

**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)

45/180

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**- D - HEPATITIS B**

  
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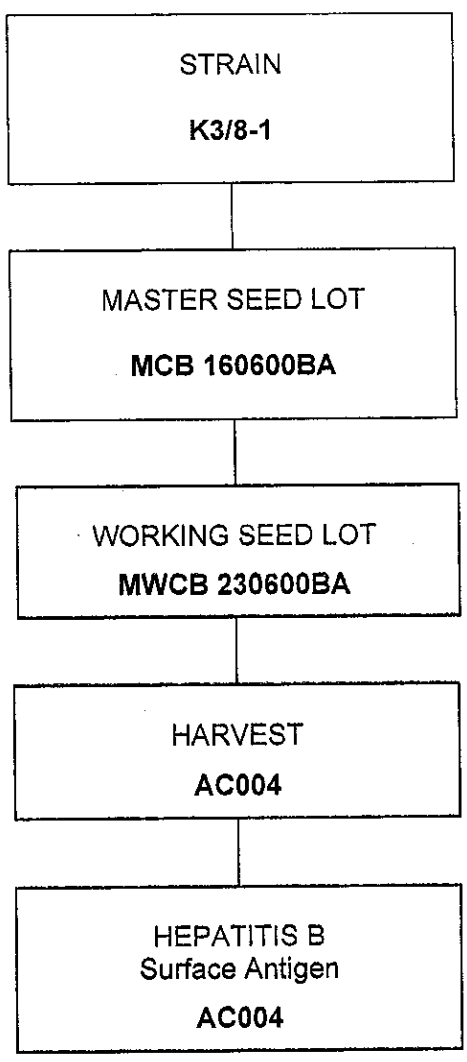




Product Quality and Disposition Department

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hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus  
influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch)

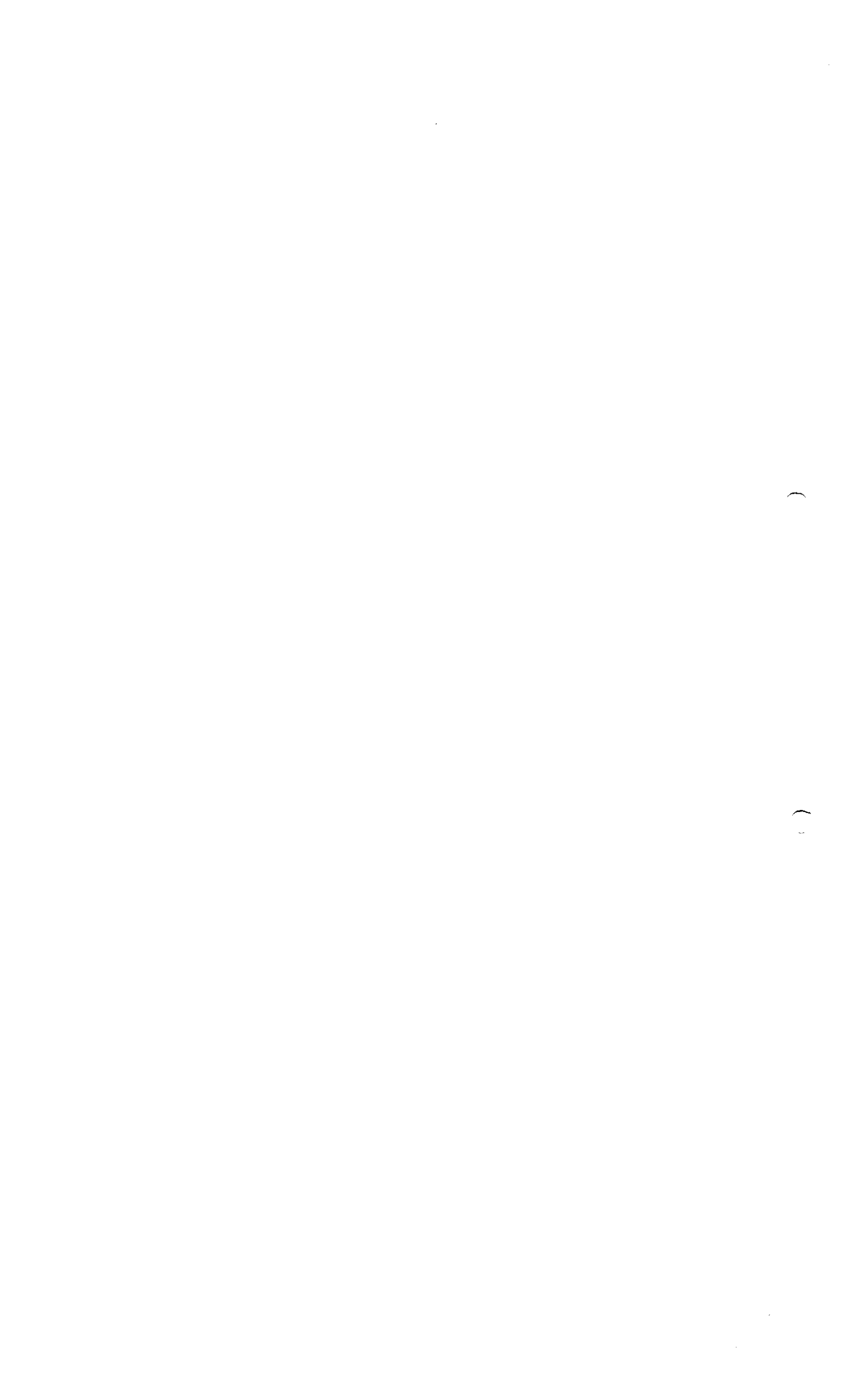
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**PRODUCTION FLOW SHEET**

