



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) 181/229

Bacterial endotoxins

Method : LAL
Date : 10.05.06
Result : 0.36 IU/µg - Conforms

Molecular size

Method : size exclusion Chromatography
Date : 22.05.06
Result : 66.4 % - Conforms

Haemophilus identification

Method : Double diffusion in gel
Date : 09.05.06
Result : Positive - Conforms

Pyrogens

Method : by intravenous injection 1 µg/ml/kg of the rabbit body weight
Date : 11.05.06
Number of rabbits : 3

Table with 5 columns: Weight of each rabbit (kg), Initial temperature in °C (A), Intravenous dose (ml), Maximum temperature in °C (B), Response (B) - (A). Rows show data for three rabbits with weights 2.5, 2.4, and 2.4 kg.

The sum of the 3 individual maximum temperature rises : 0.36 °C
Result : Conforms

Handwritten signatures and stamps for Roxana Montemilone (Directora Técnica) and Christian Domínguez (Cooperador) at Sanofi Pasteur S.A.





Product Quality and Disposition Department

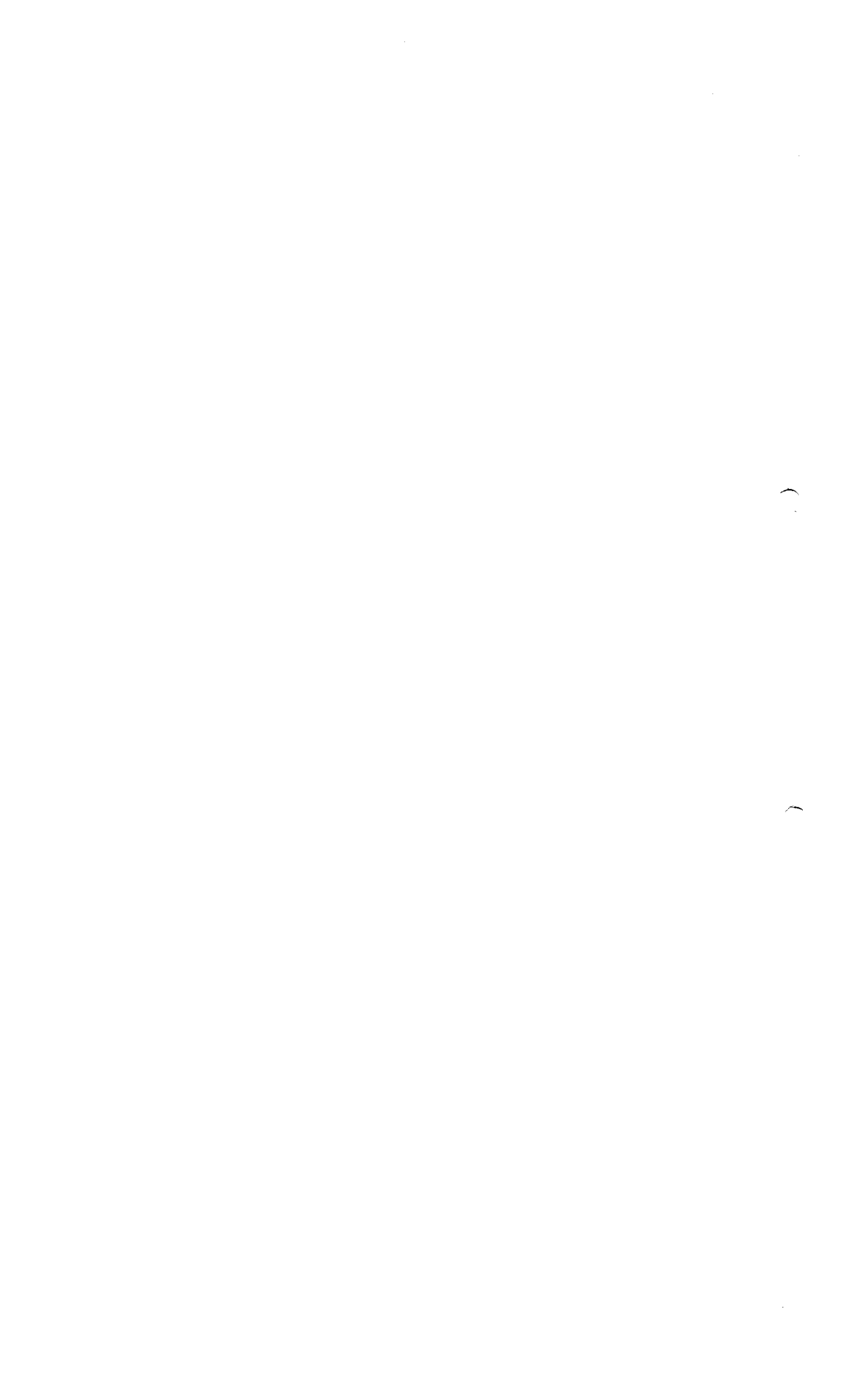
Diphtheria, tetanus, pertussis (acellular, component),
 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
 influenzae type b conjugate vaccine (adsorbed) lot n° S4312 (clinical batch)

182/229

HAEMOPHILUS AH POLYSACCHARIDE DERIVATIVE

TESTS	SPECIFICATIONS
RESIDUAL CYANIDES CONTENT	≤ 5 µg/ml
POLYSACCHARIDE CONTENT (PHOSPHORUS CONTENT)	For calculation
PERCENTAGE OF POLYSACCHARIDE LINKED ADH	1.8 - 2.9 %
FREE ADH/TOTAL ADH RATIO	≤ 0.1
MOLECULAR SIZE : - Polysaccharide eluted before K _d 0.20 - K _d of polysaccharide	≤ 15 % 0.40 - 0.60
HAEMOPHILUS IDENTIFICATION	Positive



