



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)

105/211

**

CONTROLS ON SINGLE VIRUS HARVEST:

Polio virus Identification

Method : Cytopathogenic effect neutralization
 Cell strain used : Hep 2
 Antiserum : LEPINE type 2
 Test on : 23.10.07
 Test off : 30.10.07
 Result : Positive

Polio virus concentration

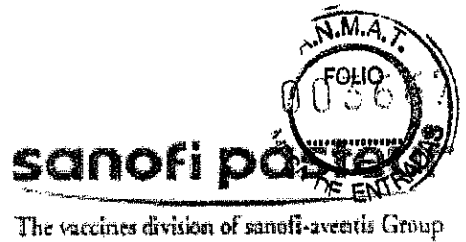
Method : Cytopathogenic effect on sensitive cells
 Cell strain used : Hep 2
 Test on : 23.10.07
 Test off : 30.10.07
 Virus concentration : 10^{9.03} CCID₅₀/ml
 Result : Conforms

Bacterial and fungal sterility

Method used	: 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	: 18.10.07	
Test off	: 05.11.07	
Result	: No microbial growth	

ROXANA MONTEMILONE DIRECTORA TÉCNICA SANOFI PASTEUR S.A.
 CHRISTIAN LUIS RODRIGUEZ RODRIGADO SANOFI PASTEUR S.A.





Product Quality and Disposition Department

The vaccines division of sanofi-aventis Group

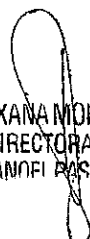

Diphtheria, tetanus, pertussis (acellular, component),
 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
 influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)

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 **

Test for Mycoplasma by culture method

Method	: Agar culture
Volume tested	: 34 ml
Media used	: Hayflick solid Hayflick semi-solid B.O.B. solid B.O.B. semi-solid
Temperature of incubation	: 36 ± 1° C
Positive controls	: <i>M. pneumoniae</i> and <i>M. orale</i> and <i>A. laidlawii</i>
<i>Primary test</i>	
Test on	: 22.04.08
Test off	: 06.05.08
<i>Subculture at 3rd day</i>	
Test on	: 25.04.08
Test off	: 13.05.08
<i>Subculture at 7th day</i>	
Test on	: 29.04.08
Test off	: 13.05.08
<i>Subculture at 14th day</i>	
Test on	: 06.05.08
Test off	: 20.05.08
<i>Subculture at 21th day</i>	
Test on	: 13.05.08
Test off	: 20.05.08
Result	: No growth of Mycoplasma


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 SUPERVISOR
 SANOFI PASTEUR S.A.



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Diphtheria, tetanus, pertussis (acellular, component),
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**

CONTROLS ON CONCENTRATED PURIFIED VIRAL SUSPENSION:

Protein content

Method : Spectrophotometry
 Date : 22.10.07
 Result : 79.56 µg/ml

D Antigen content

Method : ELISA
 Reference standard : 28.1.99
 Date : 29.10.07
 Result : 2 212 D Antigen U/ml

Bacterial and fungal sterility

Method used	: 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	: 19.10.07	
Test off	: 05.11.07	
Result	: No microbial growth	

Purity

Result : 0.036 µg of protein / D Antigen U

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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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**

SINGLE HARVEST - Type : 2
Lot Number : FA287531

CELL:

Working Cell Bank : FA106611
Date of thawing ampoule of WCB : 20.09.07
Thawing ampoule amount : One ampoule

VIRUS:

Virus Working Seed lot : FA091251
Date of inoculation : 15.10.07
Date of virus harvest : 18.10.07

CRUDE HARVEST:

Storage : from 19.10.07 to 22.10.07
Volume : 1 504 L
Storage temperature : +5°C ± 3°C

SINGLE HARVEST:


Date of single harvest : 22.10.07


CONCENTRATION AND PURIFICATION OF THE SINGLE HARVEST

DEAE SpheroDEX large column : Ion exchange chromatography on 23.10.07
CL-6-B Sepharose column : Gel filtration on 24.10.07
DEAE SphéroDEX small column : Ion exchange chromatography on 24.10.07

