



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)

21/211

**

TETANUS TOXOID
Lot n° FA298985

Strain	: 1472-C
Working seed-lot number	: FA246159
Storage temperature	: + 5°C ± 3°C
Approved storage period	: 112 days
Volume	: 419.25 liters

Single harvests :

Medium used	: Massachussets
Date of reconstitution of seed lot ampoule(s)	: 28.01.08
Date of inoculation	: 31.01.08
Date of harvest	: 07.02.08
Date of inactivation (formaldehyde)	: 25.02.08

CONTROLS

Identity test

Method : tetanus flocculation
Date : 10.03.08
Result : positive

Bacterial purity

Method : Gram coloration
Date: 06.02.08
Result : conforms

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22/211

**

TETANUS TOXOID
Lot n° FA301562

Strain	: 1472-C
Working seed-lot number	: FA229010
Storage temperature	: + 5°C ± 3°C
Approved storage period	: 112 days
Volume	: 446.50 liters

Single harvests :

Medium used	: Massachussets
Date of reconstitution of seed lot ampoule(s)	: 04.02.08
Date of inoculation	: 07.02.08
Date of harvest	: 14.02.08
Date of inactivation (formaldehyde)	: 03.03.08

CONTROLS

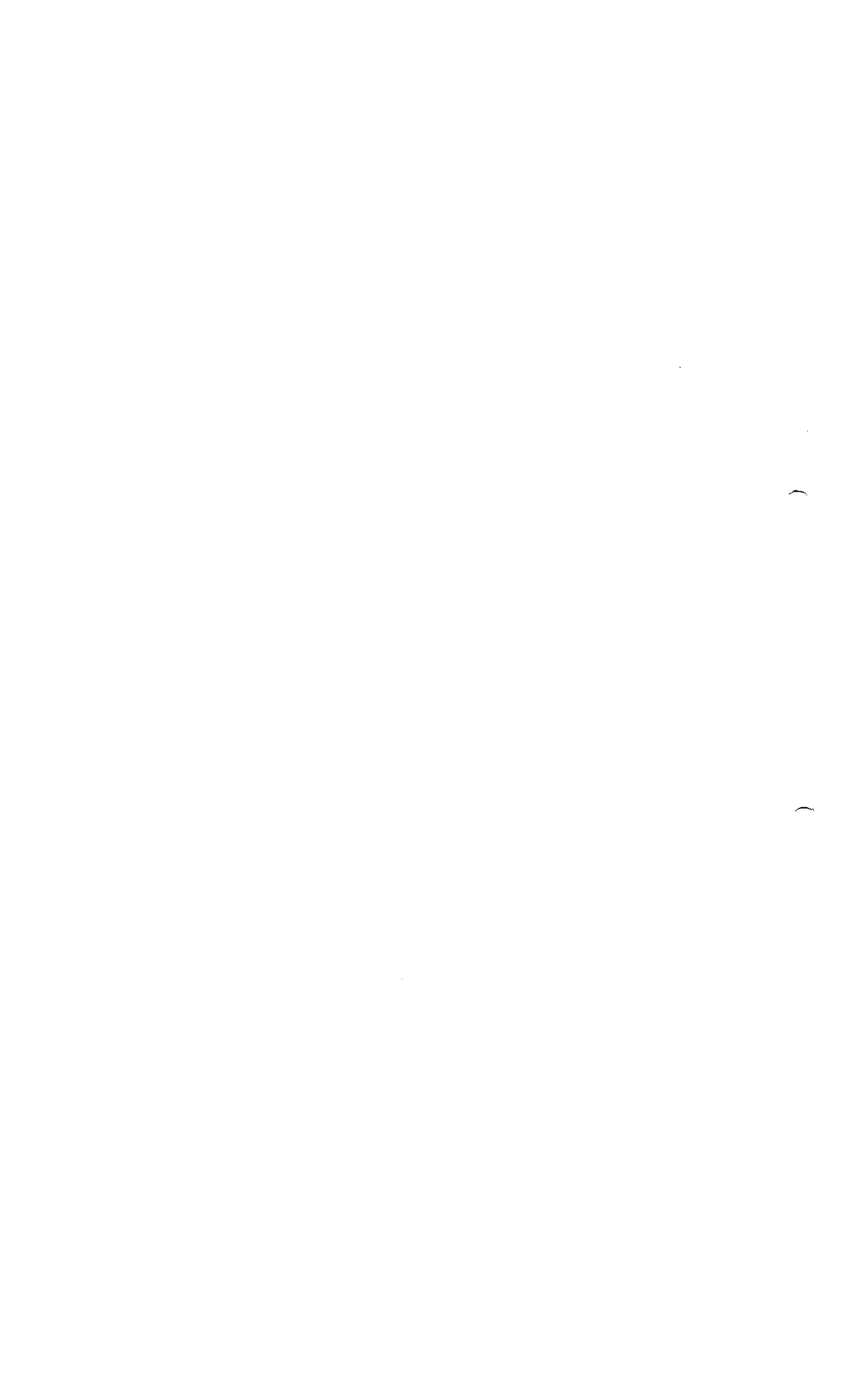
Identity test

Method : tetanus flocculation
Date : 18.03.08
Result : positive

Bacterial purity

Method : Gram coloration
Date: 13.02.08
Result : conforms

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23/211

**

TETANUS TOXOID
Lot n° FA302464

Strain	: 1472-C
Working seed-lot number	: FA229010
Storage temperature	: + 5°C ± 3°C
Approved storage period	: 112 days
Volume	: 419.45 liters

Single harvests :

Medium used	: Massachussets
Date of reconstitution of seed lot ampoule(s)	: 15.02.08
Date of inoculation	: 21.02.08
Date of harvest	: 28.02.08
Date of inactivation (formaldehyde)	: 17.03.08