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hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus  
influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch) 47/180  
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HANSENULA POLYMORPHA  
Strain : K3/8-1

**STRAIN**

Name : Hansenula Polymorpha RB11  
Origin : Rhein Biotech  
Received : February 15<sup>th</sup>, 2000

HANSENULA POLYMORPHA  
Master seed lot n° MCB 160600BA

Strain : IM K3/8-1  
Preparation date : June 16<sup>th</sup>, 2000  
Release date : June 29<sup>th</sup>, 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**

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Diphtheria, tetanus, pertussis (acellular, component),  
hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus  
influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch) 48/180

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**HANSENULA POLYMORPHA**  
**Working seed lot n°MWCB 230600BA**

Master seed lot : MCB 160600BA  
Preparation date : June 23<sup>rd</sup>, 2000  
Release date : June 29<sup>th</sup>, 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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influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch) 49/180  
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**HEPATITIS B SURFACE ANTIGEN**  
**Lot n° AC004**

Strain : K3/8-1  
Working seed lot number : MWCB 230600BA  
Date of reconstitution of working seed lot vials: November 13<sup>th</sup>, 2007  
Temperature incubation : 28 to 32°C

**Harvest**

Date of inoculation in last stage of fermentation : November 16<sup>th</sup>, 2007  
Date of harvest : November 19<sup>th</sup>, 2007  
Volume : 50 liters


**CONTROLS**


**Microbiological purity**

Method : Direct Inoculation  
Result : Absence of microbiological contamination

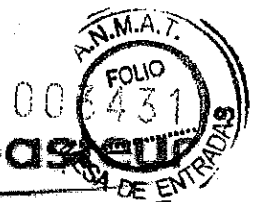
**Identification of Hansenula Polymorpha**

Method : Biochemical and microscopic Tests  
Result : Conforms

  
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hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus  
influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch) 50/180  
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**HEPATITIS B SURFACE ANTIGEN**

TESTS	SPECIFICATIONS
APPEARANCE	Opalescent, slightly cloudy and yellowish solution
pH MEASUREMENT	6.5 - 7.5
PROTEIN CONTENT	1.05 – 1.80 mg/mL
IDENTIFICATION OF HBsAg	Positive
	Main bands of molecular weight around 23 kD, 46 kD and eventually 65 kD
HBsAg CONTENT	≥0.7 mg of HBsAg/mg of total proteins
CARBOHYDRATES CONTENT	≤50 µg of carbohydrates/mg of total proteins
LIPIDS CONTENT	0.5 – 0.9 mg lipids/mg of total proteins
PURITY	≥95%
PERCENTAGE OF HBsAg FREE MONOMER	≤3%
BACTERIAL AND FUNGAL STERILITY TEST	Absence of microbial growth
BACTERIAL ENDOTOXINS	<10 EU/dose (i.e. 10 EU/10µg of proteins)

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Diphtheria, tetanus, pertussis (acellular, component),  
 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus  
 influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch)

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**HEPATITIS B SURFACE ANTIGEN**  
  
