

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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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



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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 : FA281302

Date of manufacture : 20.07.07
 Storage temperature : ≤ -35°C
 Expiry date : 24 MAY 2009
 Quantity : 87.590 g

CONTROLS :

Phosphorus content

Method : Chen
 Date : 31.07.07
 Result : 8.20 % - Conforms

Ribose content


Method : Mejbaum
 Date : 02.08.07
 Result : 41.7 % - 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 : 0.10 % - Conforms


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**

Bacterial endotoxins

Method : LAL
 Date : 10.09.07
 Result : 1.41 IU/µg – Conforms

Molecular size

Method : size exclusion Chromatography
 Date : 06.08.07
 Result : 65.2 % - 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 : 30.07.07
 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)
1.9	38.81	1.90	38.91	0.10
2.1	39.02	2.10	39.30	0.28
2.0	39.16	2.00	39.55	0.39

The sum of the 3 individual maximum temperature rises : 0.77 °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 : FA316546

Date of manufacture : 16.06.08
Storage temperature : ≤ -35°C
Expiry date : 16 JUNE 2010
Volume : 2.136 liters

CONTROLS :

Residual cyanides content

Method : Gas Chromatography, cyanide or cyanate ions are transformed into cyanogen chloride by chlorination
Date : 01.07.08
Result : <0.004 µg/ml - **Conforms**

Polysaccharide content

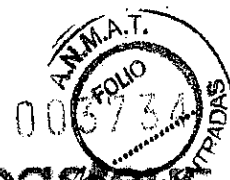
Method : Calculated from the theoretical phosphorus content of PRP
Date : 23.06.08
Result : 9.7 mg/ml

Percentage of polysaccharide linked ADH (Adipic DiHydrazide acid)

Method : Colorimetry
Date : 05.09.08
Result : 2.68 % - **Conforms**

Free ADH/total ADH ratio : 0.032 - Conforms

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Molecular size

Method	: size exclusion Chromatography
Date	: 22.07.08
Result : - Percentage of polysaccharide eluted before K_d 0.20	: 1.40 % - Conforms
- K_d of polysaccharide	: 0.52 - Conforms

Haemophilus identification

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

	
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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 : FA327148

Date of manufacture : 09.10.08
 Storage temperature : ≤ -35°C
 Expiry date : 09 OCTOBER 2011
 Volume : 22.347 liters

CONTROLS :

pH

Method : Glass electrode
 Date : 20.10.08
 Result : 7.04 - Conforms

Phosphorus content



Method : Chen
 Date : 20.10.08
 Result : 15.6 µg/ml

Polysaccharide content

Method : Calculated from the theoretical phosphorus
 content of PRP
 Date : 22.10.08
 Result : 185.71 µg/ml

Sucrose content

Method : quantitative analysis of glucose before and after enzymatic
 hydrolysis
 Date : 23.10.08
 Result : 85.57 g/l



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Protein/Polysaccharide ratio : 2.2 - Conforms

Residual EDAC content (Ethyl-DimethylAminopropyl-Carbodiimide)

Method : Wilcheck
 Date : 27.10.08
 Result : <10.0 µmol/l - Conforms

Residual phenol content

Method : Colorimetry
 Date : 22.10.08
 Result : <1.0 µg/ml - Conforms

Free Polysaccharide content


Method : High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection method (HPAEC-PAD)
 Date : 22.01.09
 Result : <4.3 % - Conforms


Molecular size

Method : size exclusion chromatography
 Date : 02.12.08
 Result : 75.4 % - Conforms

Free tetanus protein content

Method : polyacrylamide gel electrophoresis SDS-PAGE
 Date : 23.10.08
 Result : <1.0% - Conforms


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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	:		13.10.08
Test off	:		27.10.08
Result	:	No microbial growth – Conforms	