CONTROLS

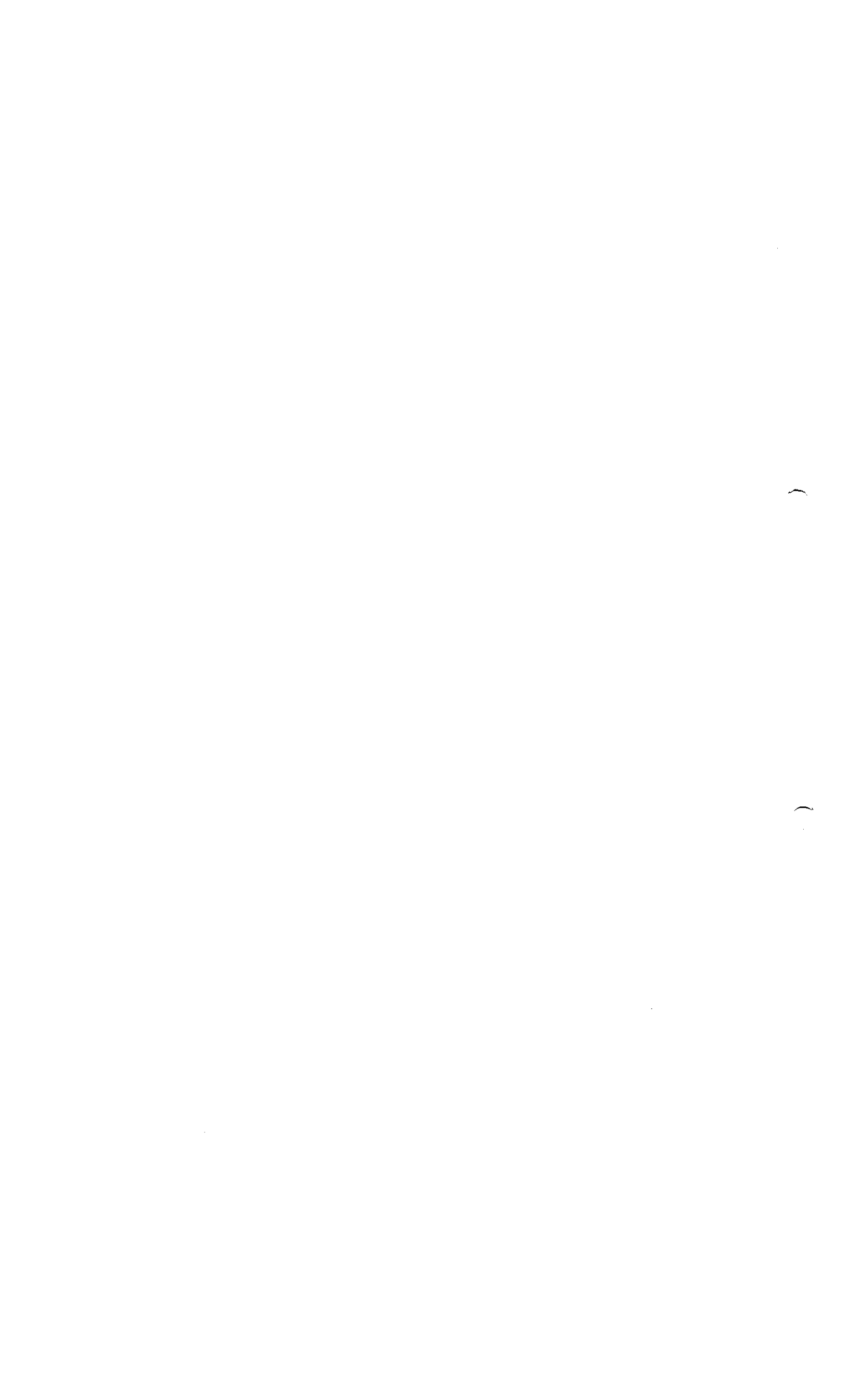
Identity test

Method : tetanus flocculation
Date : 27.03.08
Result : positive

Bacterial purity

Method : Gram coloration
Date: 27.02.08
Result : conforms

	
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Product Quality and Disposition Department

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

24/211

**

PURIFIED TETANUS TOXOID
FA309471

TESTS	SPECIFICATIONS
PROTEIN NITROGEN CONTENT	For antigenic purity calculation
FREE FORMALDEHYDE CONTENT	For formulation (used for formulation in final bulk product where appropriate)
FLOCCULATING TITER	For antigenic purity calculation
ANTIGENIC PURITY	≥ 1000 Lf/mg of protein nitrogen
BACTERIAL AND FUNGAL STERILITY	No microbial growth
ABSENCE OF TOXIN (SPECIFIC TOXICITY) AND IRREVERSIBILITY OF TOXOID	No tetanus symptom and at least 93 % of survival


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Diphtheria, tetanus, pertussis (acellular, component),
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25/211

PURIFIED TETANUS TOXOID
 Lot n° FA309471

Working seed Lot n° : FA246159 – FA229010

Tetanus toxoid Lot number :	FA298985	FA301562	FA302464
Volume used (liters) :	419.25	446.5	419.45

Volume : 62.500 liters
 Storage temperature : + 5°C ± 3°C
 Date of manufacture : 19.05.2008
 Expiry date : 19 MAY 2011
 Approved storage period : 3 years

CONTROLS

Protein nitrogen content


Method : Kjeldahl after trichloroacetic acid precipitation
 Date : 27.05.08
 Result : 2.60 mg/ml

Free formaldehyde content


Method : Nash
 Date : 28.05.08
 Result : 37.23 µg/ml

Flocculating titer

Method : Flocculation in comparison with in house reference
 Date : 23.05.08
 Result : 4500 Lf/ml



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Antigenic purity

Date : 04.07.08
 Result : 1731 Lf/mg of protein nitrogen - Conforms



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 influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch) 26/211

Bacterial and fungal sterility

Method	:	Membrane filtration	
Media	:	Thioglycollate	Soybean casein digest
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
Number of inoculated Steritest	:	1	1
Test on	:	21.05.08	
Test off	:	04.06.08	
Result	:	No microbial growth - Conforms	