**Lot number : AC004**

Date of manufacture : December 26<sup>th</sup>, 2007  
 Storage temperature : + 5°C ± 3°C  
 Expiry date : November 2010  
 (shelf life used at the time of the release of the batch)  
 Quantity: : 3,65745 g of proteins

**CONTROLS :**

**Appearance**

Method : Visual inspection  
 Date : March 17<sup>th</sup>, 2008  
 Result : Opalescent, slightly cloudy and yellowish solution

**pH**

Method : Glass electrode  
 Date : March 17<sup>th</sup>, 2008  
 Result : 6,9 - Conforms

**Protein content**

Method : Lowry  
 Date : February 26<sup>th</sup>, 2008  
 Result : 1,46 mg/ml - Conforms

**Identification of HBsAg**

Method : ELISA  
 Date : February 27<sup>th</sup>, 2008  
 Result : Positive

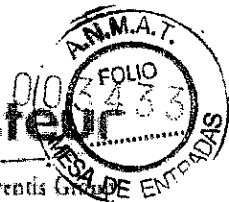
Method : SDS - PAGE  
 Date : January 27<sup>th</sup>, 2008  
 Result : Conforms

  
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Diphtheria, tetanus, pertussis (acellular, component),  
 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus  
 influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch)

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**HBsAg Content**

Method : ELISA  
 Date : February 27<sup>th</sup>, 2008  
 Result : 1,1 mg/mg of total proteins – **Conforms**

**Carbohydrates Content**

Method : Colorimetric assay  
 Date : February 28<sup>th</sup>, 2008  
 Result : 33 µg/mg of total proteins – **Conforms**

**Lipids Content**

Method : Colorimetric assay  
 Date : February 28<sup>th</sup>, 2008  
 Result : 0,8 mg/mg of total proteins – **Conforms**

**Purity Content**

Method : SDS - PAGE  
 Date : February 27<sup>th</sup>, 2008  
 Result : **96 %**

**Percentage of HBsAg free monomer**

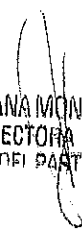
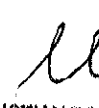
Method : SDS - PAGE  
 Date : February 28<sup>th</sup>, 2008  
 Result : Less than 3 % - **Conforms**

**Bacterial and fungal sterility**

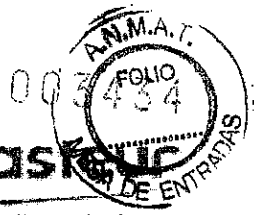
Method	:	Membrane filtration
Media	:	Thioglycollate medium   Soybean-Casein Digest medium
Temperature	:	30-35° C   20-25° C
Inoculum per Steritest	:	10 ml   10 ml
Volume of medium per Steritest	:	100 ml   100 ml
Test on	:	January 29 <sup>th</sup> , 2008
Test off	:	February 12 <sup>th</sup> , 2008
Result	:	No microbial growth - <b>Conforms</b>

**Bacterial Endotoxins**

Method : LAL – Gel clot  
 Date : January 31<sup>st</sup>, 2008  
 Result : <1 EU/20µg of proteins- **Conforms**


  
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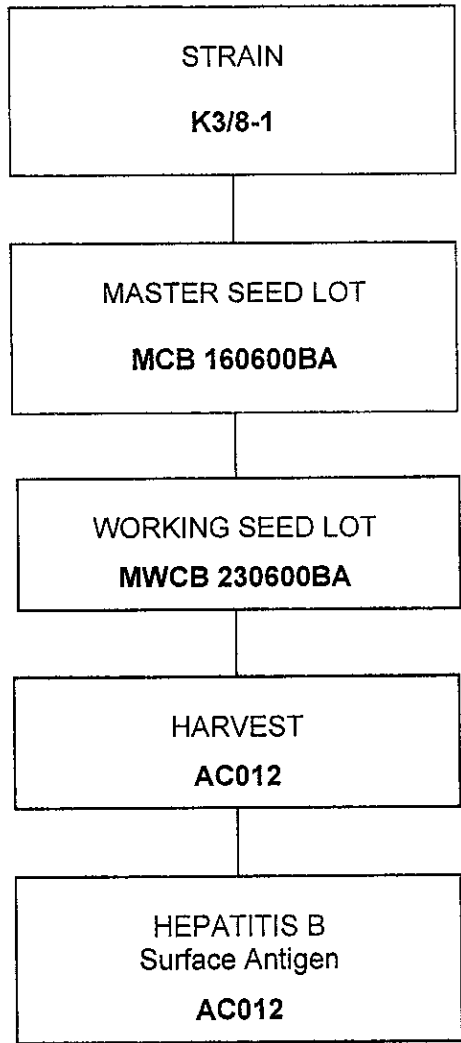