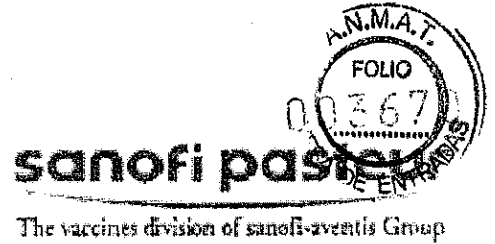
CONCENTRATED PURIFIED VIRAL SUSPENSION :

Manufacture date : 24.10.07
Volume : 19 600 ml
Storage temperature : +5°C ± 3°C


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DIRECTOR FEDERAL
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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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 **

CONTROLS ON CELLS:

Observation


Method : Studied through a microscope
 Cells : Vero, 142nd passage
 Number of cells tested : 320*10⁶ cells
 Test on : 15.10.07
 Test off : 29.10.07
 Result : Absence of cytopathic effect

Test for haemadsorbing viruses

Method : visual inspection
 Number of vials tested : 8
 Type of red blood cells : Guinea-pig
 Incubation : 4 vials, 30 minutes at 5°C ± 3°C
 : 4 vials, 30 minutes at 20-25°C
 Date : 29.10.07
 Result : Absence of hemadsorption

Simian cells identification

Method : Isoenzymes analysis by IsoElectric Focusing
 Iso-enzymes analysed : Lactate DeHydrogenase
 Phospho Gluconate Dehydrogenase
 Glucose Phosphate Isomerase
 Reference cells : Vero, MRC5, WI38, Hep 2
 Date : 29.01.08
 Result : Positive identification



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 DIRECTORA TÉCNICA COORDINADOR
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 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
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 **

CONTROLS ON SUPERNATANTS:

Test for extraneous agents

Vero cells

Method : Studied through a microscope
 Type of cells : Vero
 Volume tested : 10 ml
 Temperature of incubation : 36 ± 1° C
 Test on : 12.11.07
 Test off : 26.11.07
 Result : Absence of cytopathic effect

Human diploid cells

Method : Studied through a microscope
 Type of cells : MRC5
 Volume tested : 10 ml
 Temperature of incubation : 36 ± 1° C
 Test on : 22.01.08
 Test off : 19.02.08
 Result : Absence of cytopathic effect

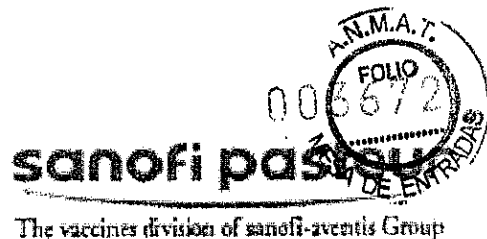
Primary monkey kidney cells

Method : Studied through a microscope
 Type of cells : Vervet Monkey Cells
 Volume tested : 10 ml
 Temperature of incubation : 36 ± 1° C
 Test on : 30.10.07
 Test off : 13.11.07
 Result : Absence of cytopathic effect



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Product Quality and Disposition Department

The vaccines division of sanofi-aventis Group

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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**

Test for Mycoplasma by culture method

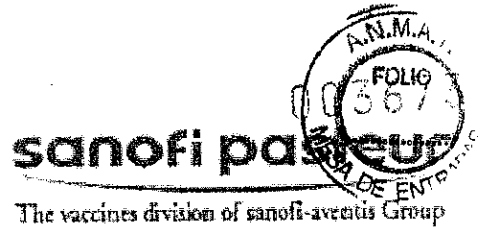
Method	: Agar culture
Volume tested	: 34 ml
Media used	: Hayflick solid Hayflick semi-solid B.O.B. solid B.O.B. semi-solid
Temperature of incubation	: 36 ± 1° C
Positive controls	: <i>M. pneumoniae</i> and <i>M. orale</i> and <i>A. laidlawii</i>
Primary test	
Test on	: 23.05.08
Test off	: 06.06.08
Subculture at 3rd day	
Test on	: 26.05.08
Test off	: 09.06.08
Subculture at 7th day	
Test on	: 30.05.08
Test off	: 13.06.08
Subculture at 14th day	
Test on	: 06.06.08
Test off	: 20.06.08
Subculture at 21th day	
Test on	: 13.06.08
Test off	: 20.06.08
Result	: No growth of Mycoplasma