 ROXANA MONTEMILOME CHRISTIAN DOMINGUEZ
 DIRECTORA TÉCNICA ASISTENTE
 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)

183/229

**HAEMOPHILUS AH
POLYSACCHARIDE DERIVATIVE**

lot number : FA300558

Date of manufacture : 08.02.08
Storage temperature : ≤ -35°C
Expiry date : 08 FEBRUARY 2010
Volume : 1.963 liters

CONTROLS :

Residual cyanides content

Method : Gas Chromatography, cyanide or cyanate ions are transformed
into cyanogen chloride by chlorination
Date : 21.02.08
Result : < 0.004 µg/ml – Conforms

Polysaccharide content

Method : Calculated from the theoretical phosphorus
content of PRP
Date : 20.02.08
Result : 11.3 mg/ml

Percentage of polysaccharide linked ADH (Adipic DiHydrazide acid)

Method : Colorimetry
Date : 06.03.08
Result : 2.46 % - Conforms

Free ADH/total ADH ratio : 0.038 - Conforms

ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.
CHRISTIAN DOMINGUEZ
SECRETARIO
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) 184/229


Molecular size

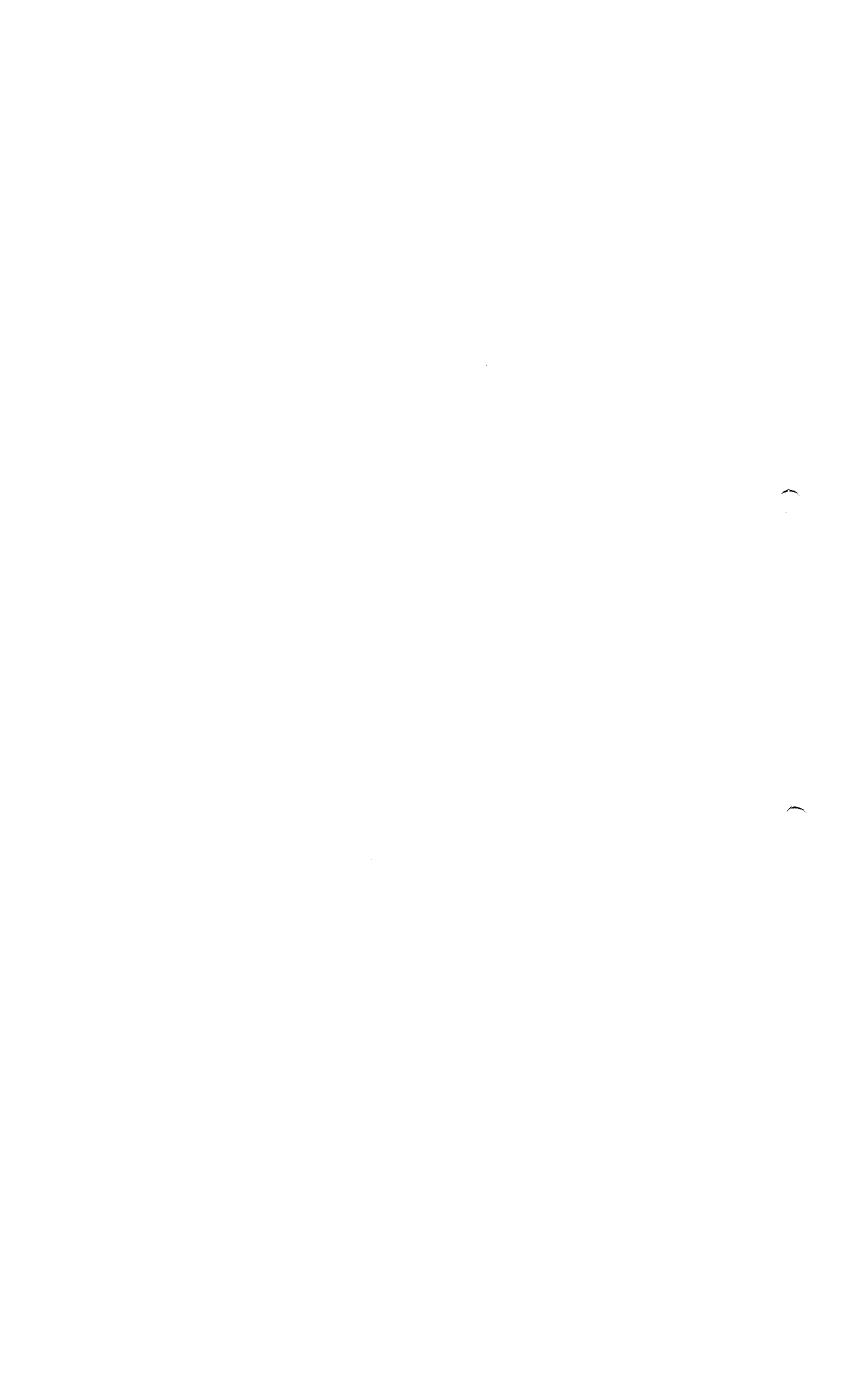
Method : size exclusion Chromatography
Date : 27.02.08
Result : - Percentage of polysaccharide : 1.54 % - Conforms
 eluted before K_d 0.20
 - K_d of polysaccharide : 0.51 - Conforms

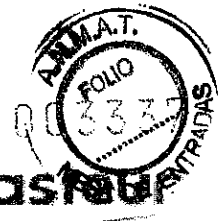
Haemophilus identification

Method : Double diffusion in gel
Date : 18.02.08
Result : Positive - Conforms


ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.


