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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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Diphtheria, tetanus, pertussis (acellular, component), hepatitis B (rDNA),poliomyelitis (inactivated) and Haemophilus influenzae type b conjugate vaccine (adsorbed) lot n° S4312 (clinical batch) 213/229

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HAEMOPHILUS POLYSACCHARIDE CONJUGATE (CONCENTRATED BULK)

lot number : **FA327102**

Date of manufacture	: **11.10.08**
Storage temperature	: ≤ -35°C
Expiry date	: **11 OCTOBER 2011**
Volume	: **29.470** liters

CONTROLS :

pH

Method	: Glass electrode
Date	: **20.10.08**
Result	: **7.01 - Conforms**

Phosphorus content

Method	: Chen
Date	: **20.10.08**
Result	: **16.9** µg/ml

Polysaccharide content

Method	: Calculated from the theoretical phosphorus content of PRP
Date	: **22.10.08**
Result	: **201.19** µg/ml

Sucrose content

Method	: quantitative analysis of glucose before and after enzymatic hydrolysis
Date	: **23.10.08**
Result	: **88.93** g/l

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Protein/Polysaccharide ratio : **2.2 - Conforms**

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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	: **<4.0 % - Conforms**

Molecular size

Method	: size exclusion chromatography
Date	: **02.12.08**
Result	: **78.3 % - 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	:	**15.10.08**	
Test off	:	**29.10.08**	
Result	:	**No microbial growth – Conforms**	

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PART 2

FINAL BULK PRODUCT

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FINAL BULK

TESTS	SPECIFICATIONS
OSMOLALITY	300 - 400 mosmol/kg
FREE FORMALDEHYDE CONTENT	≤30 µg/mL
BACTERIAL AND FUNGAL STERILITY	No microbial growth
HISTAMINE-SENSITIZING ACTIVITY	≥95% survival
NON ADSORBED POLYRIBOSYL RIBITOL PHOSPHATE (PRP) DEPOLYMERIZED PRP	≥8.0 µg/dose <20.0%
PERCENT ADSORPTION – DIPHTHERIA TOXOID	≥23% At time of release, this acceptance criterion was not defined
PERCENT ADSORPTION – HEPATITIS B	≥64% At time of release, this acceptance criterion was not defined
DIPHTHERIA POTENCY	Activity ≥30 IU/dose Lower confidence limit (P = 0.95) of the estimated potency ≥ 20 IU/dose
TETANUS POTENCY	Lower confidence limit (P = 0.95) of the estimated potency ≥ 40 IU/dose
IMMUNOGENICITY PERTUSSIS IN MICE	Anti-PT and anti-FHA antibody GMTs for the vaccine are not significantly lower than those of the reference vaccine (analysis of variance) at the level 5%.
D-ANTIGEN CONTENT	TYPE 1 : 20.0 – 43.0 DU/DOSE TYPE 2 : 5.0 – 9.0 DU/DOSE TYPE 3 : 17.0 – 36.0 DU/DOSE
HEPATITIS B IN VITRO RELATIVE POTENCY (IVRP)	≥0.70 At time of release, this acceptance criterion was not defined

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FINAL BULK

Lot number : FDV01398

Date of manufacture : **05.11.2009**
Storage temperature : + 5°C ± 3°C
Expiry date : **05 August 2010**

Composition

INGREDIENT	Lot number	Volume (ml)
Purified Diphtheria Toxoid	**FA293607**	**2 728**
Purified Tetanus Toxoid	**FA269112**	**1 000**
Adsorbed Pertussis Toxoid	**FA329595**	**18 058**
Adsorbed FHA Pertussis	**FA308424**	**17 402**
Concentrated Polio Trivalent (5C)	**FA327604**	**50 020**
Hepatitis B Surface Antigene C	**AC002** **AC007** **AC011**	**1 142** **1 138** **1 100**
Haemophilus Polysaccharide Conjugate	**FA316432** **FA327102**	**16 258** **14 912**
Aluminium hydroxide	**FA365289**	**34 239**
Buffer solution (Disodium hydrogen phosphate and potassium dihydrogen phosphate)	**FA363235**	**20 001**
Essential Amino acids solution	**FA363507**	**25 050**
Buffer solution (trometamol and saccharose)	**FA363236**	**6 266**
Water for injections	**ABS2E307A** **ABS2E308A**	**40 761**
APPROXIMATE TOTAL VOLUME		**242 100**

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CONTROLS :

Osmolality

Method	: Measure of the freezing point reduction
Date	: **19.11.2009**
Result	: **338** mosmol/kg - **Conforms**

Free formaldehyde content

Method	: Spectrophotometry / colorimetric assay
Date	: **17.11.2009**
Result	: **3.23** µg/ml - **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	:	**13.11.2009**	
Test off	:	**27.11.2009**	
Result	:	**No microbial growth – Conforms**	

Histamine-sensitizing activity

Injection (Day 0)

Animals and sex	: mice CD1 - Female
Number of animals	: **20**
Route	: Intraperitoneal
Dose tested per mouse	: 2 human doses (1ml)
Date of injection	: **23.12.2009**

Histamine challenge (Day 5)

Challenge dose per mouse	: 2 mg histamine base
Route	: Intraperitoneal
Date of injection	: **28.12.2009**

Reading date (Day 6)	: **29.12.2009**
Result	: **100** % of survival – **Conforms**