Absence of toxin (specific toxicity)

Animals	:	Guinea-pigs
Number of animals	:	5
Lf of toxoid injected per animal	:	2000
Route of injection	:	Subcutaneous
Test on	:	04.06.08
Test off	:	25.06.08
Result	:	No tetanus symptom and at least 93% of survival - Conforms

Irreversibility of toxoid

Concentration of the toxoid solution	:	20 Lf/ml	20 Lf/ml
Incubation period	:		
Temperatures	:	5°C ± 3°C	37°C
Date of beginning	:	03.06.08	
Date of end	:	15.07.08	

Test in animals

Number of guinea pigs injected	:	5	5
Route	:	Subcutaneous	
Volume injected	:	5 ml	5 ml
Date of inoculation	:	15.07.08	
Date of end of observation	:	05.08.08	
Result	:	No tetanus symptom and at least 93% of survival - Conforms	

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

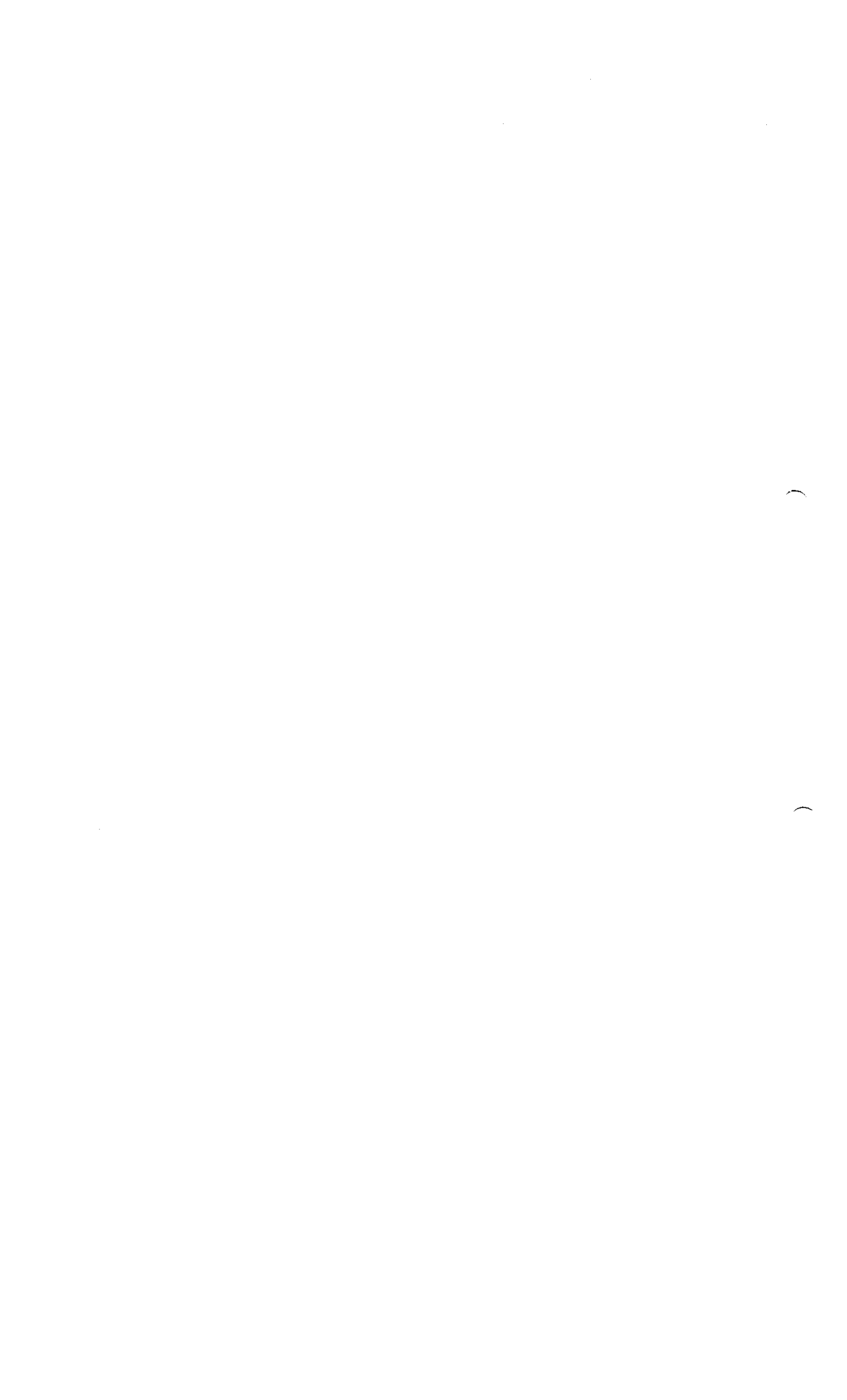
27/211

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- C - 2 COMPONENT ACELLULAR PERTUSSIS


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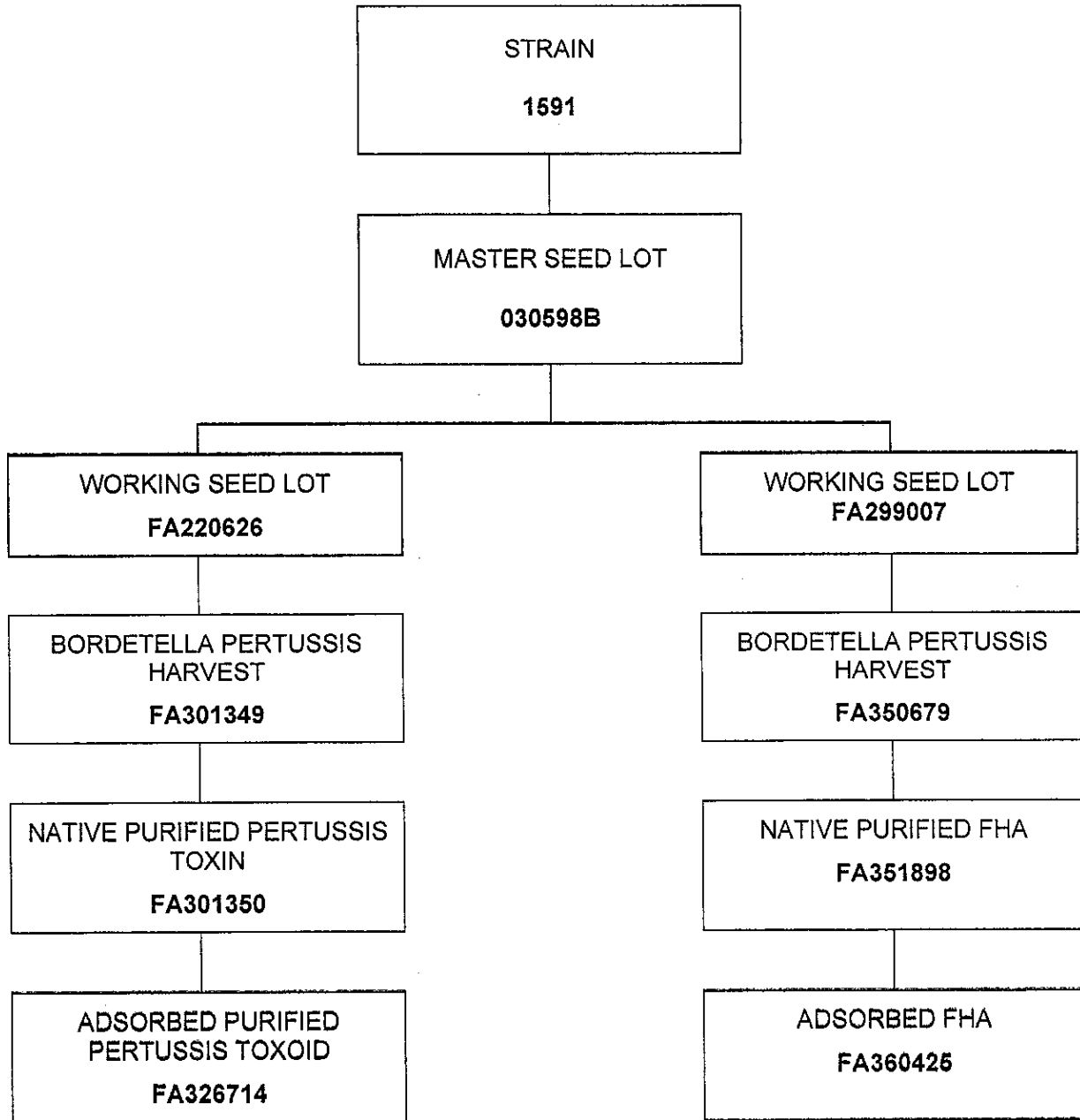


