

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) 50/211**

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HEPATITIS B SURFACE ANTIGEN

Lot n° AC005

Strain : K3/8-1
Working seed lot number : MWCB 230600BA
Date of reconstitution of working seed lot vials: November 20th, 2007
Temperature incubation : 28 to 32°C

Harvest

Date of inoculation in last stage of fermentation : November 23rd, 2007
 Date of harvest : November 26th, 2007
 Volume : 50 liters

CONTROLS

Microbiological purity

Method : Direct inoculation
 Result : Absence of microbiological contamination

Identification of Hansenula Polymorpha

Method : Biochemical and microscopic Tests
 Result : Conforms


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HANSENULA POLYMORPHA
Working seed lot n°MWCB 230600BA

Master seed lot	: MCB 160600BA
Preparation date	: June 23 rd , 2000
Release date	: June 29 th , 2001

CONTROLS

- Purity . **Conforms**
- Identification : **Conforms**
- Full integration of the vector : **Conforms**
- Number of copies of the plasmid in the genome : **Conforms**
- Integrated cDNA gene sequence : **Conforms**
- Sequencing of the regions 3' and 5' : **Conforms**
- Determination of the population of yeast cells containing the cloned gene: **Conforms**

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

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HANSENULA POLYMORPHA
Strain : K3/8-1

STRAIN


Name : Hansenula Polymorpha RB11
Origin : Rhein Biotech
Received : February 15th, 2000


HANSENULA POLYMORPHA
Master seed lot n° MCB 160600BA

Strain : IM K3/8-1
Preparation date : June 16th, 2000
Release date : June 29th, 2001

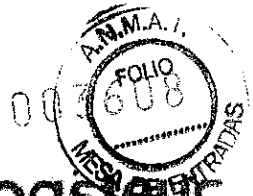
CONTROLS

- Purity : **Conforms**
- Identification : **Conforms**
- Full integration of the vector : **Conforms**
- Number of copies of the plasmid in the genome : **Conforms**
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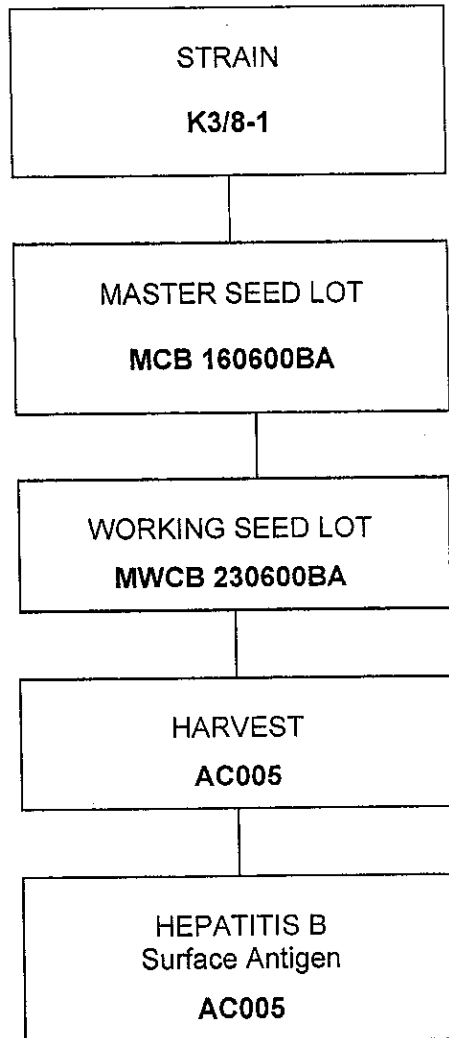
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PRODUCTION FLOW SHEET

[Signature]
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COORDINADOR
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- D - HEPATITIS B


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ADSORBED PURIFIED FHA :

CONTROLS :

Aluminium content

Method : Complexometry
 Date : 23.09.09
 Result : 1.09 mg Al/mg of protein – **Conforms**

pH

Method : Glass electrode
 Date : 21.09.09
 Result : 6.84 - **Conforms**

FHA identification


Method : Double Diffusion in Gel
 Date : 08.10.09
 Result : **Positive - Conforms**


