

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)**
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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

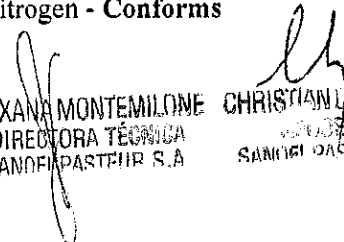
OD280/OD260 ratio : 1.83 - Conforms

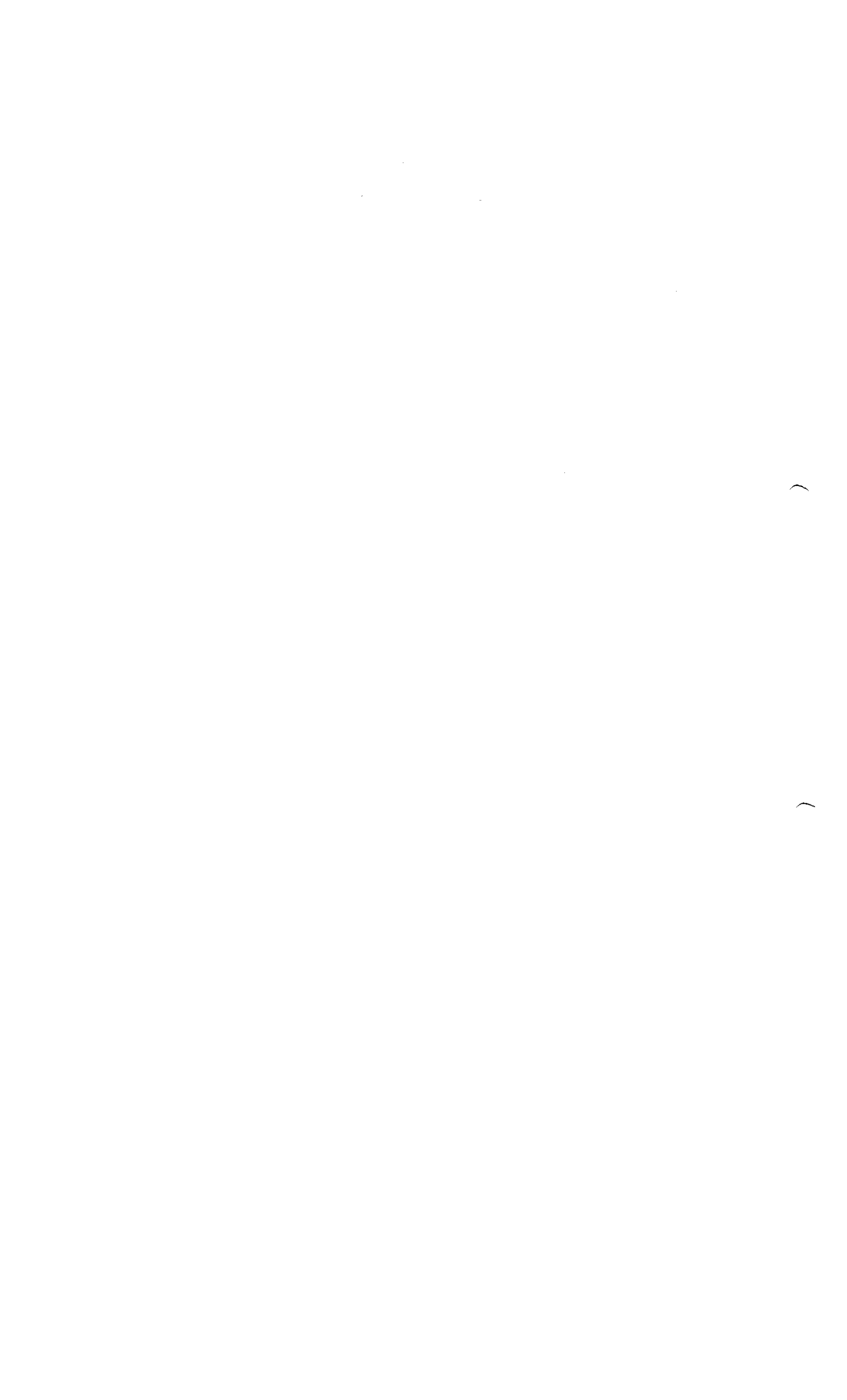
Flocculating titre

