



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° S4312 (clinical batch)

134/229

CONTROLS ON CONCENTRATED PURIFIED VIRAL SUSPENSION:

Protein content

Method : Spectrophotometry
Date : 04.12.07
Result : 91.88 µg/ml

D Antigen content


Method : ELISA
Reference standard : 28.1.99
Date : 05.12.07
Result : 6 443 D Antigen U/ml

Bacterial and fungal sterility

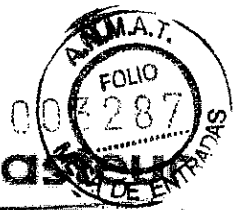
Method used	:	Membrane filtration	
Media	:	Thioglycollate medium	Soybean Casein Digest medium
Temperatures	:	30-35° C	20-25° C
Volume tested	:	20 ml	
Inoculum per Steritest	:	10 ml	10 ml
Volume of medium per Steritest	:	100 ml	100 ml
Test on	:	04.12.07	
Test off	:	18.12.07	
Result	:	No microbial growth	

Purity

Result : 0.014 µg of protein / D Antigen U



 ROXANA MONTEMILONE DIRECTORA TÉCNICA SANOFI PASTEUR S.A.
 CHRISTIAN DOMÍNGUEZ GERENTE SANOFI PASTEUR S.A.





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SINGLE HARVEST - Type : 3
Lot Number : FA292974

CELL:

Working Cell Bank : FA106611
Date of thawing ampoule of WCB : 15.11.07
Thawing ampoule amount : One ampoule

VIRUS:

Virus Working Seed lot : FA109086
Date of inoculation : 10.12.07
Date of virus harvest : 13.12.07

CRUDE HARVEST:

Storage : from 14.12.07 to 17.12.07
Volume : 1 489 L
Storage temperature : +5°C ± 3°C

SINGLE HARVEST:

Date of single harvest : 17.12.07

CONCENTRATION AND PURIFICATION OF THE SINGLE HARVEST

DEAE SpheroDex large column : Ion exchange chromatography on 18.12.07
CL-6-B Sepharose column : Gel filtration on 19.12.07
DEAE SphéroDex small column : Ion exchange chromatography on 19.12.07

CONCENTRATED PURIFIED VIRAL SUSPENSION :

Manufacture date : 19.12.07
Volume : 19 600 ml
Storage temperature : +5°C ± 3°C

ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.

CHRISTIAN DOMINIQUEZ
PRODUCIDO
SANOFI PASTEUR S.A.



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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 : 10.12.07
 Test off : 24.12.07
 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 : 24.12.07
 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 : 14.02.08
 Result : Positive identification



ROXANA MONTEMILONE **CHRISTIAN DOMINGUEZ**
 DIRECTORA TÉCNICA APODERADO
 SANOFI PASTEUR S.A. SANOFI PASTEUR S.A.





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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