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Diphtheria, tetanus, pertussis (acellular, component),  
hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus  
influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch)


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PRODUCTION FLOW SHEET

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

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**HANSENULA POLYMORPHA**  
Strain : K3/8-1

**STRAIN**

Name : Hansenula Polymorpha RB11  
Origin : Rhein Biotech  
Received : February 15<sup>th</sup>, 2000

**HANSENULA POLYMORPHA**  
Master seed lot n° MCB 160600BA

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

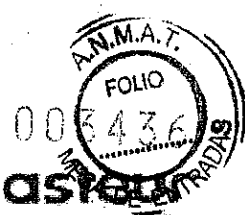
**CONTROLS**

- 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**

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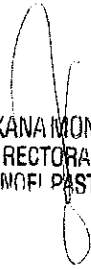
HANSENULA POLYMORPHA  
Working seed lot n°MWCB 230600BA


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

CONTROLS

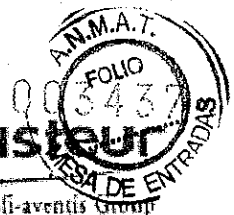
- Purity . **Conforms**
- Identification : **Conforms**
- Full integration of the vector : **Conforms**
- Number of copies of the plasmid in the genome : **Conforms**
- Integrated cDNA gene sequence : **Conforms**
- Sequencing of the regions 3' and 5' : **Conforms**
- Determination of the population 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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influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch) 56/180

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**HEPATITIS B SURFACE ANTIGEN**  
**Lot n° AC012**

Strain : K3/8-1  
Working seed lot number : MWCB 230600BA  
Date of reconstitution of working seed lot vials: March 25<sup>th</sup>, 2008  
Temperature incubation : 28 to 32°C

**Harvest**

Date of inoculation in last stage of fermentation : March 28<sup>th</sup>, 2008  
Date of harvest : March 31<sup>st</sup>, 2008  
Volume : 50 liters

**CONTROLS**

**Microbiological purity**

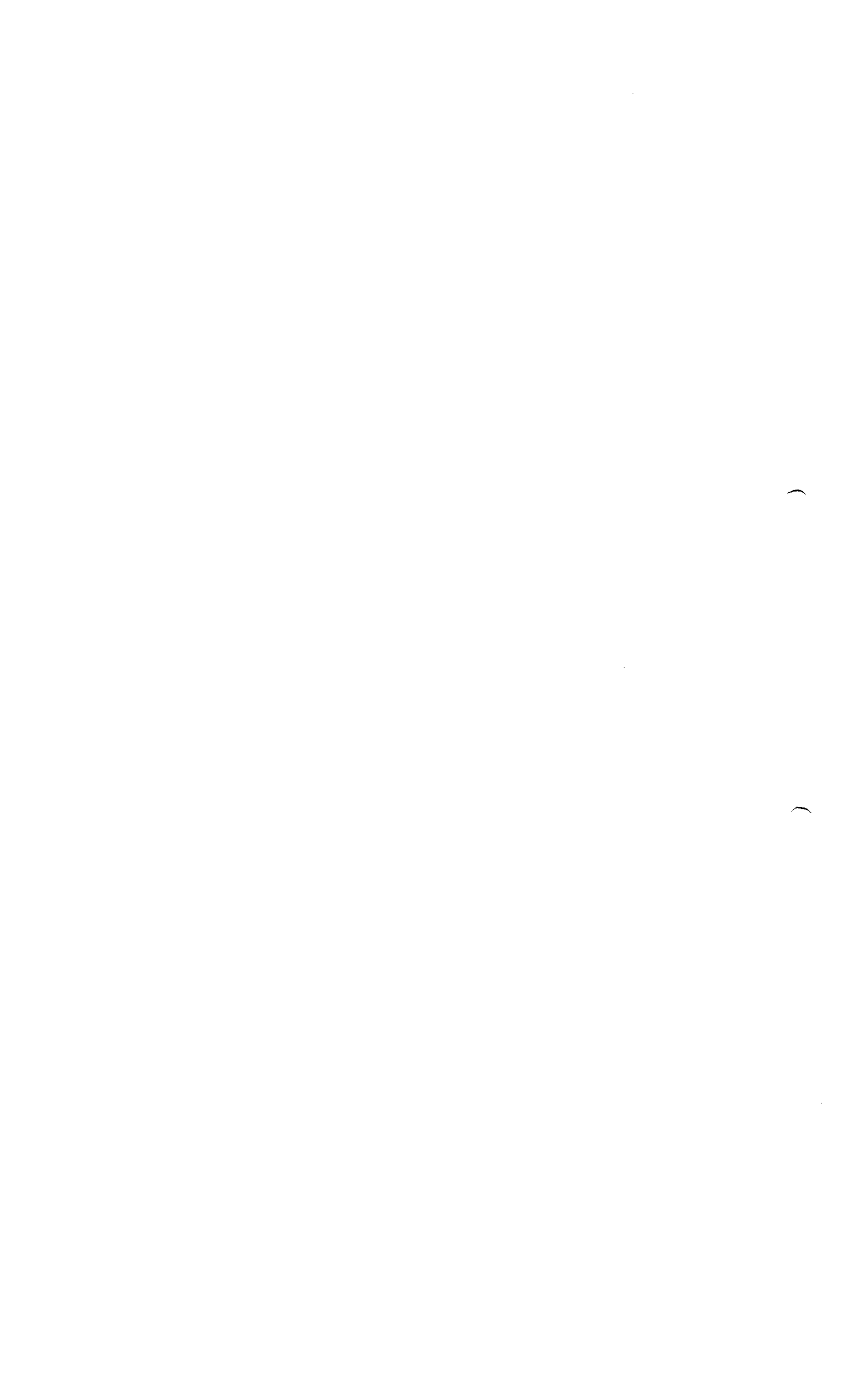
Method : Direct Inoculation  
Result : Absence of microbiological contamination