CHRISTIAN DOMÍNGUEZ
GERENTE
SANOFI PASTEUR S.A.





sanofi pasteur

Product Quality and Disposition Department



The vaccines division of sanofi-aventis Group

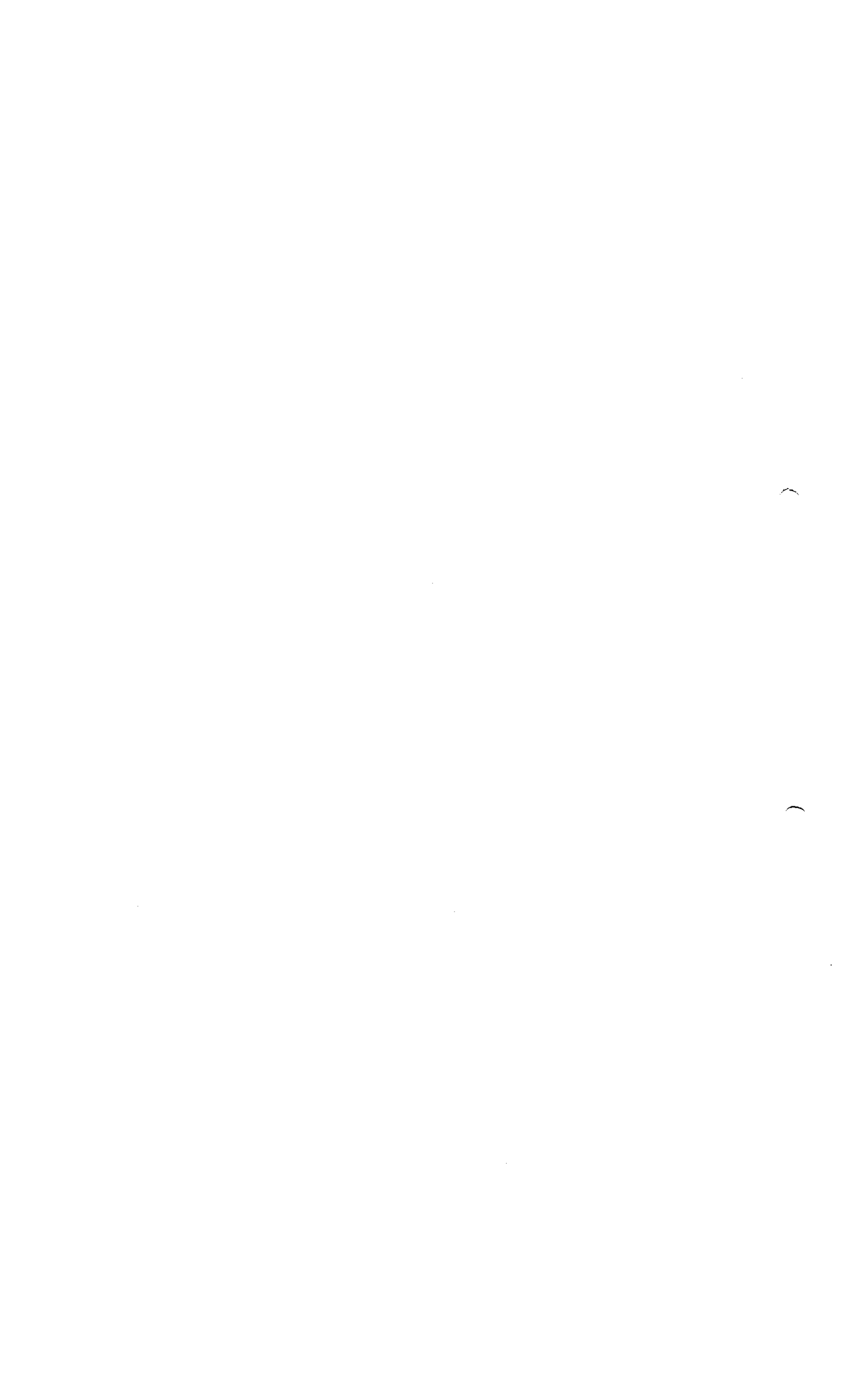
Diphtheria, tetanus, pertussis (acellular, component), hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus influenzae type b conjugate vaccine (adsorbed) lot n° S4312 (clinical batch)

185/229

HAEMOPHILUS POLYSACCHARIDE CONJUGATE (CONCENTRATED BULK)

TESTS	SPECIFICATIONS
PH	6.5 - 7.5
PHOSPHORUS CONTENT	No specification
POLYSACCHARIDE CONTENT	No specification
SUCROSE CONTENT	No specification
PROTEIN/POLYSACCHARIDE RATIO (after dialysis)	1.8 - 3.0
RESIDUAL EDAC CONTENT	< 10 µmol/l
RESIDUAL PHENOL CONTENT	< 1 µg/ml
FREE POLYSACCHARIDE CONTENT	< 20 %
MOLECULAR SIZE : Percentage of polysaccharide eluted before K _d 0.20	≥ 60 %
FREE TETANUS PROTEIN CONTENT	< 1 %
BACTERIAL AND FUNGAL STERILITY	No microbial growth



 ROXANA MONTEMILOME CHRISTIAN DOMINGUEZ
 DIRECTORA TÉCNICA INGENIERO
 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)

186/229

HAEMOPHILUS POLYSACCHARIDE CONJUGATE (CONCENTRATED BULK)

lot number : FA316432

Date of manufacture : 13.06.08
Storage temperature : ≤ -35°C
Expiry date : 13 JUNE 2011
Volume : 27.345 liters