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

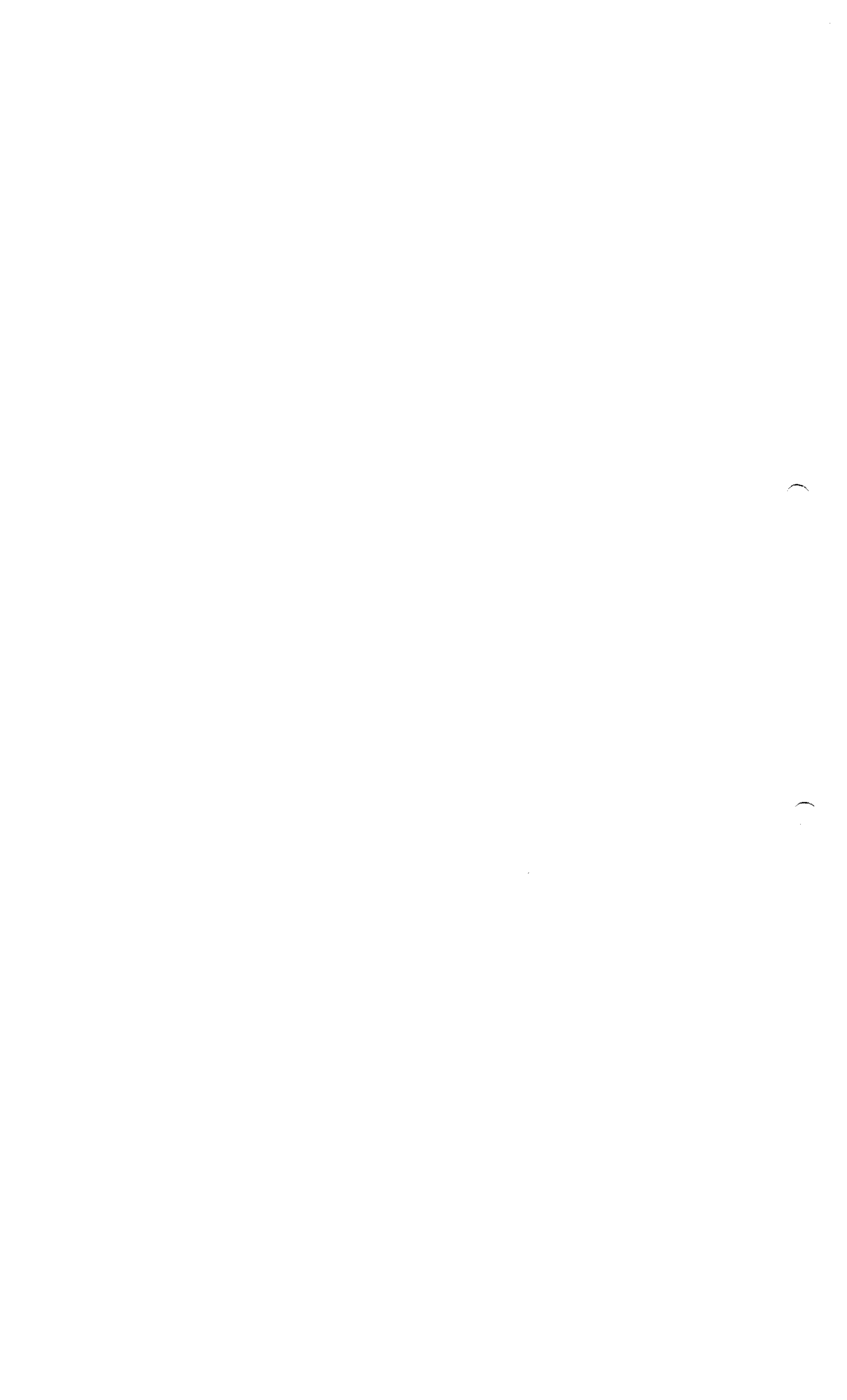
Weight of each rabbit (kg)	Initial temperature in °C (A)	Intravenous dose (ml)	Maximum temperature in °C (B)	Response (B) - (A)
2.6	38.89	2.60	38.92	0.03
2.4	39.04	2.40	39.05	0.01
2.8	38.84	2.80	39.00	0.17