**Identification of *Hansenula Polymorpha***

Method : Biochemical and microscopic Tests  
Result : Conforms

  
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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)

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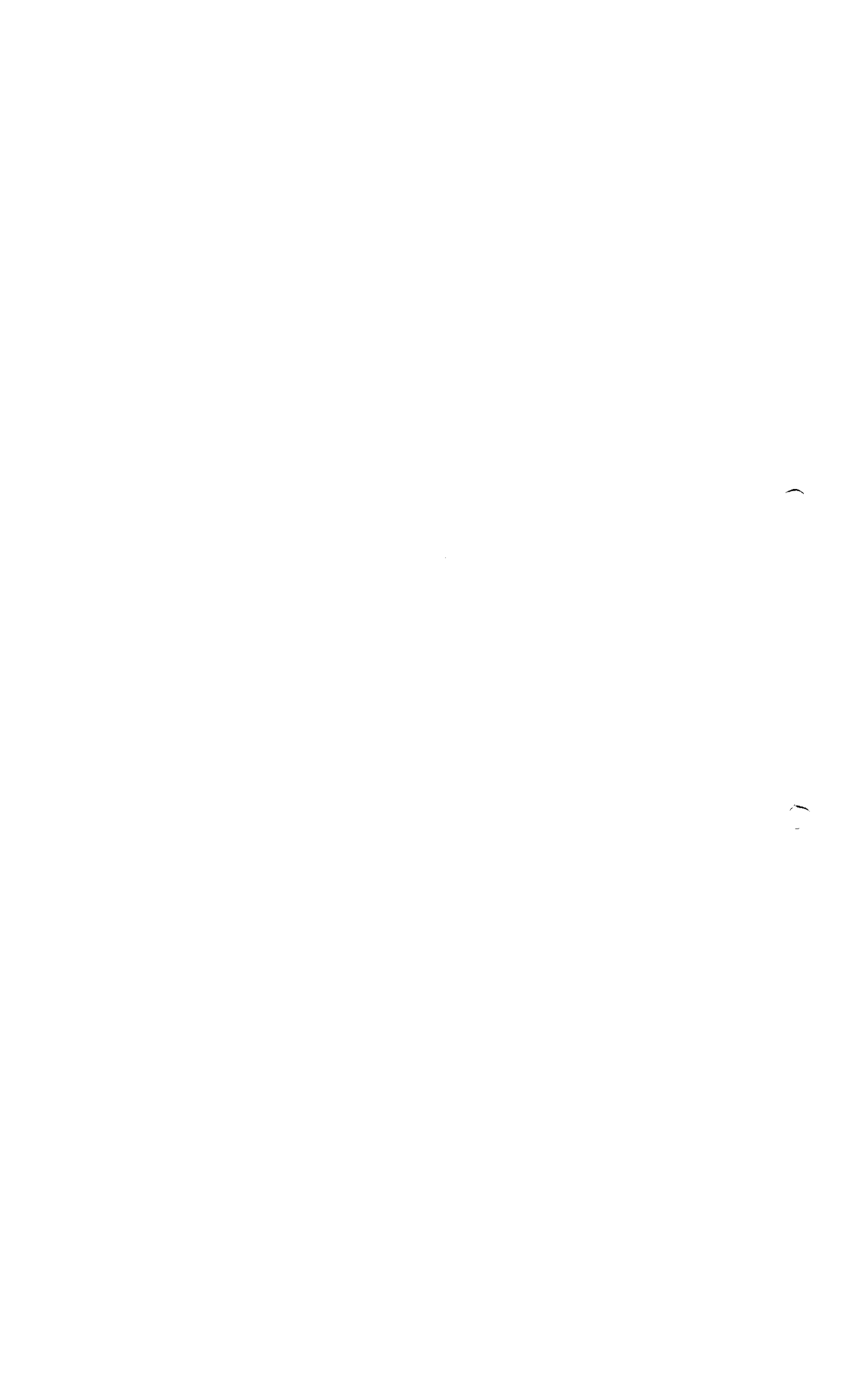
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**HEPATITIS B SURFACE ANTIGEN**