CONTROLS :

pH

Method : Glass electrode
Date : 25.06.08
Result : 7.08 - Conforms

Phosphorus content

Method : Chen
Date : 30.06.08
Result : 15.5 µg/ml

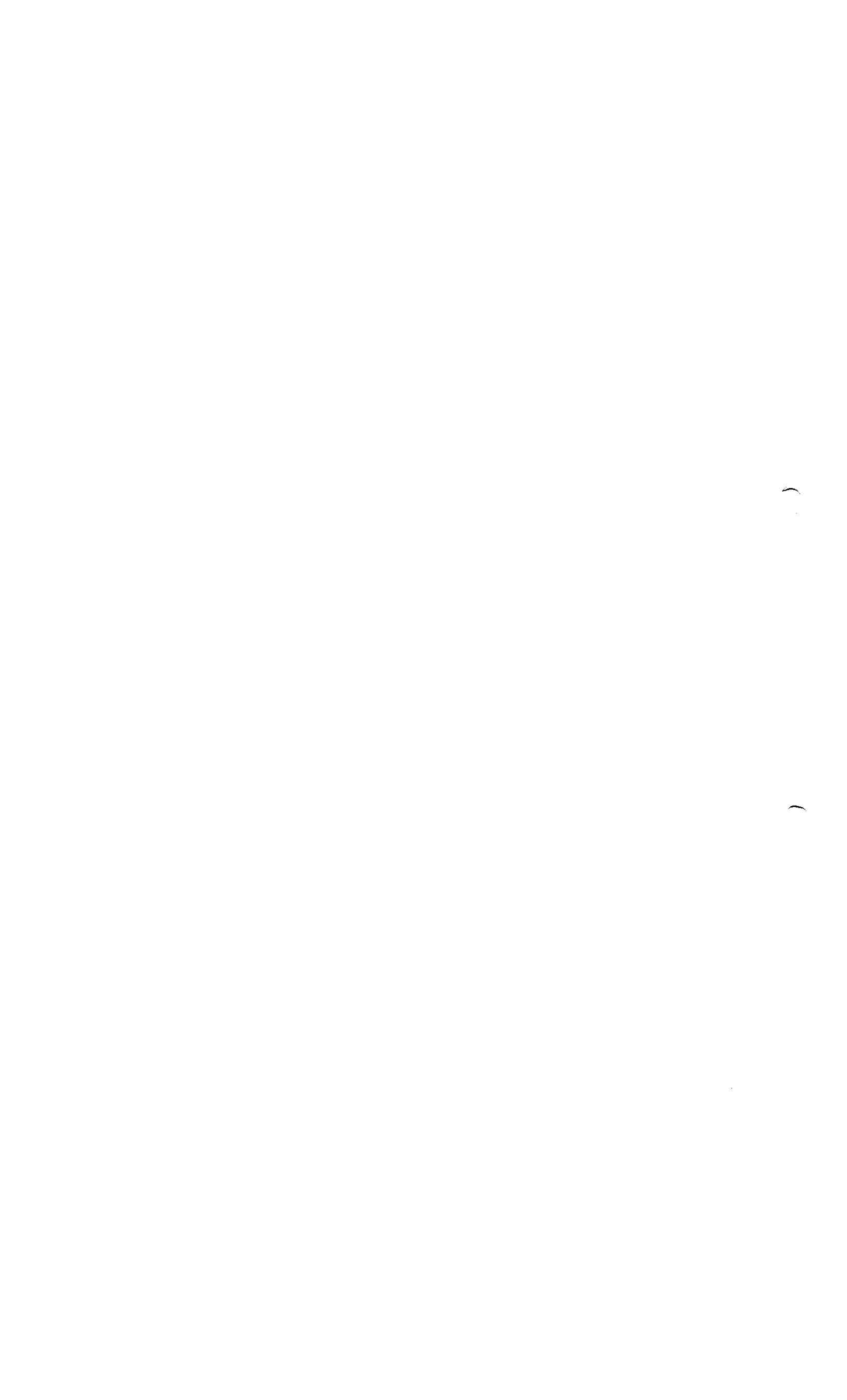
Polysaccharide content

Method : Calculated from the theoretical phosphorus content of PRP
Date : 01.07.08
Result : 184.52 µg/ml

Sucrose content

Method : quantitative analysis of glucose before and after enzymatic hydrolysis
Date : 03.07.08
Result : 81.39 g/l

ROXANA MONTEMILONE DIRECTORA TÉCNICA SANOFI PASTEUR S.A.
CHRISTIAN DOMINGUEZ CODERADO 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)

187/229

Protein/Polysaccharide ratio : 2.7 - Conforms

Residual EDAC content (Ethyl-DimethylAminopropyl-Carbodiimide)

Method : Wilcheck
Date : 02.07.08
Result : < 10.00 µmol/l - Conforms

Residual phenol content

Method : Colorimetry
Date : 03.07.08
Result : < 1.00 µg/ml - Conforms

Free Polysaccharide content

Method : High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection method (HPAEC-PAD)
Date : 07.07.08
Result : < 4.0 % - Conforms