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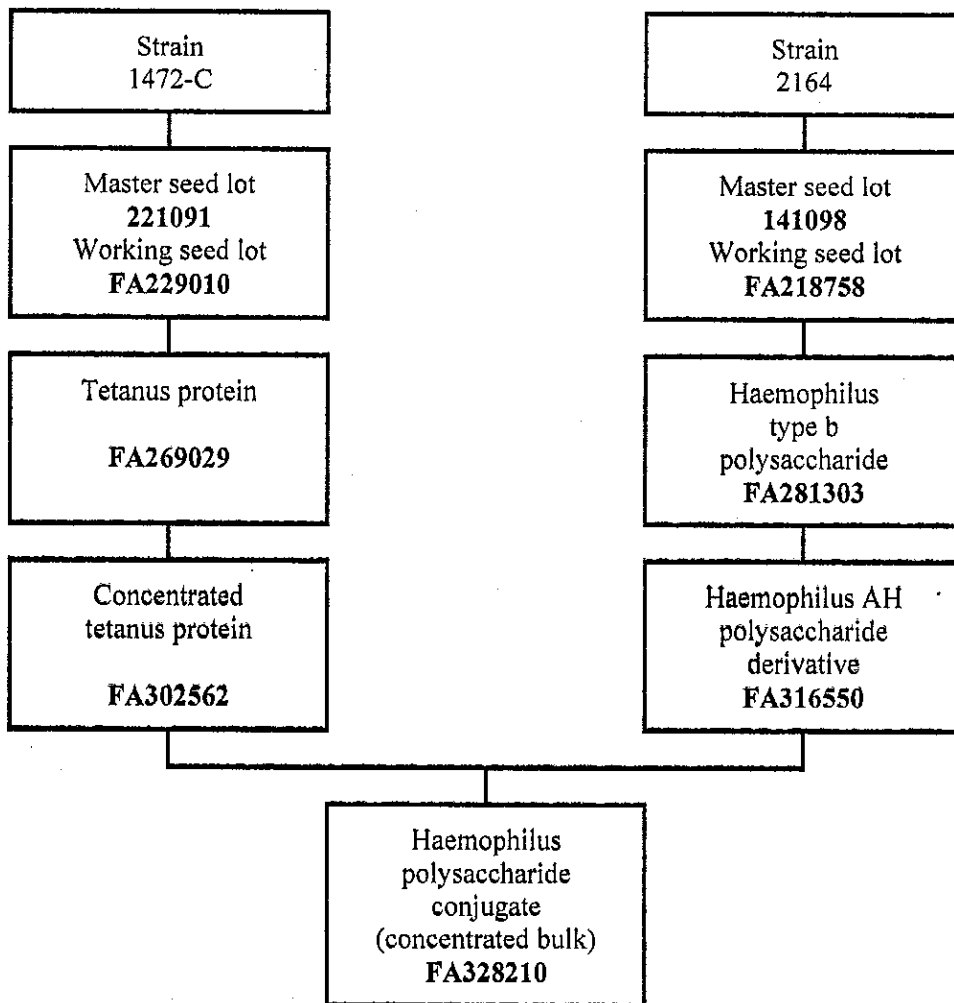
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PRODUCTION FLOW SHEET