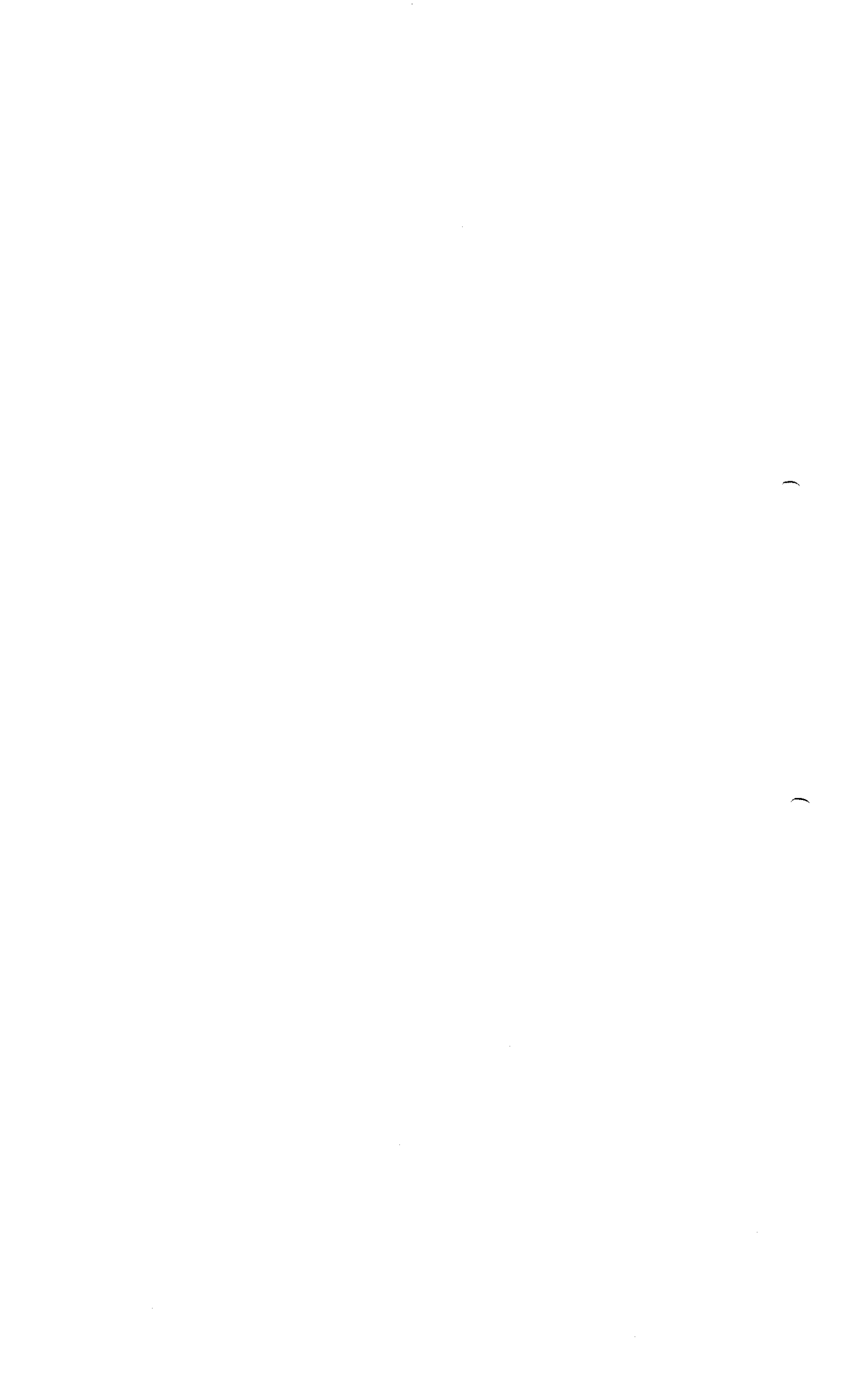
Method	: Studied through a microscope
Type of cells	: MRC5
Volume tested	: 10 ml
Temperature of incubation	: 36 ± 1° C
Test on	: 20.02.08
Test off	: 19.03.08
Result	: Absence of cytopathic effect

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


ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.


CHRISTIAN DOMÍNGUEZ
ENCUADERADO
SANOFI PASTEUR S.A.



