

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° S4313 (clinical batch)

146/180

- F - HAEMOPHILUS type b CONJUGATE

352_CC5_SPI_AE.dot /19.09.11


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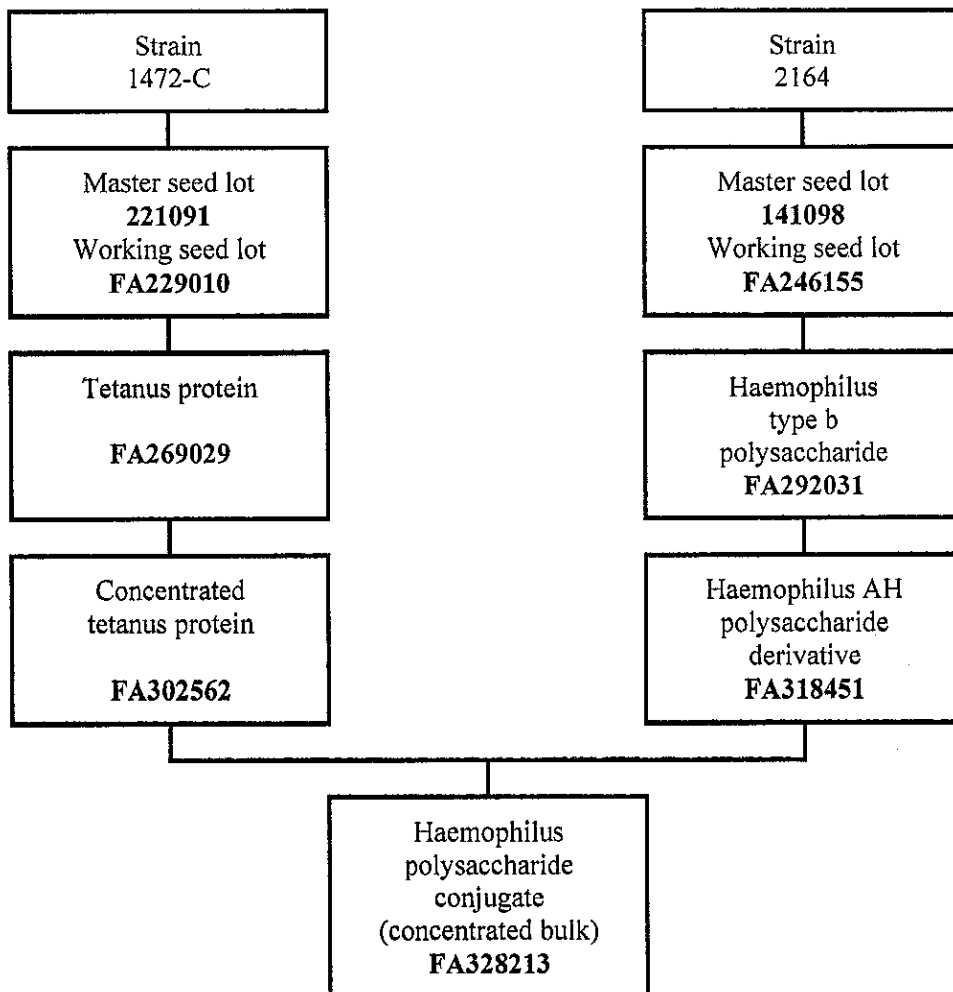
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PRODUCTION FLOW SHEET



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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