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 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
 influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)

28/211

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PRODUCTION FLOW SHEET




 ROXANA MONTEMILONE CHRISTIAN DUMINIEL

 DIRECTORA TÉCNICA GERENTE

 SANOFI PASTEUR S.A. 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)

30/211

**


BORDETELLA PERTUSSIS
Working seed lot n° FA220626


Master seed lot : 030598B
Preparation date : 30.09.05
Release date : 07.12.05

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.


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

31/211

**

BORDETELLA PERTUSSIS
Working seed lot n° FA299007

Master seed lot : 030598B
Preparation date : 18.01.08
Release date : 26.09.08

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.


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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)

32/211

**

Bordetella pertussis harvest
Lot n° FA301349

Strain : 1591
Working seed lot number : FA220626
Date of reconstitution of working seed lot ampoules: 29.02.08
Medium used : SS-SAT
Temperature incubation : 36°C +/- 1°C

Harvest

Date of inoculation : 05.03.08
Date of harvest : 07.03.08
Volume : 1235 liters

CONTROLS

Bacterial purity

Method : Gram staining
Result : Gram negative bacilli

Bordetella pertussis harvest
Lot n° FA350679

Strain : 1591
Working seed lot number : FA299007
Date of reconstitution of working seed lot ampoules: 15.05.09
Medium used : SS-SAT
Temperature incubation : 36°C +/- 1°C

Harvest

Date of inoculation : 20.05.09
Date of harvest : 22.06.09
Volume : 1232 liters

CONTROLS

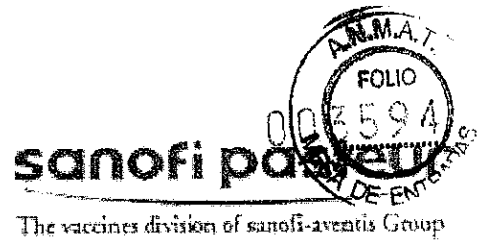
Bacterial purity

Method : Gram staining

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**

Result : Gram negative bacilli


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**

NATIVE PURIFIED PERTUSSIS TOXIN

TESTS	SPECIFICATIONS
PROTEIN CONTENT	For calculation
SDS-PAGE	Electrophoresis profile comparable to the profile of the reference (5 bands between 30 and 14 kDa)
PERTUSSIS TOXIN IDENTIFICATION	Positive
CLUSTERING ACTIVITY ON CHO CELLS	Cytopathic activity (CPU/μg of protein) ≥ lower confidence limit (P = 0.95) of cytopathic activity of the reference.
HAEMAGGLUTINATING ACTIVITY	20 000 – 500 000 HAU/mg of protein IHA with cholesterol : ≤ 20 %


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

35/211

**

NATIVE PURIFIED PERTUSSIS TOXIN

Lot number : FA301350

Date of manufacture : 07.04.2008
Storage temperature : + 5°C ± 3°C
Expiry date : 07 APRIL 2012
Volume : 13.000 liters

CONTROLS :

Protein content

Method : Spectrophotometry
Date : 10.04.08
Result : 1046.48 µg/ml

SDS-page

Method : Polyacrylamide Gel Electrophoresis
Date : 24.04.08
Result : Electrophoresis profile comparable to the profile
of the reference (5 bands between 30 and 14 kDa)
- Conforms.