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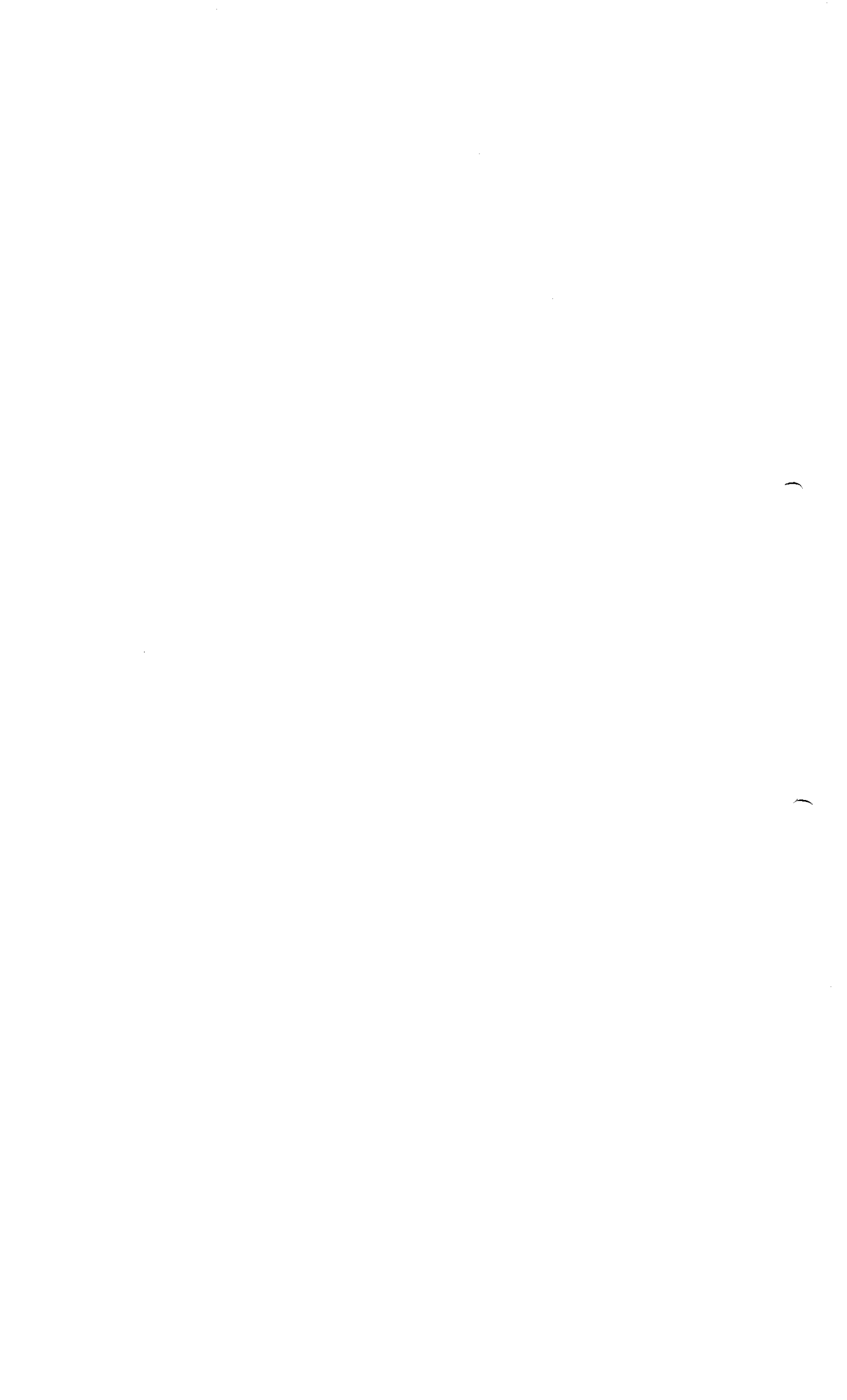
Test for Mycoplasma by culture method

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	: 24.11.08
Test off	: 08.12.08
<i>Subculture at 3rd day</i>	
Test on	: 27.11.08
Test off	: 11.12.08
<i>Subculture at 7th day</i>	
Test on	: 01.12.08
Test off	: 15.12.08
<i>Subculture at 14th day</i>	
Test on	: 08.12.08
Test off	: 22.12.08
<i>Subculture at 21th day</i>	
Test on	: 15.12.08
Test off	: 22.12.08
Result	: No growth of Mycoplasma

Test for Mycoplasma by epifluorescence in cell culture

Method	: DNA fluorescence staining
Cell used	: Vero, 146 th passage
Volume tested	: 1 ml
Temperature of incubation	: 36 ± 1° C
Positive control	: <i>M. hyorhins</i> and <i>M. orale</i>
Test on	: 20.03.08
Test off	: 27.03.08
Result	: No Mycoplasma detected by epifluorescence in cell culture

	
ROXANA MONTEMILOME DIRECTORA TÉCNICA SANOFI PASTEUR S.A.	CHRISTIAN DOMINGUEZ FEDERADO SANOFI PASTEUR S.A.