Residual dermonecrotic activity

Method : Specific toxicity
 Test on : 28.09.09
 Test off : 01.10.09
 Result : **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	:	16.09.09	
Test off	:	30.09.09	
Result	:	No microbial growth - Conforms	


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ADSORBED PURIFIED FHA
Lot Number : FA360425

Date of manufacture : 11.09.2009
 Storage temperature : + 5°C + 3°C
 Expiry date : 11 SEPTEMBER 2013
 Volume : 44.3 liters

PURIFIED FHA IN SOLUTION :

CONTROLS :

Protein content

Method : Spectrophotometry
 Date : 08.09.09
 Result : 836.79 µg/ml - Conforms

Bacterial endotoxins


Method : LAL
 Date : 16.09.09
 Result : <5.98 IU/mg of protein


Antigenicity

Method : ELISA
 Date : 08.10.09

	EU/mg of protein
Lower confidence limit (P=0.95) of the specific activity of SP batches	38.5
Product	154.4

Result : **Conforms**


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ADSORBED PURIFIED FHA

TESTS	SPECIFICATIONS
PURIFIED FHA IN SOLUTION	
PROTEIN CONTENT	350 - 2 100 µg/ml
BACTERIAL ENDOTOXINS	≤ 150 UI/mg of protein
ANTIGENICITY	Specific activity (EU/mg of proteins) ≥ lower confidence limit (P = 0.95) of the specific activity of SP batches
ADSORBED PURIFIED FHA	
ALUMINIUM CONTENT	0.6 - 1.4 mg Al/mg of protein
pH	6.2 - 8.2
FHA IDENTIFICATION	Positive
RESIDUAL DERMONECROTIC ACTIVITY	No necrotic reaction (++++) or marked haemorrhagic reaction (+++) in any of the suckling mice 72h after injection - conforms
BACTERIAL AND FUNGAL STERILITY	No microbial growth


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NATIVE PURIFIED FHA

Lot number : FA351898

Date of manufacture : **25.06.2009**
 Storage temperature : **+ 5°C ± 3°C**
 Expiry date : **25 JUNE 2013**
 Volume : **43.67 liters**

CONTROLS :

Protein content

Method : Spectrophotometry
 Date : **02.07.09**
 Result : **1289.59 mg/ml**

SDS-page

Method : Polyacrylamide Gel Electrophoresis
 Date : **21.07.09**
 Result : **Electrophoresis profile comparable to the profile
 of the reference (one major band at about 220
 kDa ≥ 70 % of total and some minor bands) -
 Conforms**

FHA identification

Method : Double Diffusion in Gel
 Date : **24.07.09**
 Result : **Positive - Conforms**

Clustering activity on Chinese Hamster Ovary cells

Method : Clusters Assay on CHO Cells
 Date : **22.07.09**
 Result : **No cytopathic effect**

Haemagglutinating activity

Method : Haemagglutination of goose red blood cells by PT
 Date : **23.07.09**
 Result : **1024326 HAU/mg**
 IHA with cholesterol : **98 % - Conforms**



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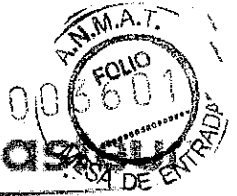
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NATIVE PURIFIED FHA

TESTS	SPECIFICATIONS
PROTEIN CONTENT	For calculation
SDS-PAGE	Electrophoresis profile comparable to the profile of the reference (one major band at about 220 kDa \geq 70 % of total and some minor bands)
FHA IDENTIFICATION	Positive
CLUSTERING ACTIVITY ON CHO CELLS	No cytopathic effect
HAEMAGGLUTINATING ACTIVITY	200 000 – 2 500 000 HAU/mg of protein IHA with cholesterol : \geq 80 %


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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	:		21.10.08
Test off	:		04.11.08
Result	:	No microbial growth - Conforms	


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