TESTS	SPECIFICATIONS
APPEARANCE	Opalescent, slightly cloudy and yellowish solution
pH MEASUREMENT	6.5 - 7.5
PROTEIN CONTENT	1.05 – 1.80 mg/mL
IDENTIFICATION OF HBsAg	Positive
	Main bands of molecular weight around 23 kD, 46 kD and eventually 65 kD
HBsAg CONTENT	≥0.7 mg of HBsAg/mg of total proteins
CARBOHYDRATES CONTENT	≤50 µg of carbohydrates/mg of total proteins
LIPIDS CONTENT	0.5 – 0.9 mg lipids/mg of total proteins
PURITY	≥95%
PERCENTAGE OF HBsAg FREE MONOMER	≤3%
BACTERIAL AND FUNGAL STERILITY TEST	Absence of microbial growth
BACTERIAL ENDOTOXINS	<10 EU/dose (i.e. 10 EU/10µg of proteins)



  
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 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus  
 influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch)

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**HEPATITIS B SURFACE ANTIGEN**  
 Lot number : AC012

Date of manufacture : May 3<sup>rd</sup>, 2008  
 Storage temperature : + 5°C ± 3°C  
 Expiry date : April 2011  
 (shelf life used at the time of the release of the batch)  
 Quantity: : 3,35779 g of proteins

**CONTROLS :**

Appearance

Method : Visual inspection  
 Date : May 8<sup>th</sup>, 2008  
 Result : **Opalescent, slightly cloudy and yellowish solution**

pH

Method : Glass electrode  
 Date : May 8<sup>th</sup>, 2008  
 Result : **6,9 - Conforms**


Protein content


Method : Lowry  
 Date : June 19<sup>th</sup>, 2008  
 Result : **1,51 mg/ml - Conforms**