Pertussis toxin identification

Method : Double Diffusion in Gel
Date : 16.04.08
Result : Positive - Conforms

Clustering activity on Chinese Hamster Ovary cells

Method : Clusters Assay on CHO Cells
Date : 07.05.08

	CPU/µg
Reference 90/518	102400
Product	204800

Result : Conforms

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

36/211

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Haemagglutinating activity

Method	: Haemagglutination of goose red blood cells by PT
Date	: 23.04.08
Result	: 76691 HAU/mg
	IHA with cholesterol : 0 % - Conforms

	
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Product Quality and Disposition Department

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ADSORBED PURIFIED PERTUSSIS TOXOID

TESTS	SPECIFICATIONS
PURIFIED PERTUSSIS TOXOID IN SOLUTION	
PROTEIN CONTENT	50 – 180 µg/ml
BACTERIAL ENDOTOXINS	≤ 3 500 UI/mg protein
RESIDUAL GLUTARALDEHYDE CONTENT	< 8 µg/ml
ANTIGENICITY	Specific activity (EU/mg of protein) ≥ lower confidence limit (p = 0.95) of the specific activity of SP batches
CLUSTERING ACTIVITY ON CHO CELLS	No cytopathic effect
IRREVERSIBILITY OF PERTUSSIS TOXOID	≥ 95% of survival
ADSORBED PURIFIED PERTUSSIS TOXOID	
ALUMINIUM CONTENT	0.6 - 1.4 mg Al/mg of protein
pH	6.2 - 8.2
PERTUSSIS IDENTIFICATION	Positive
BACTERIAL AND FUNGAL STERILITY	No microbial growth


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 influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch) 38/211

**

ADSORBED PURIFIED PERTUSSIS TOXOID

Lot number : FA326714

Date of manufacture : 17.10.2008
 Storage temperature : + 5°C ± 3°C
 Expiry date : 17 OCTOBER 2012
 Volume : 35.801 liters

PURIFIED PERTUSSIS TOXOID IN SOLUTION :

CONTROLS :

Protein content

Method : Spectrophotometry
 Date : 15.10.08
 Result : 118.12 µg/ml - Conforms

Bacterial endotoxins

Method : LAL
 Date : 27.10.08
 Result : <4.23 IU/mg of protein

Residual glutaraldehyde content


Method : Spectrophotometry
 Date : 28.10.08
 Result : <2 µg/ml – Conforms


Antigenicity

Method : ELISA
 Date : 31.10.08

	EU/mg of protein
Lower confidence limit (P=0.95) of the specific activity of SP batches	24.5
Product	50.3

Result : Conforms


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 APST
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Clustering activity on Chinese Hamster Ovary cells

Method : Clusters Assay on CHO Cells
Date : 07.01.09
Result : No cytopathic effect

Irreversibility of pertussis toxoid

Injection (Day 0)

Animals and sex : mice CD1 - Female
Route : Intraperitoneal
Dose tested per mouse : 2 human doses (1ml)
Date of injection : 21.11.08

Histamine challenge (Day 5)

Challenge dose per mouse : 2 mg histamine base
Route : Intraperitoneal
Date of injection : 26.11.08

Reading date (Day 6)

Result (+5°C and +37°C) : 27.11.08
: ≥ 95% of survival - Conforms

ADSORBED PURIFIED PERTUSSIS TOXOID :

CONTROLS :

Aluminium content

Method : Complexometry
Date : 28.10.08
Result : 0.98 mgAl/mg of protein - Conforms

pH

Method : Glass electrode
Date : 27.10.08
Result : 6.69 - Conforms

Pertussis identification

Method : Double diffusion in Gel
Date : 30.10.08
Result : Positive - Conforms

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