Test for Mycoplasma by epifluorescence in cell culture

Method	: DNA fluorescence staining
Cell used	: Vero, 152 nd passage
Volume tested	: 1 ml
Temperature of incubation	: 36 ± 1° C
Positive control	: <i>M. hyorhinis</i> and <i>M. orale</i>
Test on	: 22.01.08
Test off	: 29.01.08
Result	: No Mycoplasma detected by epifluorescence in cell culture

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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° S4314 (clinical batch)

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**

CONTROLS ON SINGLE VIRUS HARVEST:

Polio virus Identification



Method : Cytopathogenic effect neutralization
 Cell strain used : Hep 2
 Antiserum : LEPINE type 2
 Test on : 29.10.07
 Test off : 05.11.07
 Result : Positive

Polio virus concentration

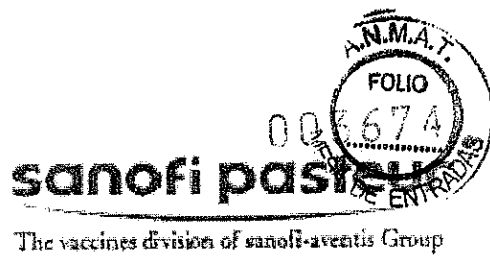
Method : Cytopathogenic effect on sensitive cells
 Cell strain used : Hep 2
 Test on : 29.10.07
 Test off : 05.11.07
 Virus concentration : $10^{8.93}$ CCID₅₀/ml
 Result : **Conforms**

Bacterial and fungal sterility

Method used	:	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	:	24.10.07	
Test off	:	07.11.07	
Result	:	No microbial growth	



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Product Quality and Disposition Department

The vaccines division of sanofi-aventis Group

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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**

Test for Mycoplasma by culture method

Method	: Agar culture
Volume tested	: 34 ml
Media used	: Hayflick solid Hayflick semi-solid B.O.B. solid B.O.B. semi-solid
Temperature of incubation	: 36 ± 1° C
Positive controls	: <i>M. pneumoniae</i> and <i>M. orale</i> and <i>A. laidlawii</i>
 <i>Primary test</i>	
Test on	: 23.05.08
Test off	: 06.06.08
<i>Subculture at 3rd day</i>	
Test on	: 26.05.08
Test off	: 09.06.08
<i>Subculture at 7th day</i>	
Test on	: 30.05.08
Test off	: 13.06.08
<i>Subculture at 14th day</i>	
Test on	: 06.06.08
Test off	: 20.06.08
<i>Subculture at 21th day</i>	
Test on	: 13.06.08
Test off	: 20.06.08
Result	: No growth of Mycoplasma



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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° S4314 (clinical batch)

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**

CONTROLS ON CONCENTRATED PURIFIED VIRAL SUSPENSION:

Protein content

Method : Spectrophotometry
Date : 08.11.07
Result : 80.00 µg/ml

D Antigen content

Method : ELISA
Reference standard : 28.1.99
Date : 05.11.07
Result : 2 124 D Antigen U/ml