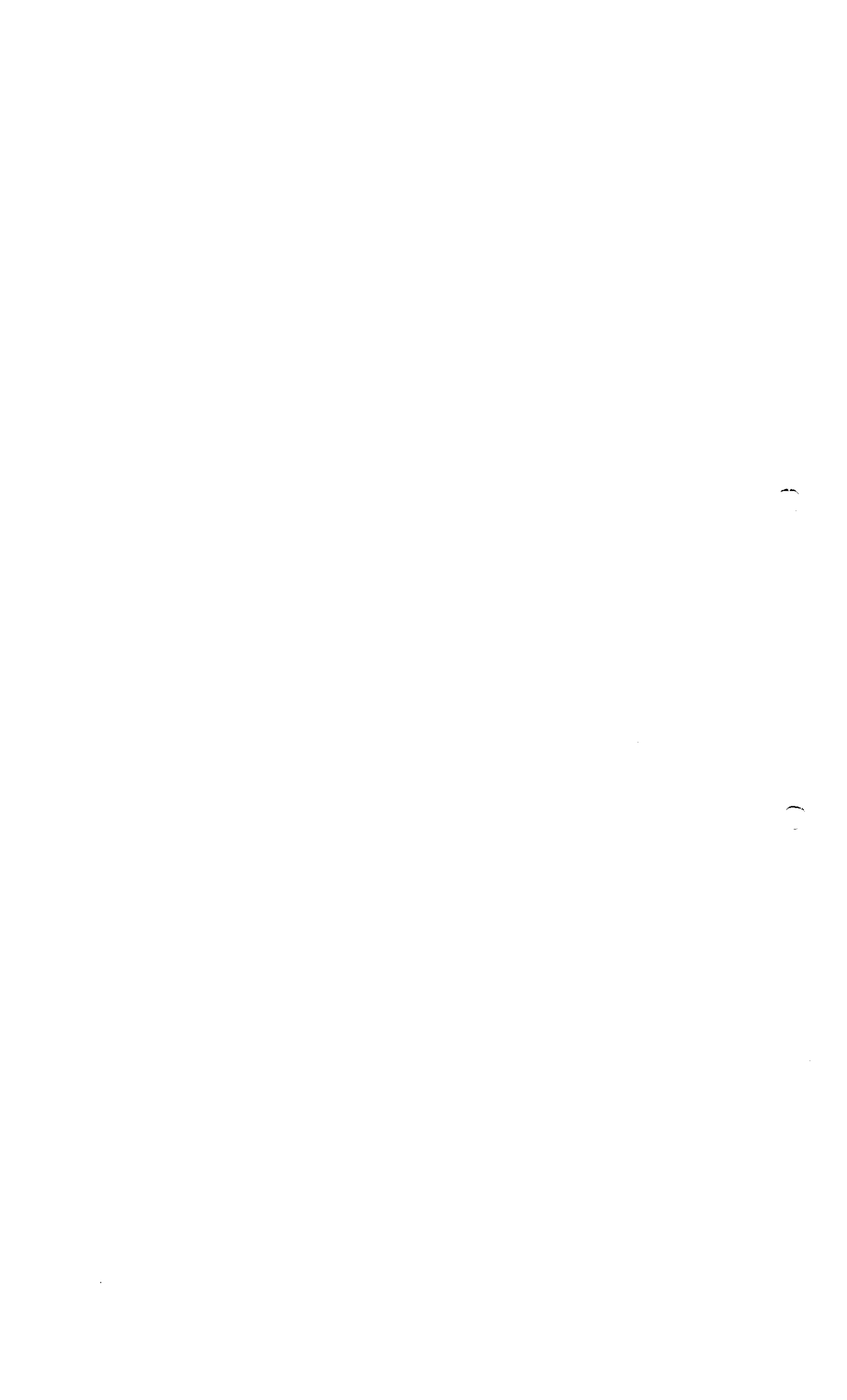
Molecular size

Method : size exclusion chromatography
Date : 24.07.08
Result : 62.3 % - Conforms

Free tetanus protein content

Method : polyacrylamide gel electrophoresis SDS-PAGE
Date : 07.08.08
Result : < 1% - Conforms

Handwritten signatures and printed names: 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° S4312 (clinical batch)

188/229

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	:	17.06.08	
Test off	:	01.07.08	
Result	:	No microbial growth – Conforms	


 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.


 CHRISTIAN DOMÍNGUEZ
 SUPERVISOR
 SANOFI PASTEUR S.A.

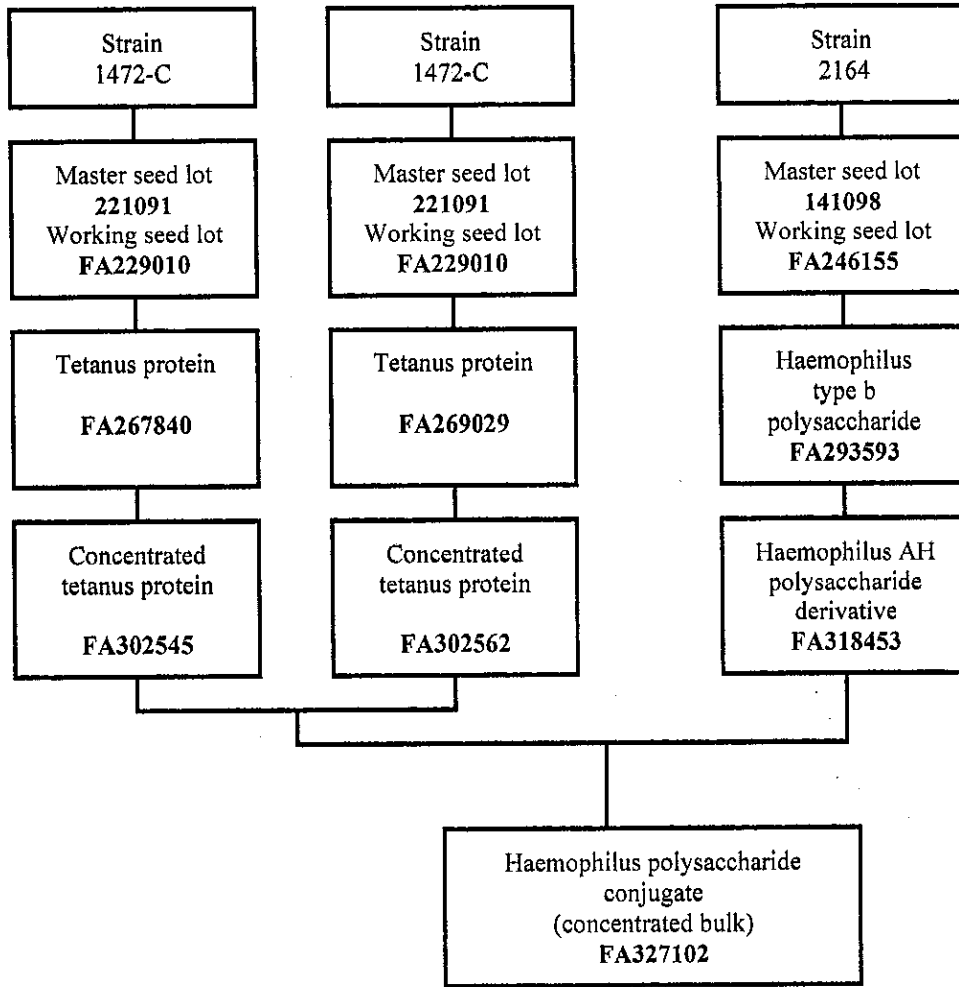


Product Quality and Disposition Department

Diphtheria, tetanus, pertussis (acellular, component),
 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
 influenzae type b conjugate vaccine (adsorbed) lot n° S4312 (clinical batch)

189/229

PRODUCTION FLOW SHEET




 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.