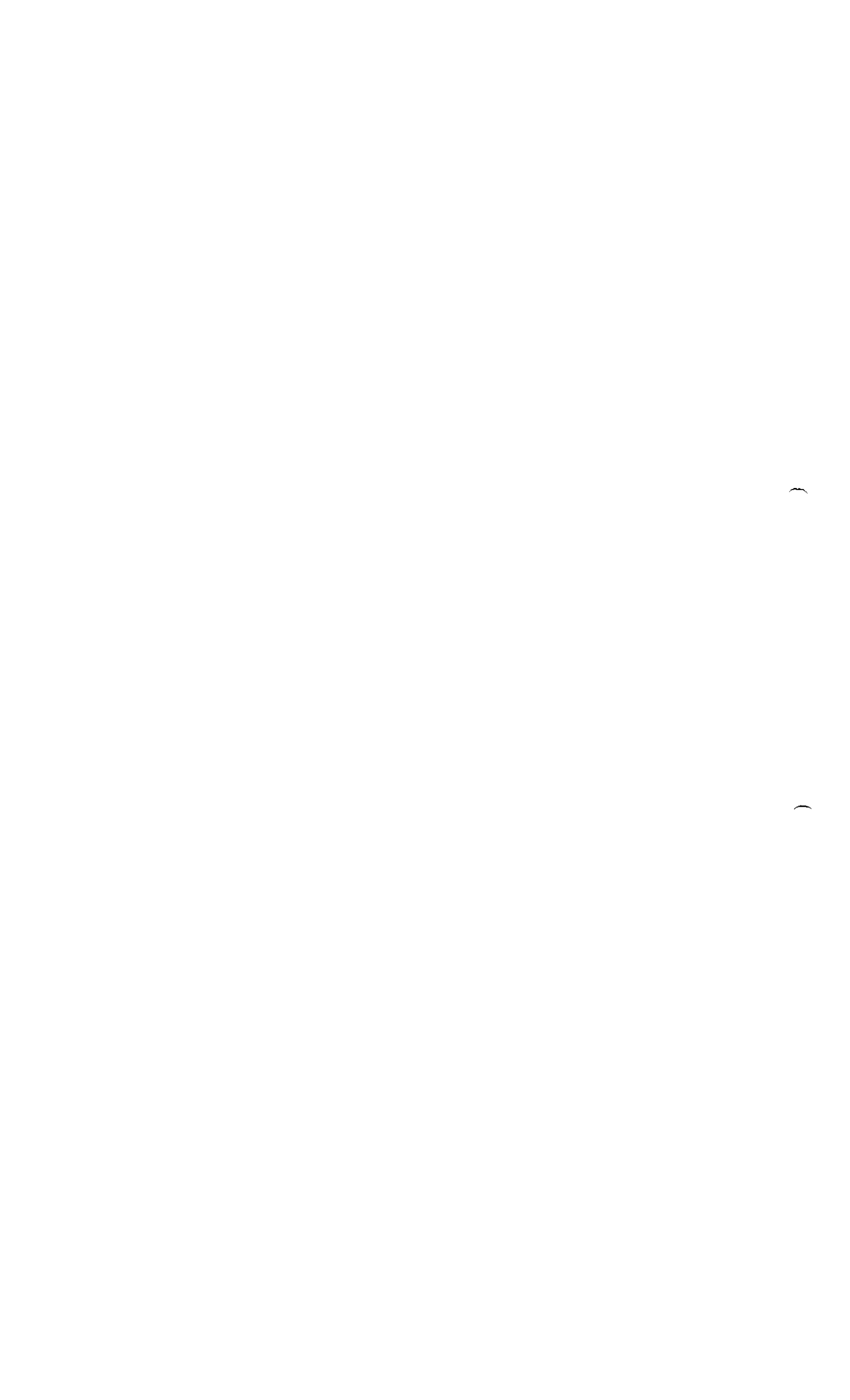
Strain number : IM 2164
Preparation date : 14.10.98
Release date : 11.09.01

CONTROLS

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


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HAEMOPHILUS INFLUENZAE

Working seed lot n° FA246155

Master seed Lot n° : 141098
Preparation date : 23.06.06
Release date : 18.04.07

CONTROLS

Purity

Identification

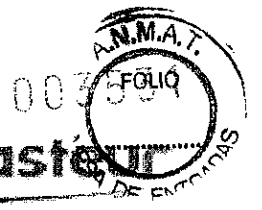
- Morphology
- Growth characteristics
- Biochemical characteristics
- Antigenic characteristics

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


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influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch) 150/180

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


TÉTANUS 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


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influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch) 151/180


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.


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 influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch)