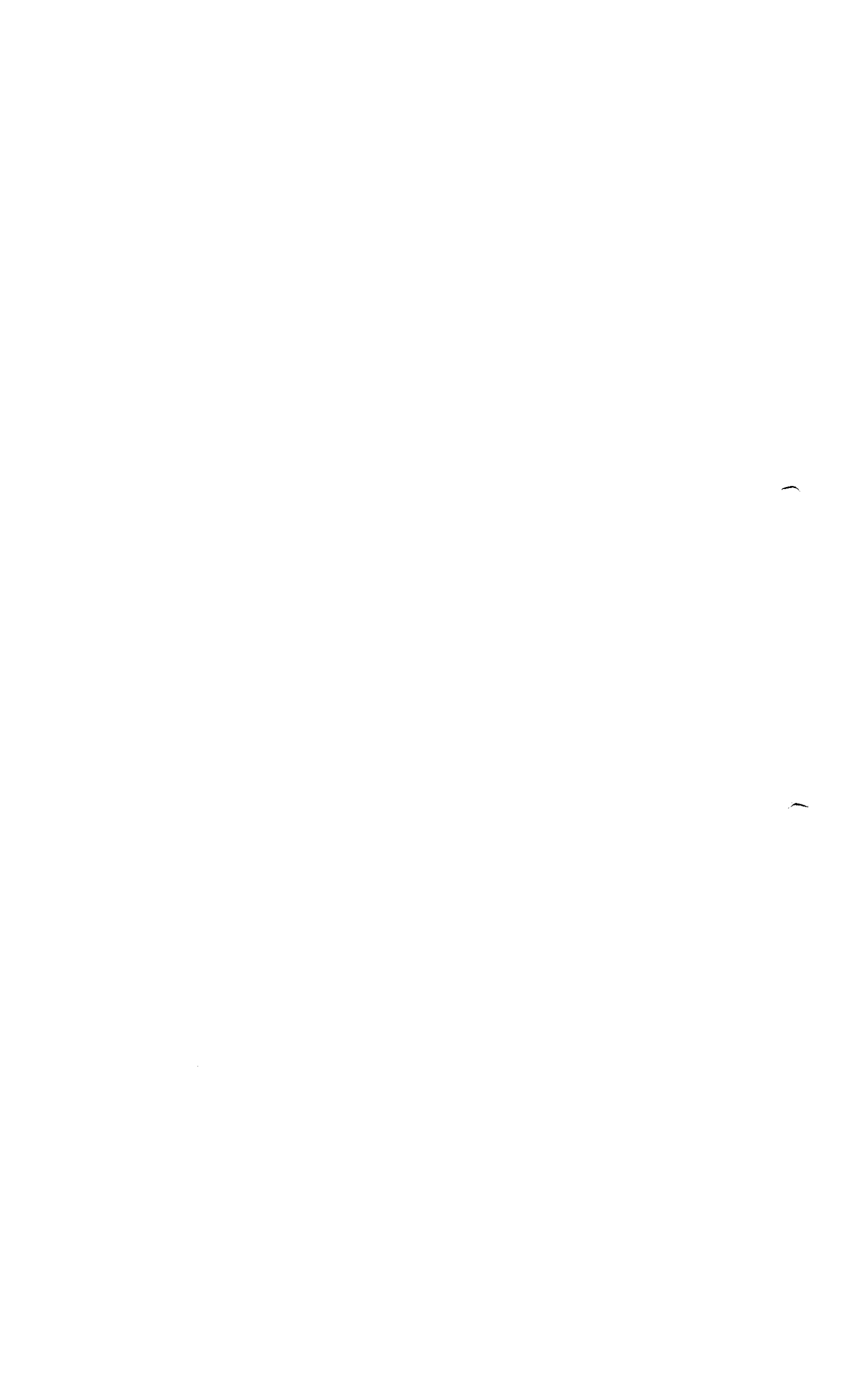
Identification of HBsAg

Method : ELISA  
 Date : August 30<sup>th</sup>, 2008  
 Result : **Positive**

Method : SDS - PAGE  
 Date : August 30<sup>th</sup>, 2008  
 Result : **Conforms**

  
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 influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch) 59/180  
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**HBsAg Content**

Method : ELISA  
 Date : August 30<sup>th</sup>, 2008  
 Result : 1,1 mg/mg of total proteins – **Conforms**

**Carbohydrates Content**

Method : Colorimetric assay  
 Date : August 30<sup>th</sup>, 2008  
 Result : 33 µg/mg of total proteins – **Conforms**

**Lipids Content**

Method : Colorimetric assay  
 Date : September 6<sup>th</sup>, 2008  
 Result : 0,7 mg/mg of total proteins – **Conforms**

**Purity Content**

Method : SDS - PAGE  
 Date : August 30<sup>th</sup>, 2008  
 Result : 97 %

**Percentage of HBsAg free monomer**


Method : SDS - PAGE  
 Date : August 30<sup>th</sup>, 2008  
 Result : Less than 3 % - **Conforms**


**Bacterial and fungal sterility**

Method	:	Membrane filtration
Media	:	Thioglycollate medium   Soybean-Casein Digest medium
Temperature	:	30-35° C   20-25° C
Inoculum per Steritest	:	10 ml   10 ml
Volume of medium per Steritest	:	100 ml   100 ml
Test on	:	May 9 <sup>th</sup> , 2008
Test off	:	May 23 <sup>rd</sup> , 2008
Result	:	No microbial growth - <b>Conforms</b>