μ


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**

HAEMOPHILUS INFLUENZAE
Working seed lot n° FA218758

Master seed Lot n° : 141098
Preparation date : 09.09.05
Release date : 21.11.05

CONTROLS

Purity

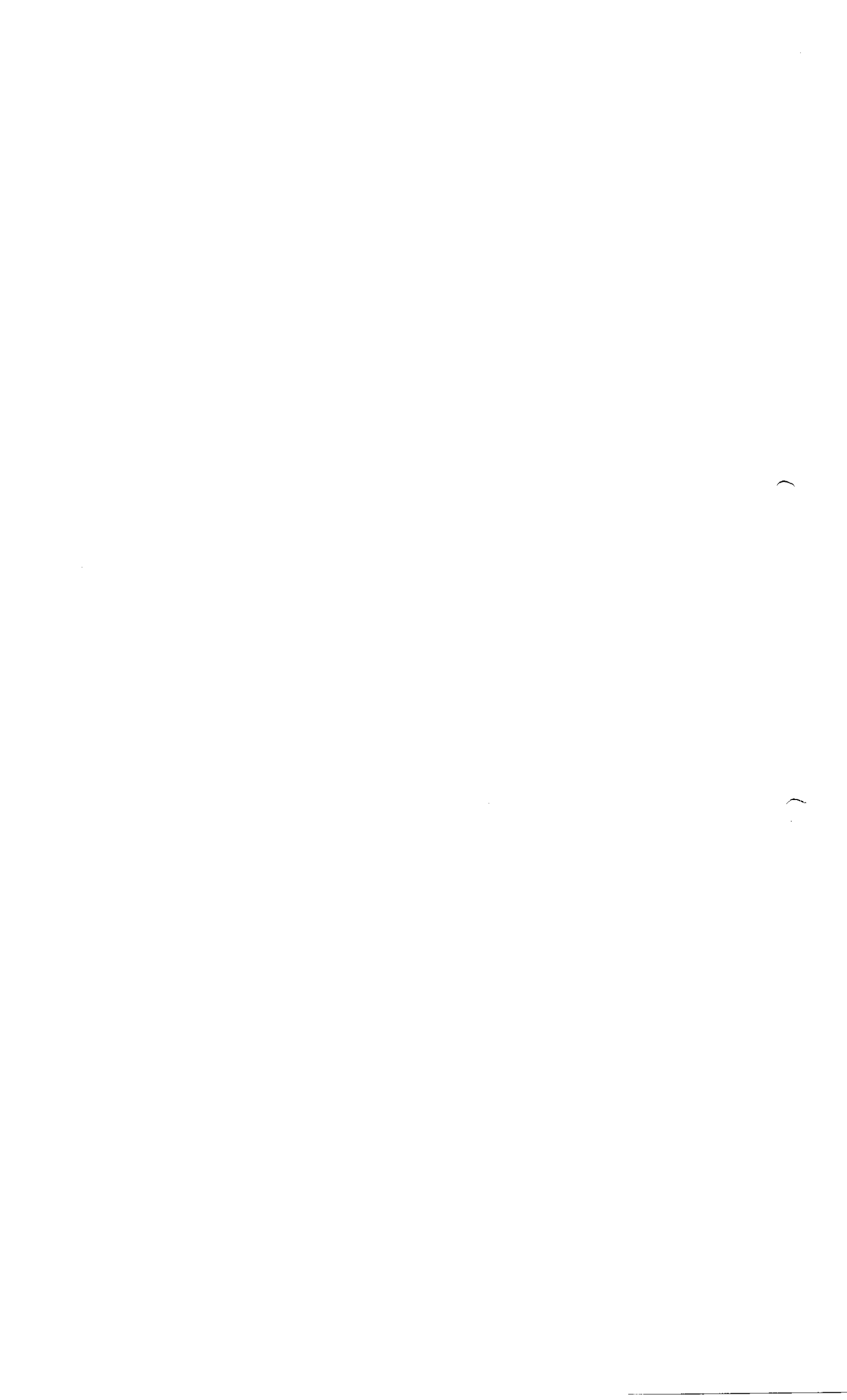
Identification

- 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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**