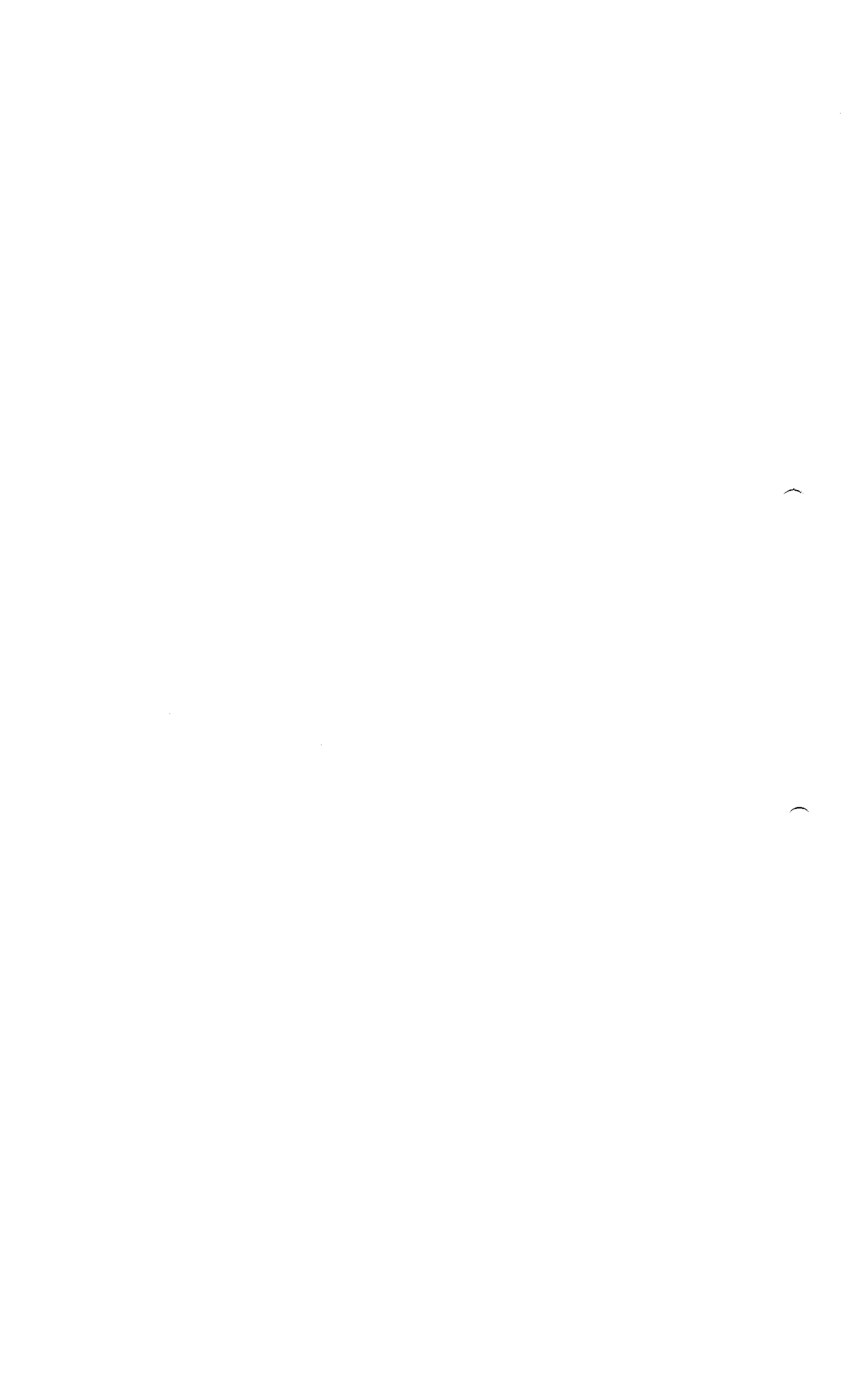
152/180

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


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CONCENTRATED TETANUS PROTEIN
lot number : FA302562

Date of manufacture : 27.03.2008
Storage temperature : + 5 °C ± 3°C
Expiry date : 27 MARCH 2009
Volume : 4.143 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