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 : FA281303

Date of manufacture : 19.05.06
 Storage temperature : ≤ - 35°C
 Expiry date : 19 MAY 2009
 Quantity : 90.810 g

CONTROLS :

Phosphorus content

Method : Chen
 Date : 31.07.07
 Result : 8.15 % - Conforms

Ribose content

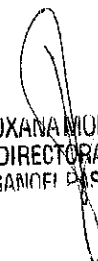
Method : Mejbaum
 Date : 02.08.07
 Result : 41.0 % - Conforms


Protein content

Method : Lowry
 Date : 03.08.07
 Result : <0.13 % - Conforms

Nucleic acid content

Method : Absorbance at 260 nm
 Date : 02.08.07
 Result : 0.01 % - Conforms


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**

Bacterial endotoxins

Method : LAL
Date : 10.09.07
Result : <0.22 IU/µg - Conforms

Molecular size

Method : size exclusion Chromatography
Date : 01.08.07
Result : 65.1 % - Conforms

Haemophilus identification


Method : Double diffusion in gel
Date : 31.07.07
Result : Positive - Conforms


Pyrogens

Method : by intravenous injection 1 µg/ml/kg of the
rabbit body weight
Date : 03.08.07
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)
3.0	38.65	3.00	38.64	0.00
2.2	38.64	2.20	38.77	0.13
2.3	39.04	2.30	39.12	0.08

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


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**

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


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**

**HAEMOPHILUS AH
POLYSACCHARIDE DERIVATIVE**

lot number : FA316550

Date of manufacture : 20.06.08
Storage temperature : $\leq -35^{\circ}\text{C}$
Expiry date : 20 JUNE 2010
Volume : 1.847 liters

CONTROLS :

Residual cyanides content

Method : Gas Chromatography, cyanide or cyanate ions are transformed into cyanogen chloride by chlorination
Date : 03.07.08
Result : <0.004 $\mu\text{g/ml}$ - Conforms

Polysaccharide content

Method : Calculated from the theoretical phosphorus content of PRP
Date : 30.06.08
Result : 9.9 mg/ml

Percentage of polysaccharide linked ADH (Adipic DiHydrazide acid)

Method : Colorimetry
Date : 18.09.08
Result : 2.38 % - Conforms

Free ADH/total ADH ratio : 0.031 - Conforms


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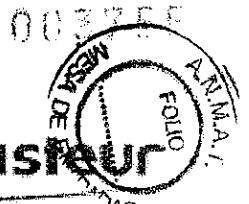
Molecular size

Method	: size exclusion Chromatography
Date	: 28.07.08
Result : - Percentage of polysaccharide eluted before K_d 0.20	: 1.70 % - Conforms
- K_d of polysaccharide	: 0.51 - Conforms

