANOFI PASTEUR S.A.