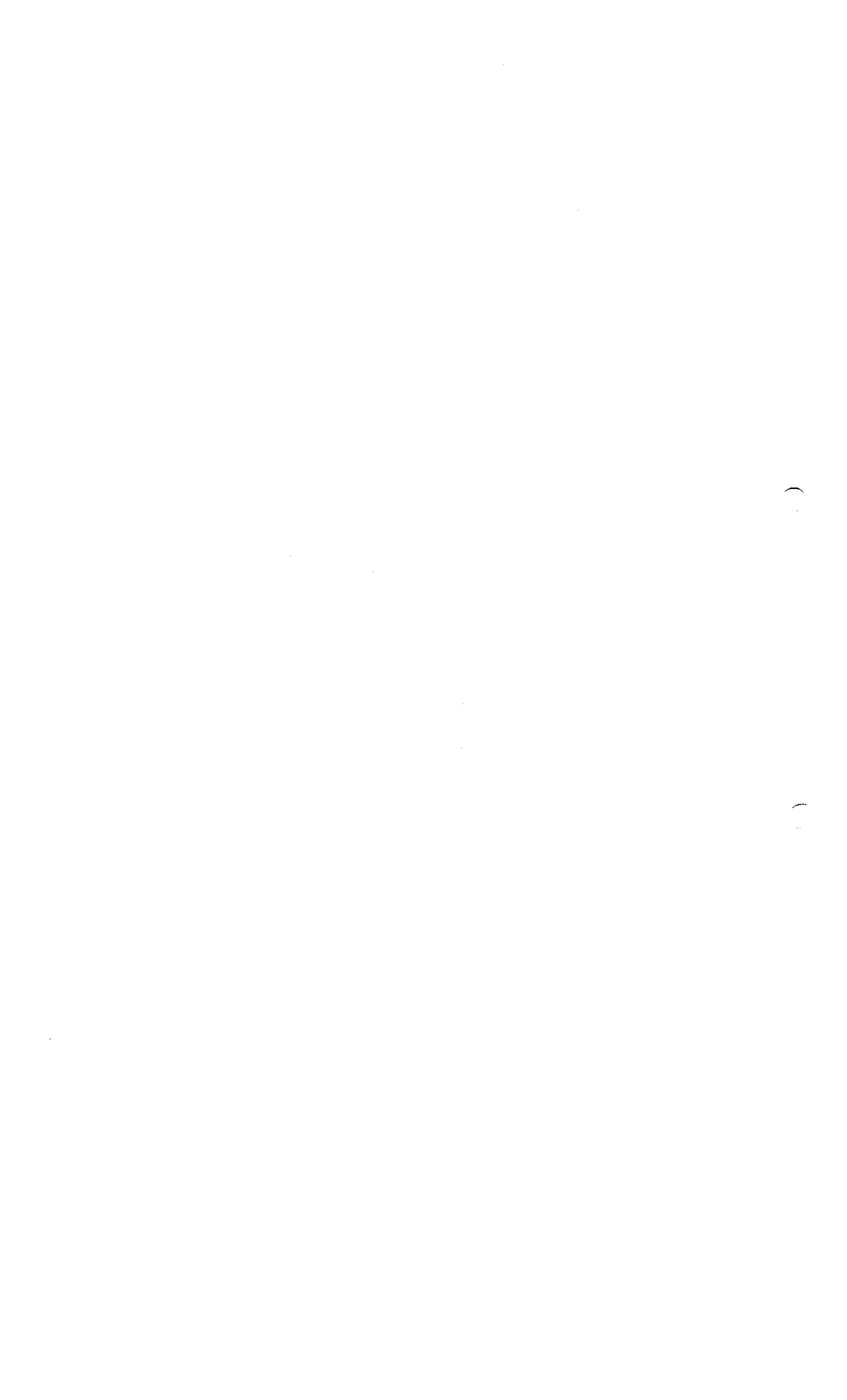
Flocculating titre

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

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Molecular size distribution

Method : size exclusion Chromatography
 Date : 22.05.07
 Result : 69.50 % 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 : 08.04.08
 Result : 4.95 mg/ml


OD280/OD260 ratio : 1.91 - Conforms

Phosphorus content


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

Residual formaldehyde content

Method : Nash
 Date : 09.04.08
 Result : 0.70 µg/mg of protein - **Conforms**



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Flocculating titre

Method : Flocculation determined from in-house reference
 Date : 31.03.08
 Result : 12000 Lf/ml

Antigenic purity

Result : 2424 Lf/mg of protein nitrogen - **Conforms**

Molecular size distribution


Method : size exclusion Chromatography
 Date : 10.04.08
 Result : 68.00 % 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	: 31.03.08	
Test off	: 14.04.08	
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**



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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	:		31.03.08
Test off	:		12.05.08

Test in animals

Number of guinea pigs injected	:	5	5
Route	:		Subcutaneous
Volume injected	:	5 ml	5 ml
Date of inoculation	:		12.05.08
Date of end of observation	:		02.06.08
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)
2.6	38.89	2.60	38.92	0.03
2.4	39.04	2.40	39.05	0.01
2.8	38.84	2.80	39.00	0.17

The sum of the 3 individual maximum temperature rises : 0.21 °C

Result : Conforms

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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


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HAEMOPHILUS type b POLYSACCHARIDE

lot number : FA292031

Date of manufacture : 29.11.07
Storage temperature : ≤ - 35°C
Expiry date : 29 NOVEMBER 2010
Quantity : 56.830 g

CONTROLS :

Phosphorus content

Method : Chen
Date : 07.01.08
Result : 8.13 % - Conforms

Ribose content



Method : Mejbaum
Date : 27.12.07
Result : 43.0 % - Conforms

Protein content

Method : Lowry
Date : 04.01.08
Result : <0.11 % - Conforms

Nucleic acid content

Method : Absorbance at 260 nm
Date : 28.12.07
Result : 0.14 % - Conforms



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Bacterial endotoxins

Method : LAL
 Date : 03.12.07
 Result : 0.81 IU/µg – Conforms

Molecular size

Method : size exclusion Chromatography
 Date : 11.12.07
 Result : 72.2 % - Conforms

Haemophilus identification


Method : Double diffusion in gel
 Date : 12.12.07
 Result : Positive - Conforms


Pyrogens

Method : by intravenous injection 1 µg/ml/kg of the
 rabbit body weight
 Date : 07.12.07
 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)
2.8	39.12	2.80	39.29	0.18
2.7	38.81	2.70	39.07	0.26
2.3	38.97	2.30	38.90	0.00

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


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