Haemophilus identification

Method	: Double diffusion in gel
Date	: 26.06.08
Result	: Positive - Conforms





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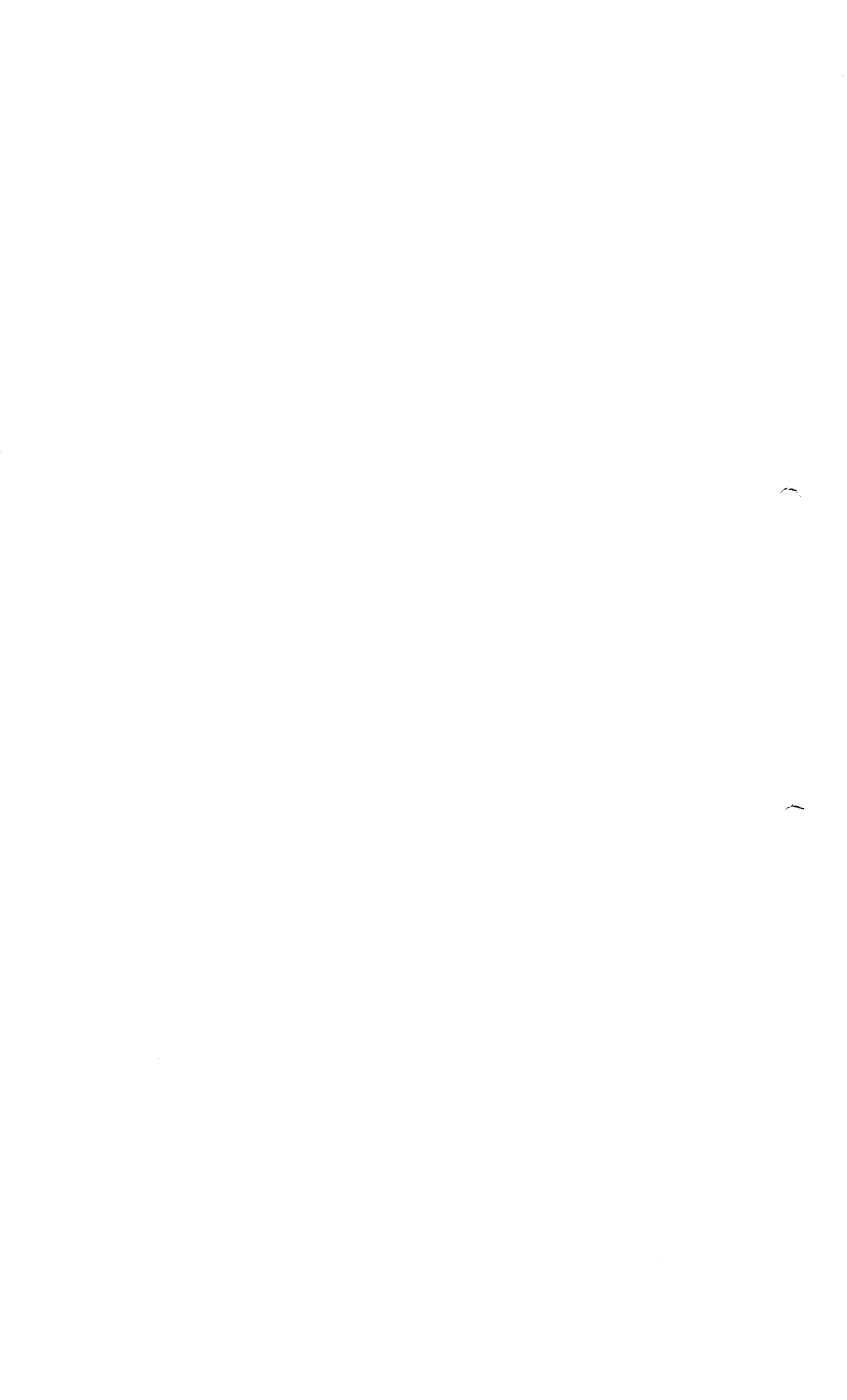
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HAEMOPHILUS POLYSACCHARIDE CONJUGATE (CONCENTRATED BULK)

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


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**

**HAEMOPHILUS POLYSACCHARIDE
 CONJUGATE (CONCENTRATED BULK)**

lot number : FA328210

Date of manufacture : 22.10.08
 Storage temperature : ≤ -35°C
 Expiry date : 22 OCTOBER 2011
 Volume : 22,517 liters

CONTROLS :

pH

Method : Glass electrode
 Date : 06.11.08
 Result : 7.02 - Conforms

Phosphorus content

Method : Chen
 Date : 03.11.08
 Result : 16.8 µg/ml

Polysaccharide content

Method : Calculated from the theoretical phosphorus
 content of PRP
 Date : 04.11.08
 Result : 200.0 µg/ml

Sucrose content

Method : quantitative analysis of glucose before and after enzymatic
 hydrolysis
 Date : 05.11.08
 Result : 84.18 g/l


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Protein/Polysaccharide ratio : 2.3 - Conforms

Residual EDAC content (Ethyl-DimethylAminopropyl-Carbodiimide)

Method : Wilcheck
Date : 07.11.08
Result : <10.0 µmol/l - Conforms

Residual phenol content

Method : Colorimetry
Date : 05.11.08
Result : <1.0 µg/ml - Conforms

Free Polysaccharide content

Method : High Performance Anion Exchange
Chromatography with Pulsed Amperometric
Detection method (HPAEC-PAD)
Date : 17.11.08
Result : <4.0 % - Conforms

Molecular size

Method : size exclusion chromatography
Date : 03.12.08
Result : 76.7 % - Conforms

Free tetanus protein content

Method : polyacrylamide gel electrophoresis SDS-PAGE
Date : 02.12.08
Result : <1.0% - Conforms

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