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

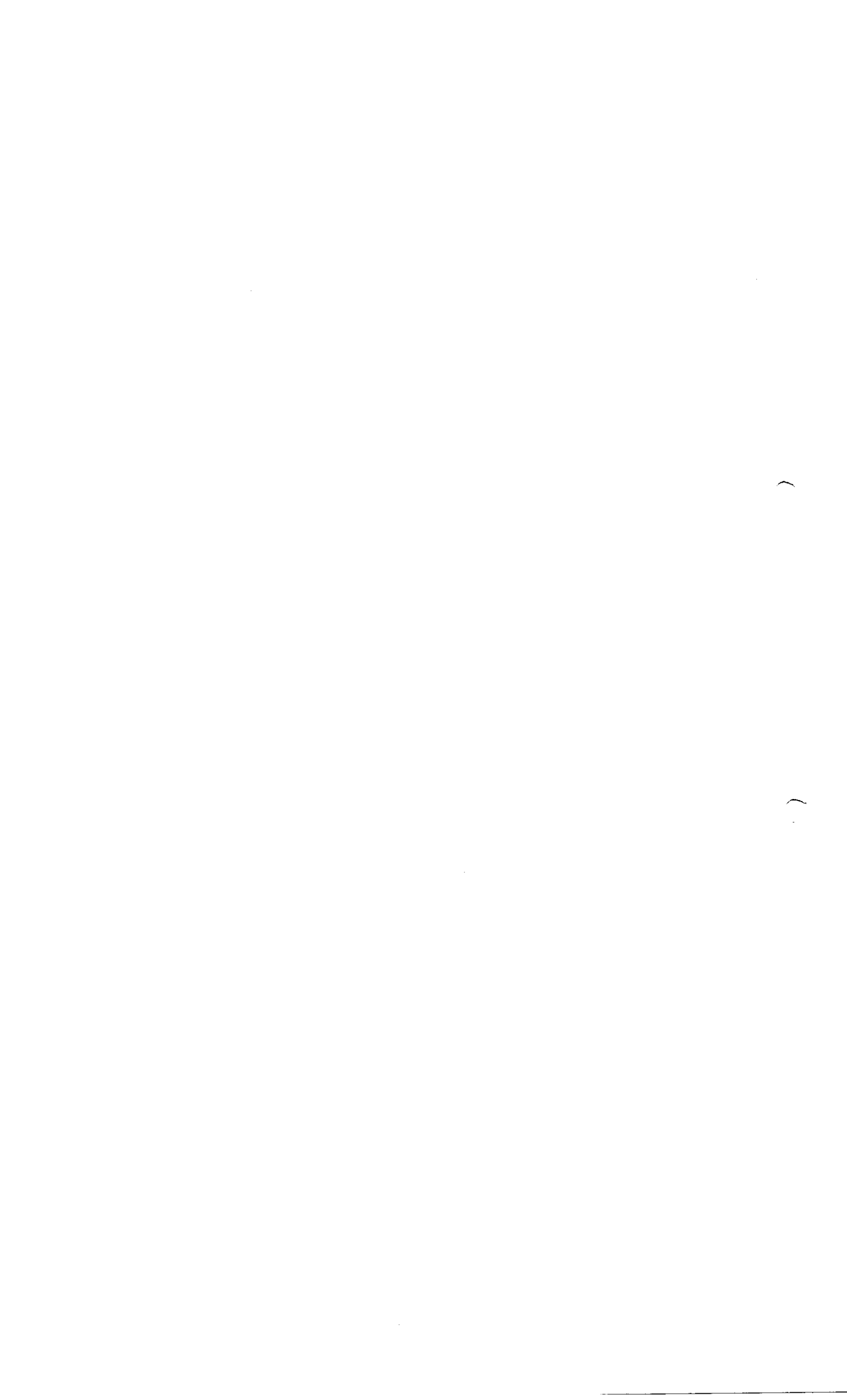
TETANUS TOXOID
Master seed lot n° 221091

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

CONTROLS

- Purity
Identification
- Morphology
 - Growth characteristics
 - Biochemical characteristics

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TETANUS TOXOID
Working seed lot n°FA229010