 CHRISTIAN DOMÍNGUEZ
 JEFE DE SECCIÓN
 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)

190/229

HAEMOPHILUS INFLUENZAE
Strain : Haemophilus Influenzae 2164

STRAIN


Name : Haemophilus influenzae type b – strain 1482
Origin : Dr John ROBBINS (FDA)
Bethesda – Maryland 20014
Received : 12.12.75
Institut Mérieux number : IM 2164


HAEMOPHILUS INFLUENZAE
Master seed lot n° 141098

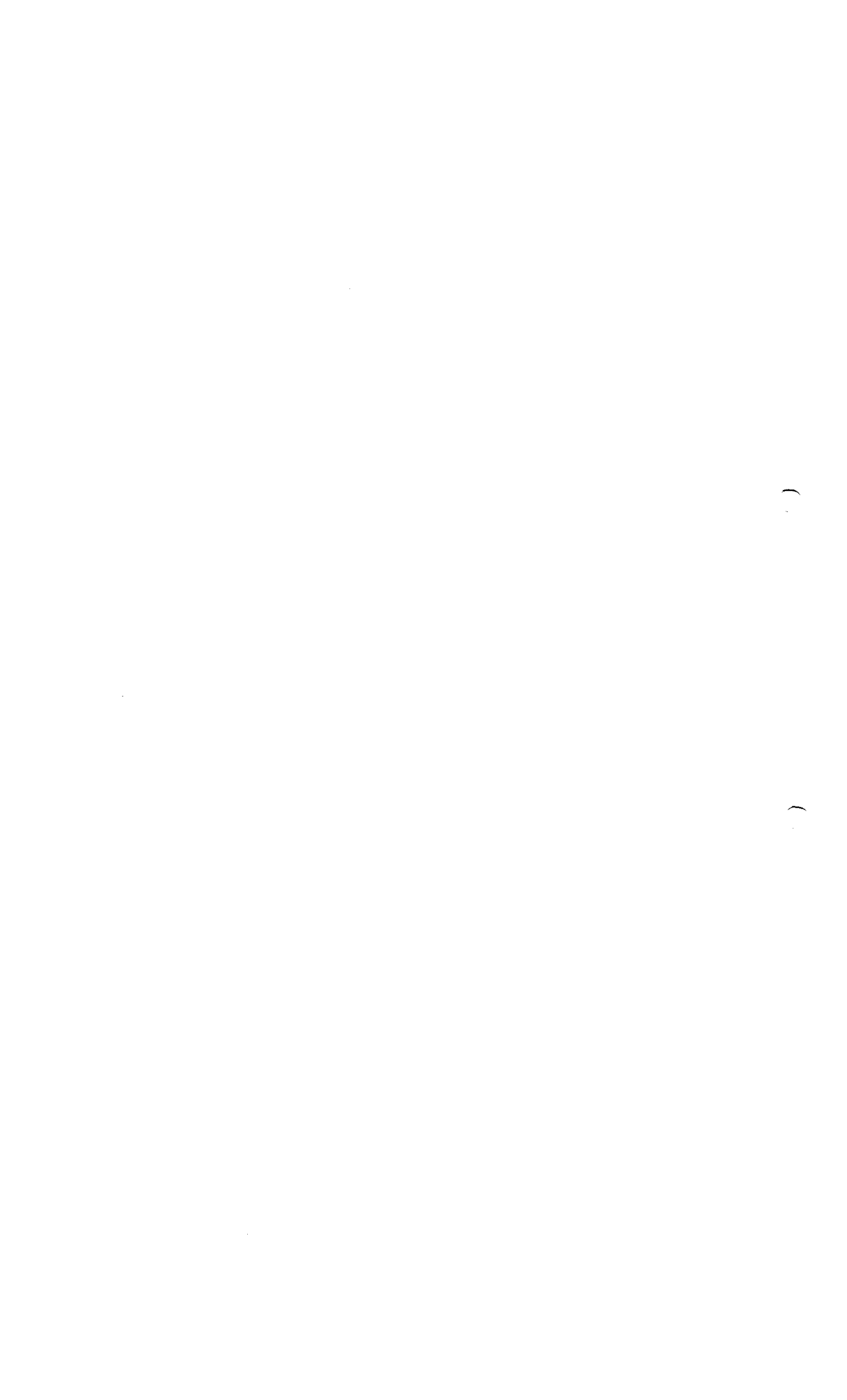
Strain number : IM 2164
Preparation date : 14.10.98
Release date : 11.09.01

CONTROLS

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


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)

191/229

HAEMOPHILUS INFLUENZAE

Working seed lot n° FA246155

Master seed Lot n° : 141098
Preparation date : 20.12.05
Release date : 19.01.09

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
JEFE DE AREA
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)

