



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° 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	: MAHONEY type 1
Test on	: 01.10.07
Test off	: 08.10.07
Result	: Positive

Polio virus concentration

Method	: Cytopathogenic effect on sensitive cells
Cell strain used	: Hep 2
Test on	: 01.10.07
Test off	: 08.10.07
Virus concentration	: 10 ^{8.93} CCID ₅₀ /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	:		20.09.07
Test off	:		04.10.07
Result	:	No microbial growth	


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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	: 28.03.08
Test off	: 11.04.08
 <i>Subculture at 3rd day</i>	
Test on	: 31.03.08
Test off	: 14.04.08
 <i>Subculture at 7th day</i>	
Test on	: 04.04.08
Test off	: 18.04.08
 <i>Subculture at 14th day</i>	
Test on	: 11.04.08
Test off	: 25.04.08
 <i>Subculture at 21th day</i>	
Test on	: 18.04.08
Test off	: 25.04.08
Result	: No growth of Mycoplasma


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CONTROLS ON CONCENTRATED PURIFIED VIRAL SUSPENSION :

Protein content

Method : Spectrophotometry
 Date : 02.10.07
 Result : 110.79 µg/ml

D Antigen content


Method : ELISA
 Reference standard : 28.1.99
 Date : 24.09.07
 Result : 9 963 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	:	21.09.07	
Test off	:	05.10.07	
Result	:	No microbial growth	

Purity

Result : 0.011 µg of protein / D Antigen U


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SINGLE HARVEST - Type : 1
Lot Number : FA285976

CELL:

Working Cell Bank : **FA106611**
 Date of thawing ampoule of WCB : **22.08.07**
 Thawing ampoule amount : **One ampoule**

VIRUS:

Virus Working Seed lot : **FA159761**
 Date of inoculation : **17.09.07**
 Date of virus harvest : **20.09.07**

CRUDE HARVEST:

Storage : **from 20.09.07 to 24.09.07**
 Volume : **1 495 L**
 Storage temperature : **+5°C ± 3°C**

SINGLE HARVEST:


Date of single harvest : **24.09.07**

CONCENTRATION AND PURIFICATION OF THE SINGLE HARVEST

DEAE SpheroDex large column : **Ion exchange chromatography on 25.09.07**
 CL-6-B Sepharose column : **Gel filtration on 26.09.07**
 DEAE SphéroDex small column : **Ion exchange chromatography on 26.09.07**

CONCENTRATED PURIFIED VIRAL SUSPENSION :

Manufacture date : **26.09.07**
 Volume : **19 600 ml**
 Storage temperature : **+5°C ± 3°C**




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CONTROLS ON CELLS:

Observation

Method	: Studied through a microscope
Cells	: Vero, 142 nd passage
Number of cells tested	: 320*10 ⁶ cells
Test on	: 17.09.07
Test off	: 01.10.07
Result	: Absence of cytopathic effect