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Polysaccharide content :

Method	: High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection method (HPAEC-PAD)
Standard	: CP09E055
Date	: **10.12.2009**
Non adsorbed PRP	: **11.3** µg/dose
Depolymerized PRP	: **9.1** % - **Conforms**

Percent adsorption – Diphtheria toxoid:

Method	: Rocket Immunoelectropheresis method
Date	: **17.11.2009**
Result	: **46** %

Percent adsorption – Hepatitis B:

Method	: ELISA
Date	: **18.11.2009**
Result	: **95** %

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Diphtheria potency

Method	: European Pharmacopoeia
Strain and sex of animals	: Guinea pigs - Dunkin-Hartley - female
Weight	: 250 - 350 g
Number of animals per dose	: 8

Immunization

Date	: **09.11.2009**
Volume tested	: 1 ml
Route	: Subcutaneous

Challenge

Date	: **07.12.2009**
Challenge dose	: 0.0512 - 0.0128 - 0.0032 - 0.0008 0.0002 and 0.00005 Lf/0.2 ml
Route	: Intradermal
Reading date	: **09.12.2009**

Results	Dilutions	Score	Log ED_{50}
Reference toxoid **BRP – D4** **97 IU/ml**	**1/16**	**29/48**	**- 1.613**
	1/32	**25/48**	
	1/64	**19/48**	
	1/128	**6/48**	
Product tested **FDV01398**	**1/17.5**	**28/48**	**- 1.553**
	1/35	**26/48**	
	1/70	**13/48**	
	1/140	**0/48**	

Potency	: **42** IU/dose
Confidence limits (P=0.95)	: **34 - 52** IU/dose

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Tetanus potency

Method	: European Pharmacopoeia
Strain and sex of animals	: Mice Swiss OF1 - Female
Age	: 5 weeks
Number of animals per dose	: 16

Immunization

Date	: **16.11.2009**
Volume	: 0.5 ml
Route	: Subcutaneous

Challenge

Date	: **14.12.2009**
Challenge dose	: **1/320**
Volume	: 0.5 ml
Route	: Subcutaneous
Reading date	: **18.12.2009**

Results	Dilutions	Score/Total	Log ED_{50}
Reference toxoid **NIBSC-T 98/552** **250 IU/ml**	**1/25**	**16/16**	**- 1.982**
	1/50	**14/16**	
	1/100	**7/16**	
	1/200	**0/16**	
Product tested **FDV01398**	**1/150**	**12/16**	**- 2.630**
	1/300	**11/16**	
	1/600	**5/16**	
	1/1200	**1/16**	

Potency : **556** IU/dose
Confidence limits (P=0.95) : **280 - 853** IU/dose

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Immunogenicity Pertussis in mice

Animals and sex	: Mice Swiss OF1- Female
Weight	: 20 - 22 g
Number of animals per group	: 10

Immunization

Date	: **12.11.2009**
Volume	: 0.5 ml
Route	: Intraperitoneal
Reference vaccine	: **97-1**
Dose per mouse	: 1/5 human dose

Bleeding date : **10.12.2009**

Titration

1. Anti-PT titers

Method	: ELISA
Pertussis toxin for coating	: **FA287985**
Standard anti-PT serum	: **BRP BATCH1**
Date	: **04.01.2010**
Results	
Reference vaccine	: **199** EU/ml (**131 - 302**)
Product tested	: **146** EU/ml (**101 - 209**)
Probability	: **0.22 (p >0.05)**

2. Anti-FHA titers

Method	: ELISA
FHA for coating	: **FA088651**
Standard anti-FHA serum	: **BRP BATCH1**
Date	: **17.12.2009**
Results	
Reference vaccine	: **295** EU/ml (**206 - 423**)
Product tested	: **303** EU/ml (**187 - 491**)
Probability	: **>0.50 (p >0.05)**

Conclusion : **Conforms**

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Diphtheria, tetanus, pertussis (acellular, component), hepatitis B (rDNA),poliomyelitis (inactivated) and Haemophilus influenzae type b conjugate vaccine (adsorbed) lot n° S4312 (clinical batch) 224/229

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D Antigen content

Method : ELISA
Standard : **11.07.07 versus EWS**
Date : **02.03.2010 to 09.03.2010**

	D Antigen unit/dose		
	Type 1	Type 2	Type 3
Date	**02.03.2010**	**02.03.2010**	**09.03.2010**
Product tested	**26.9**	**5.5**	**26.3**

Result : **Conforms**

Hepatitis B in vitro Relative Potency

Method : ELISA
Date : **18.11.2009**
Result : **Conforms**

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PART 3

FINAL PRODUCT

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FINAL PRODUCT

TESTS	SPECIFICATIONS
APPEARANCE	Whitish and cloudy suspension
pH	6.5 - 7.5
EXTRACTABLE VOLUME	At least the nominal volume
ALUMINIUM CONTENT	0.40 - 0.80 mg/dose
BACTERIAL AND FUNGAL STERILITY	No microbial growth
PYROGEN TEST	Conforms to Ph. Eur. criterion
ABNORMAL TOXICITY TEST	No death nor sickness sign At time of release, the test was performed
IDENTITY OF EACH VALENCE : DIPHTHERIA, TETANUS, PERTUSSIS, POLIOMYELITIS, HEPATITIS B, HAEMOPHILUS	Positive

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