**Bacterial Endotoxins**

Method : LAL – Gel clot  
 Date : May 14<sup>th</sup>, 2008  
 Result : ≤1 EU/20µg of proteins- **Conforms**

  
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**- E - INACTIVATED POLIOMYELITIS TRIVALENT**

  
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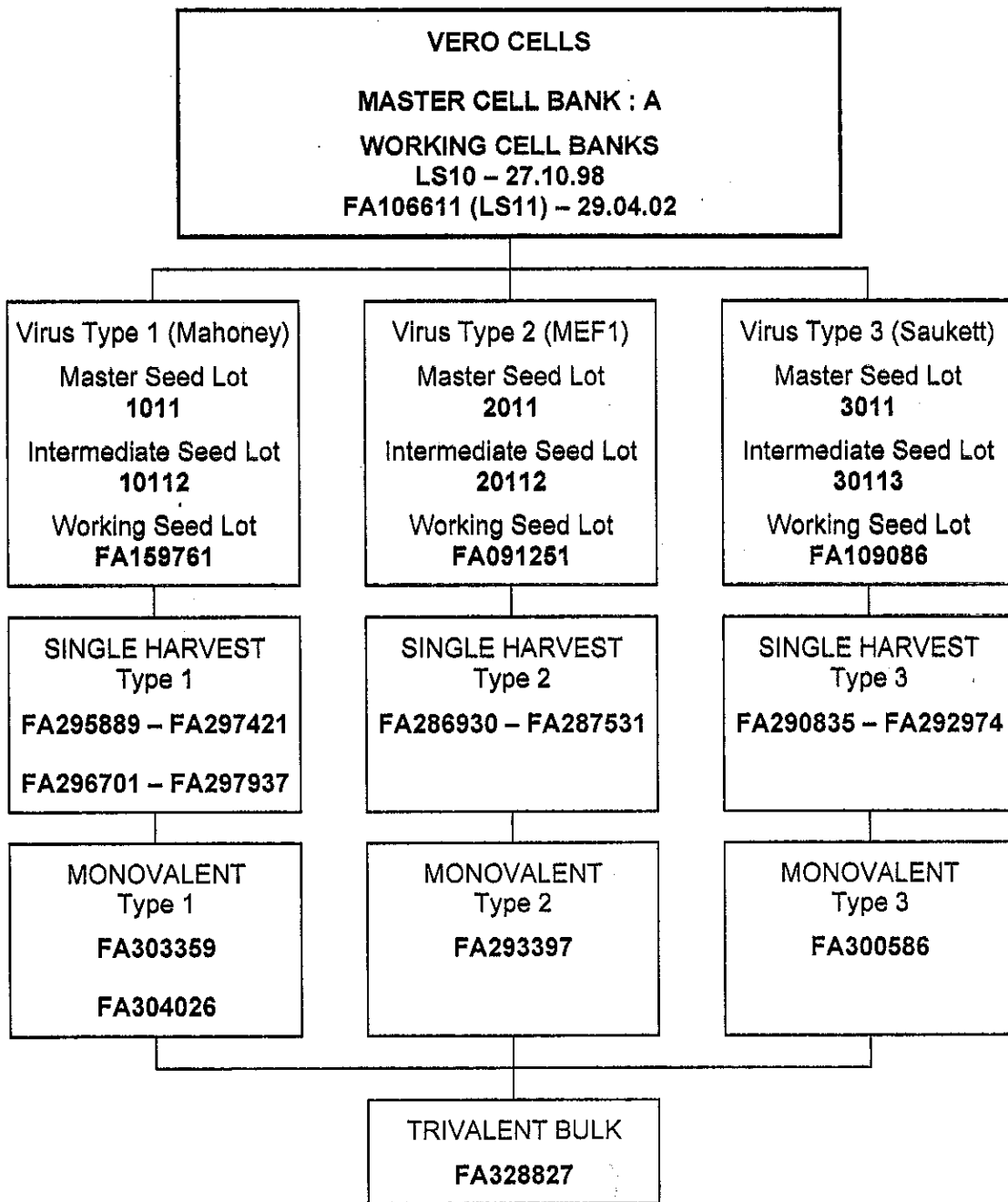



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