Test for haemadsorbing viruses

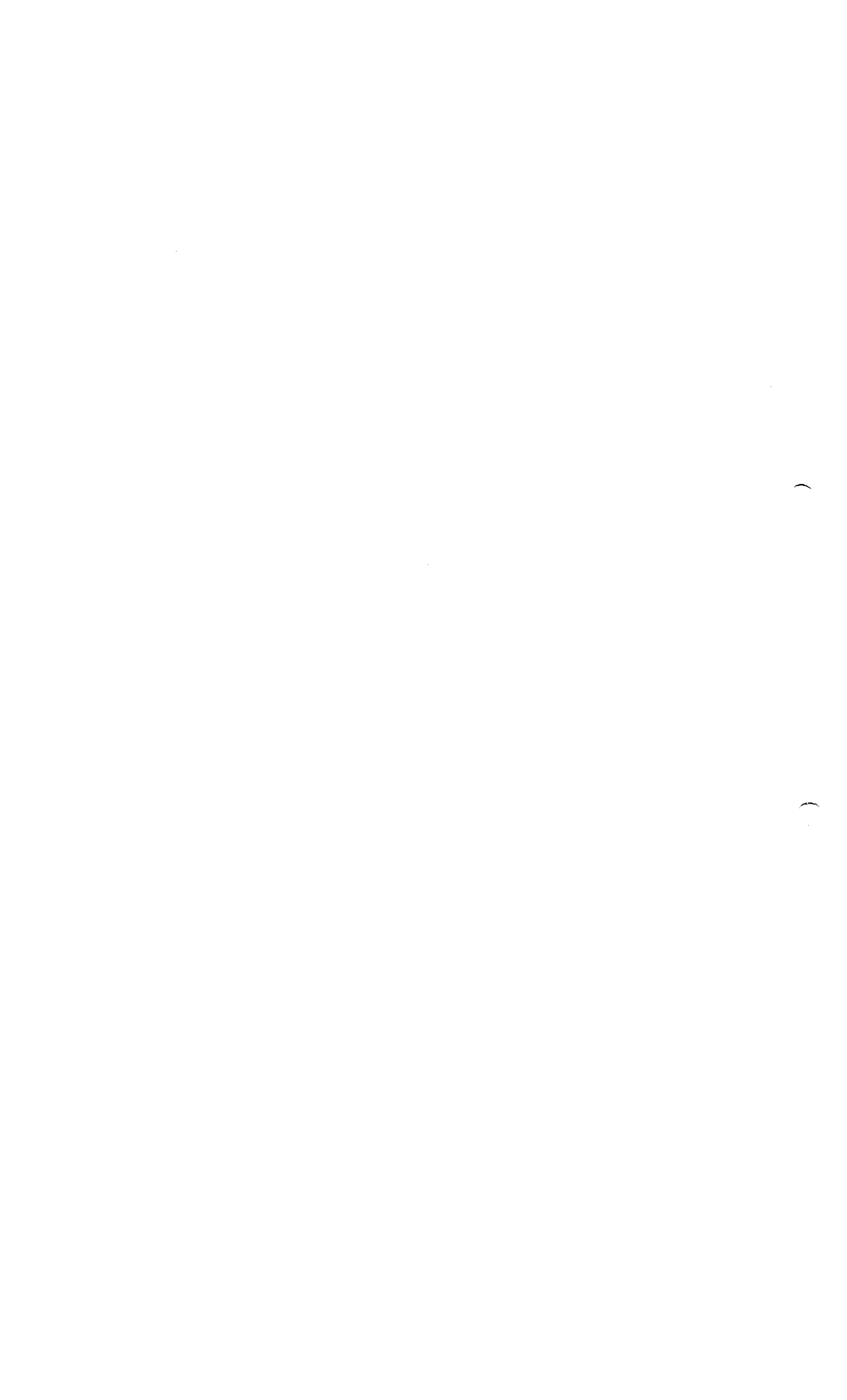
Method	: visual inspection
Number of vials tested	: 8
Type of red blood cells	: Guinea-pig
Incubation	: 4 vials, 30 minutes at 5°C ± 3°C
	: 4 vials, 30 minutes at 20-25°C
Date	: 01.10.07
Result	: Absence of hemadsorption

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	: 10.12.07
Result	: Positive identification


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Test off	: 18.04.08
<i>Subculture at 14th day</i>	
Test on	: 11.04.08
Test off	: 25.04.08
<i>Subculture at 21th day</i>	
Test on	: 18.04.08
Test off	: 25.04.08
Result	: No growth of Mycoplasma

Test for Mycoplasma by epifluorescence in cell culture

Method	: DNA fluorescence staining
Cell used	: Vero, 150 th passage
Volume tested	: 1 ml
Temperature of incubation	: 36 ± 1° C
Positive control	: <i>M. hyorhinis</i> and <i>M. orale</i>
Test on	: 17.01.08
Test off	: 24.01.08
Result	: No Mycoplasma detected by epifluorescence in cell culture

	
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