192/229

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

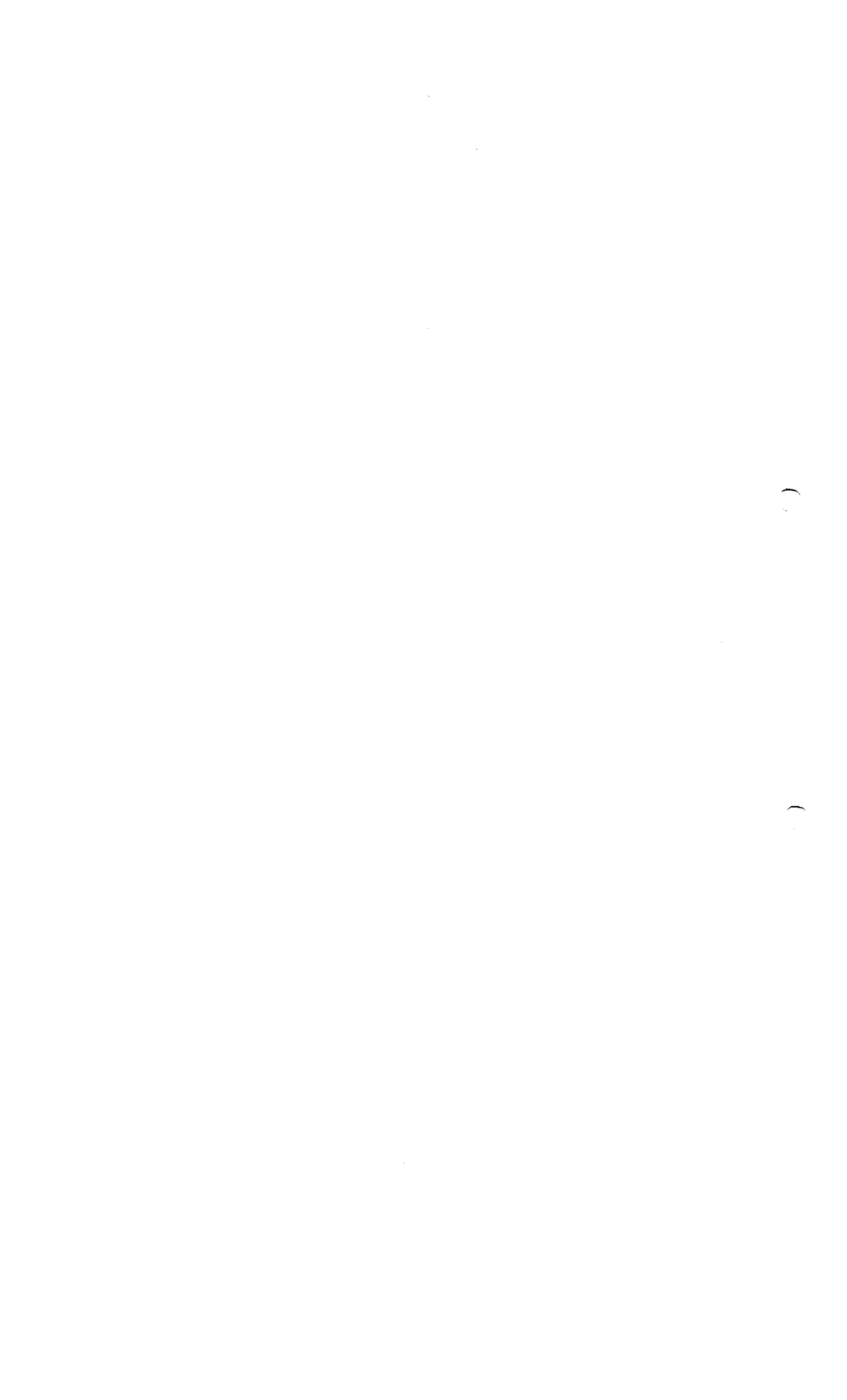
Strain : IM 1472-C
Preparation date : 22.10.91
Release date : 14.11.91

CONTROLS

- Purity
Identification
- Morphology
 - Growth characteristics
 - Biochemical characteristics

ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.

CHRISTIAN DOMÍNGUEZ
PREPARADO
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)

193/229


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
 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)

195/229

CONCENTRATED TETANUS PROTEIN
lot number : FA302545

Date of manufacture : 19.03.2008
Storage temperature : + 5 °C ± 3°C
Expiry date : 19 MARCH 2009
Volume : 3.572 liters

TETANUS PROTEIN CONTROLS :

Protein nitrogen content (PN)

Method : Kjeldahl after trichloroacetic acid precipitation
Date : 12.04.07
Result : 0.110 mg/ml

Total nitrogen content (TN)