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

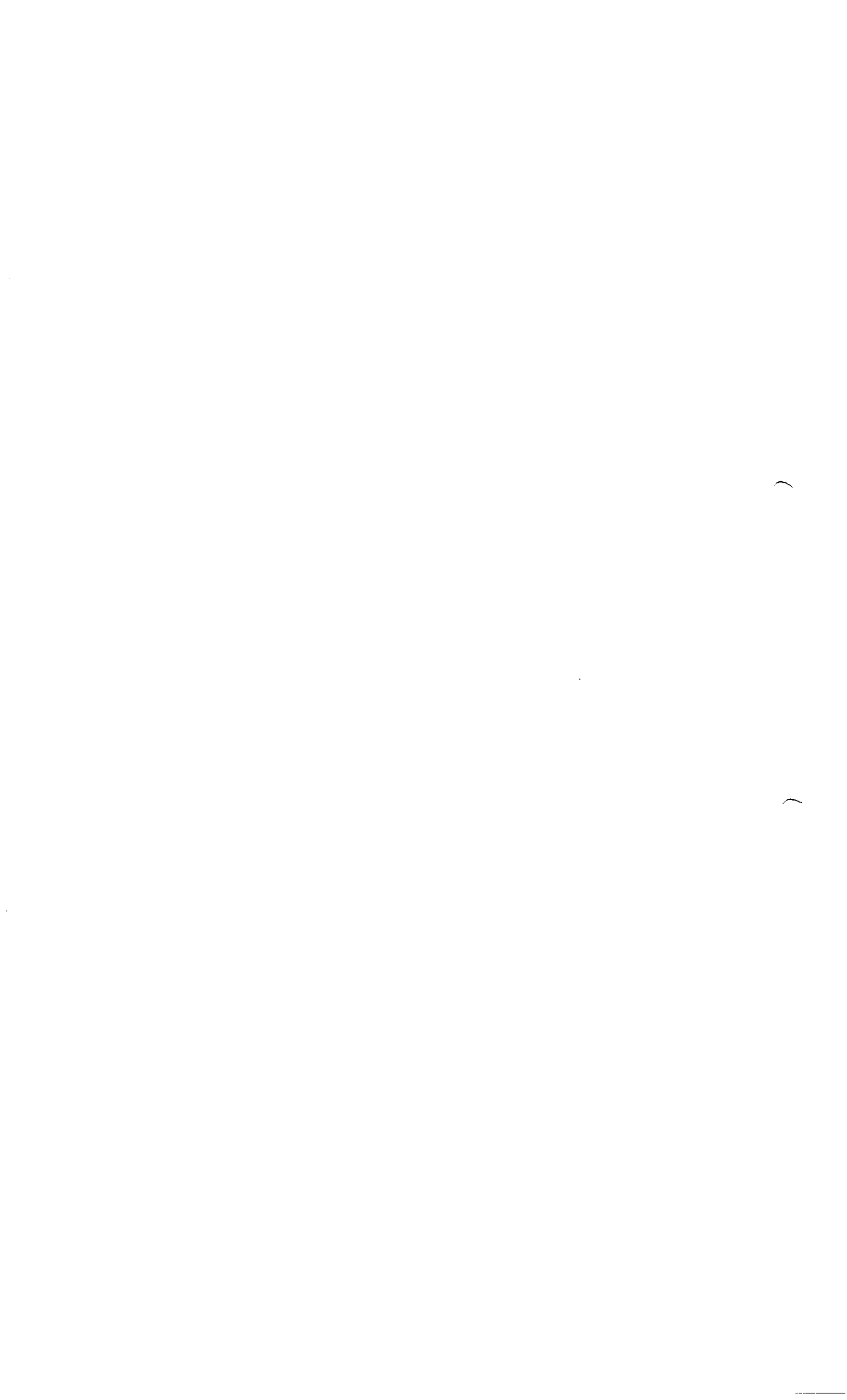
CONTROLS

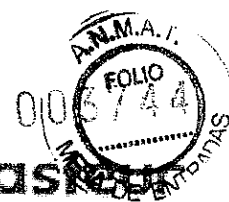
- Purity
Identification
- Morphology
 - Growth characteristics
 - Biochemical characteristics

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


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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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