Bacterial and fungal sterility

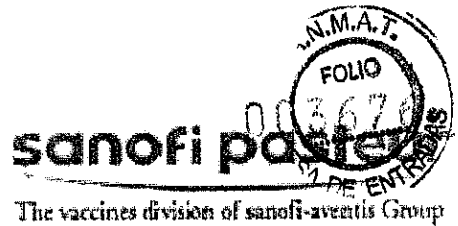
Method used	: Membrane filtration	
Media	: Thioglycollate medium	: Soybean Casein Digest medium
Temperatures	: 30-35° C	: 20-25° C
Volume tested	: 20 ml	
Inoculum per Steritest	: 10 ml	: 10 ml
Volume of medium per Steritest	: 100 ml	: 100 ml
Test on	: 30.10.07	
Test off	: 13.11.07	
Result	: No microbial growth	

Purity

Result : 0.0377 µg of protein / D Antigen U


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 CHRISTIAN DUMÉNIL
 DIRECTOR GENERAL
 SANOFI PASTEUR S.A.



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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**

SINGLE HARVEST - Type : 3
Lot Number : FA290835

CELL:

Working Cell Bank : FA106611
Date of thawing ampoule of WCB : 25.10.07
Thawing ampoule amount : One ampoule

VIRUS:

Virus Working Seed lot : FA109086
Date of inoculation : 19.11.07
Date of virus harvest : 22.11.07

CRUDE HARVEST:

Storage : from 23.11.07 to 26.11.07
Volume : 1 524 L
Storage temperature : +5°C ± 3°C

SINGLE HARVEST:

Date of single harvest : 26.11.07

CONCENTRATION AND PURIFICATION OF THE SINGLE HARVEST

DEAE SpheroDex large column : Ion exchange chromatography on 27.11.07
CL-6-B Sepharose column : Gel filtration on 28.11.07
DEAE SpheroDex small column : Ion exchange chromatography on 28.11.07

CONCENTRATED PURIFIED VIRAL SUSPENSION :

Manufacture date : 28.11.07
Volume : 19 600 ml
Storage temperature : +5°C ± 3°C

Handwritten signatures and stamps for Roxana Montemilone and Christian Duminez, Directors at Sanofi Pasteur S.A.



Product Quality and Disposition Department

The vaccines division of sanofi-aventis Group

Diphtheria, tetanus, pertussis (acellular, component),
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influenzae type b conjugate vaccine (adsorbed) lot n° S4314 (clinical batch)

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**

CONTROLS ON CELLS:

Observation


Method : Studied through a microscope
Cells : Vero, 142nd passage
Number of cells tested : 320*10⁶ cells
Test on : 19.11.07
Test off : 03.12.07
Result : Absence of cytopathic effect


Test for haemadsorbing viruses

Method : visual inspection
Number of vials tested : 8
Type of red blood cells : Guinea-pig
Incubation : 4 vials, 30 minutes at 5°C ± 3°C
 : 4 vials, 30 minutes at 20-25°C
Date : 03.12.07
Result : Absence of hemadsorption

Simian cells identification

Method : Isoenzymes analysis by IsoElectric Focusing
Iso-enzymes analysed : Lactate DeHydrogenase
 Phospho Gluconate Dehydrogenase
 Glucose Phosphate Isomerase
Reference cells : Vero, MRC5, WI38, Hep 2
Date : 10.06.08
Result : Positive identification


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Product Quality and Disposition Department

Diphtheria, tetanus, pertussis (acellular, component),
hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
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**

CONTROLS ON SUPERNATANTS:

Test for extraneous agents

Vero cells

Method : Studied through a microscope
Type of cells : Vero
Volume tested : 10 ml
Temperature of incubation : 36 ± 1° C
Test on : 14.01.08
Test off : 28.01.08
Result : Absence of cytopathic effect

Human diploid cells

Method : Studied through a microscope
Type of cells : MRC5
Volume tested : 10 ml
Temperature of incubation : 36 ± 1° C
Test on : 12.02.08
Test off : 11.03.08
Result : Absence of cytopathic effect

Primary monkey kidney cells

Method : Studied through a microscope
Type of cells : Vervet Monkey Cells
Volume tested : 10 ml
Temperature of incubation : 36 ± 1° C
Test on : 15.01.08
Test off : 29.01.08
Result : Absence of cytopathic effect

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