Method : Kjeldahl
Date : 12.04.07
Result : 0.122 mg/ml

PN/TN ratio : 0.90 - Conforms

OD280/OD260 ratio : 1.88 - Conforms

Flocculating titre

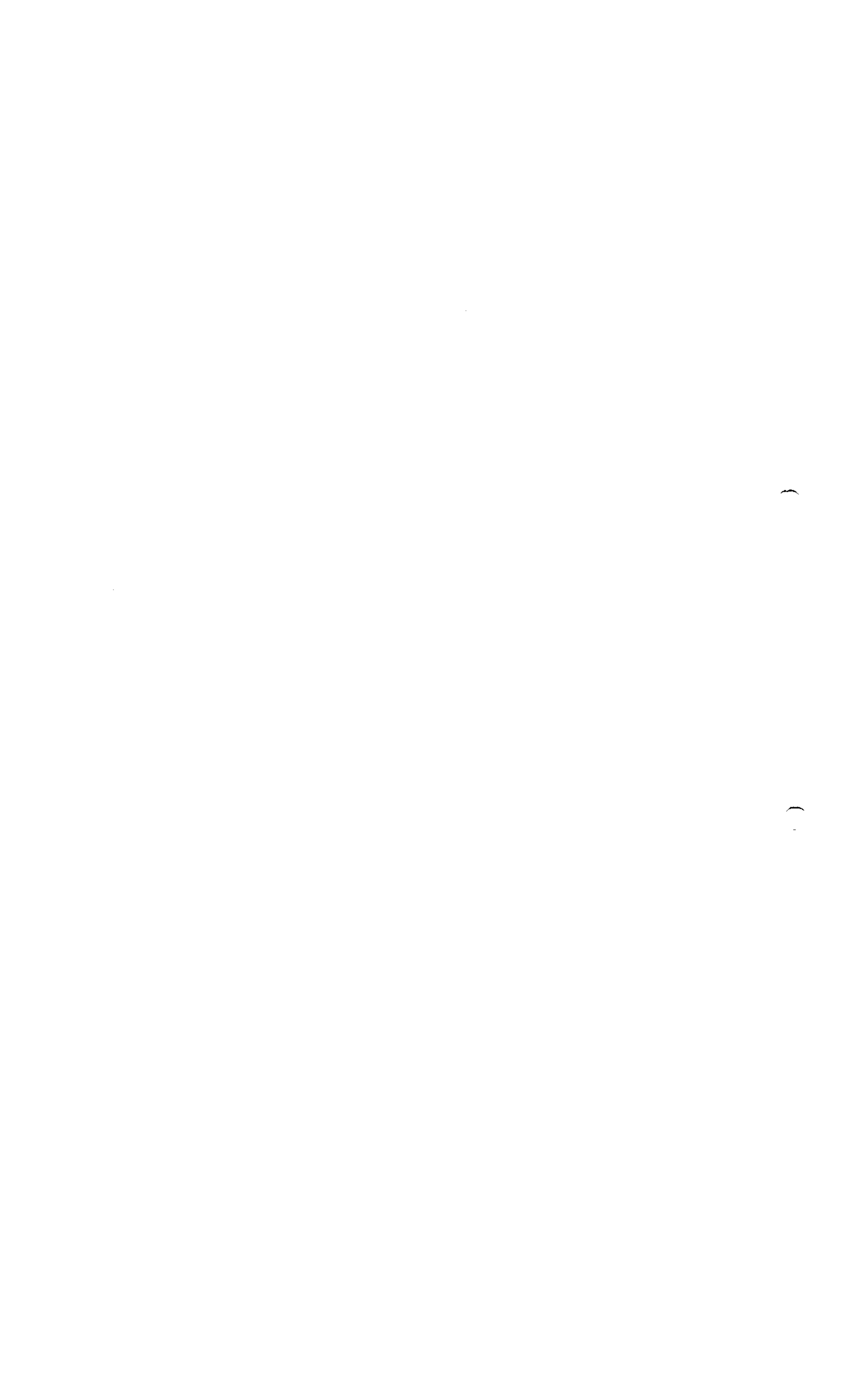
Method : Flocculation determined from in-house reference
Date : 20.04.07
Result : 270 Lf/ml

Antigenic purity

Result : 2455 Lf/mg of protein nitrogen - Conforms

ROXANA MONTEMILONE
DIRECTORA TECNICA
SANOFI PASTEUR S.A.

CHRISTIAN DOMINGUEZ
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)

196/229

Molecular size distribution

Method : size exclusion Chromatography
Date : 17.04.07
Result : 66.80 % 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	:	16.04.07	
Test off	:	30.04.07	
Result	:	No microbial growth - Conforms	

CONCENTRATED TETANUS PROTEIN CONTROLS :

Protein nitrogen content

Method : Kjeldahl after trichloroacetic acid precipitation
Date : 31.03.08
Result : 5.25 mg/ml

OD280/OD260 ratio : 1.89 - **Conforms**

Phosphorus content

Method : Chen
Date : 09.04.08
Result : <0.04 µg/mg of protein - **Conforms**

Residual formaldehyde content

Method : Nash
Date : 26.03.08
Result : 0.48 µg/mg of protein - **Conforms**

ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.

CHRISTIAN DOMÍNGUEZ
COOPERATIVO
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)

197/229

Flocculating titre

Method : Flocculation determined from in-house reference
Date : 25.03.08
Result : 12000 Lf/ml

Antigenic purity

Result : 2286 Lf/mg of protein nitrogen - **Conforms**

Molecular size distribution

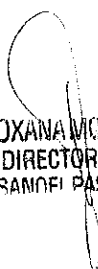
Method : size exclusion Chromatography
Date : 10.04.08
Result : 65.10 % of monomers – **Conforms**


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	:	20.03.08
Test off	:	03.04.08
Result	:	No microbial growth - Conforms

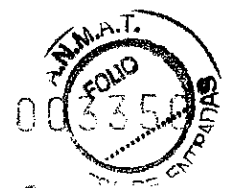
Specific toxicity test

Animals : Guinea-pigs
Number of animals : 5
Lf of toxoid injected per animal : 2000
Route : Subcutaneous
Test on : 03.04.08
Test off : 24.04.08
Result : **Conforms**


 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) 198/229

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 : 26.03.08
 Test off : 07.05.08

Test in animals

Number of guinea pigs injected : 5 5
 Route : Subcutaneous
 Volume injected : 5 ml 5 ml
 Date of inoculation : 07.05.08
 Date of end of observation : 28.05.08
 Result : Conforms

Pyrogens

Method : by intravenous injection of 2 µg/ml/kg of the rabbit body weight
 Date : 02.05.08
 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)
2.9	38.32	2.90	38.65	0.33
2.7	38.91	2.70	38.95	0.05
2.8	39.07	2.80	39.15	0.09

The sum of the 3 individual maximum temperature rises : 0.46 °C
 Result : 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° S4312 (clinical batch) 199/229

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

[Signature]
ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.

[Signature]
CHRISTIAN DOMÍNGUEZ
ACREDITADO
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)

200/229


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
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)

201/229

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
 GERENTE
 SANOFI PASTEUR S.A.