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CONTROLS ON SINGLE VIRUS HARVEST :

Polio virus identification


Method : Cytopathogenic effect neutralization
 Cell strain used : Hep 2
 Antiserum : LEPINE type 3
 Test on : 21.07.08
 Test off : 29.07.08
 Result : Positive

Polio virus concentration


Method : Cytopathogenic effect on sensitive cells
 Cell strain used : Hep 2
 Test on : 21.07.08
 Test off : 29.07.08
 Virus concentration : $10^{8.40}$ 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	:	19.12.07	
Test off	:	03.01.08	
Result	:	No microbial growth	



 ROXANA MONTEMILOME
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.



 CHRISTIAN DOMINGUEZ
 SUPERADORO
 SANOFI PASTEUR S.A.





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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	: 24.11.08
Test off	: 08.12.08
<i>Subculture at 3rd day</i>	
Test on	: 27.11.08
Test off	: 11.12.08
<i>Subculture at 7th day</i>	
Test on	: 01.12.08
Test off	: 15.12.08
<i>Subculture at 14th day</i>	
Test on	: 08.12.08
Test off	: 22.12.08
<i>Subculture at 21th day</i>	
Test on	: 15.12.08
Test off	: 22.12.08
Result	: No growth of Mycoplasma

	
ROXANA MONTEMILONE DIRECTORA TÉCNICA SANOFI PASTEUR S.A.	CHRISTIAN DOMÍNGUEZ FEDERADO SANOFI PASTEUR S.A.



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CONTROLS ON CONCENTRATED PURIFIED VIRAL SUSPENSION:

Protein content

Method : Spectrophotometry
 Date : 27.12.07
 Result : 88.90 µg/ml

D Antigen content


Method : ELISA
 Reference standard : 28.1.99
 Date : 14.01.08
 Result : 7 403 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	: 24.12.07	
Test off	: 07.01.08	
Result	: No microbial growth	

Purity

Result : 0.012 µg of protein / D Antigen U


 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.



 CHRISTIAN DOMÍNGUEZ
 COORDINADOR
 SANOFI PASTEUR S.A.

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MONOVALENT BULK (TYPE 1, 2 AND 3)

TESTS	ACCEPTANCE CRITERIA
RESIDUAL FORMALDEHYDE CONTENT	80 ± 30 µg/ml
PROTEIN CONTENT	For purity calculation
D ANTIGEN CONTENT	For purity calculation
PURITY	≤ 50 ng of protein/D antigen unit
BACTERIAL AND FUNGAL STERILITY TEST	No microbial growth
KINETICS OF INACTIVATION DAY 0 DAY 24H DAY 48H DAY 72H DAY 96H SLOPE CALCULATION (D0, D0+24H,D0+48H)	The infectious titer drops in linear mode Type 1 : within -0.1187 and -0.1758 Type 2 : within -0.0955 and -0.1890 Type 3 : within -0.1090 and -0.1933
TEST FOR EFFECTIVE INACTIVATION : DAY 9 DAY 12	No cytopathic effect during amplification process No cytopathic effect during amplification process



 ROXANA MONTEMILONE
 DIRECTORA TÉCNICA
 SANOFI PASTEUR S.A.



 CHRISTIAN DOMÍNGUEZ
 APODERADO
 SANOFI PASTEUR S.A.

