

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) 106/229

**CONTROLS ON CONCENTRATED PURIFIED VIRAL SUSPENSION:**

**Protein content**

Method : Spectrophotometry  
 Date : 25.05.07  
 Result : 75.11 µg/ml

**D Antigen content**

Method : ELISA  
 Reference standard : 28.1.99  
 Date : 23.05.07  
 Result : 1 891 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	:	24.05.07	
Test off	:	07.06.07	
Result	:	No microbial growth	

**Purity**

Result : 0.0397 µg of protein / D Antigen U

  
 ROXANA MONTEMILONE  
 DIRECTORA TÉCNICA  
 SANOFI PASTEUR S.A.

  
 CHRISTIAN DOMINGUEZ  
 ENDEPADO  
 SANOFI PASTEUR S.A.



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**SINGLE HARVEST - Type : 2**  
**Lot Number : FA286930**

**CELL:**

Working Cell Bank : **FA106611**  
 Date of thawing ampoule of WCB : **13.09.07**  
 Thawing ampoule amount : **One ampoule**

**VIRUS:**

Virus Working Seed lot : **FA091251**  
 Date of inoculation : **08.10.07**  
 Date of virus harvest : **11.10.07**

**CRUDE HARVEST:**

Storage : from **12.10.07** to **15.10.07**  
 Volume : **1 506 L**  
 Storage temperature : **+5°C ± 3°C**

**SINGLE HARVEST:**



Date of single harvest : **15.10.07**

**CONCENTRATION AND PURIFICATION OF THE SINGLE HARVEST**

DEAE Sphérodex large column : Ion exchange chromatography on **16.10.07**  
 CL-6-B Sepharose column : Gel filtration on **17.10.07**  
 DEAE Sphérodex small column : Ion exchange chromatography on **17.10.07**

**CONCENTRATED PURIFIED VIRAL SUSPENSION :**

Manufacture date : **17.10.07**  
 Volume : **19 800 ml**  
 Storage temperature : **+5°C ± 3°C**



  
**ROXANA MONTEMILONE**      **CHRISTIAN DOMINGUEZ**  
 DIRECTORA TÉCNICA      PRODEHADO  
 SANOFI PASTEUR S.A.      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\*10^6 cells
Test on : 08.10.07
Test off : 22.10.07
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 : 22.10.07
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

Handwritten signatures and stamps for ROXANA MONTEMILONE and CHRISTIAN DOMINGUEZ, DIRECTORA TECNICA and GERENTE, 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	: 12.11.07
Test off	: 26.11.07
Result	: <b>Absence of cytopathic effect</b>

*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	: <b>Absence of cytopathic effect</b>

*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	: <b>Absence of cytopathic effect</b>

 ROXANA MONTEMILONE DIRECTORA TÉCNICA SANOFI PASTEUR S.A.	 CHRISTIAN DOMÍNGUEZ APODERADO SANOFI PASTEUR S.A.
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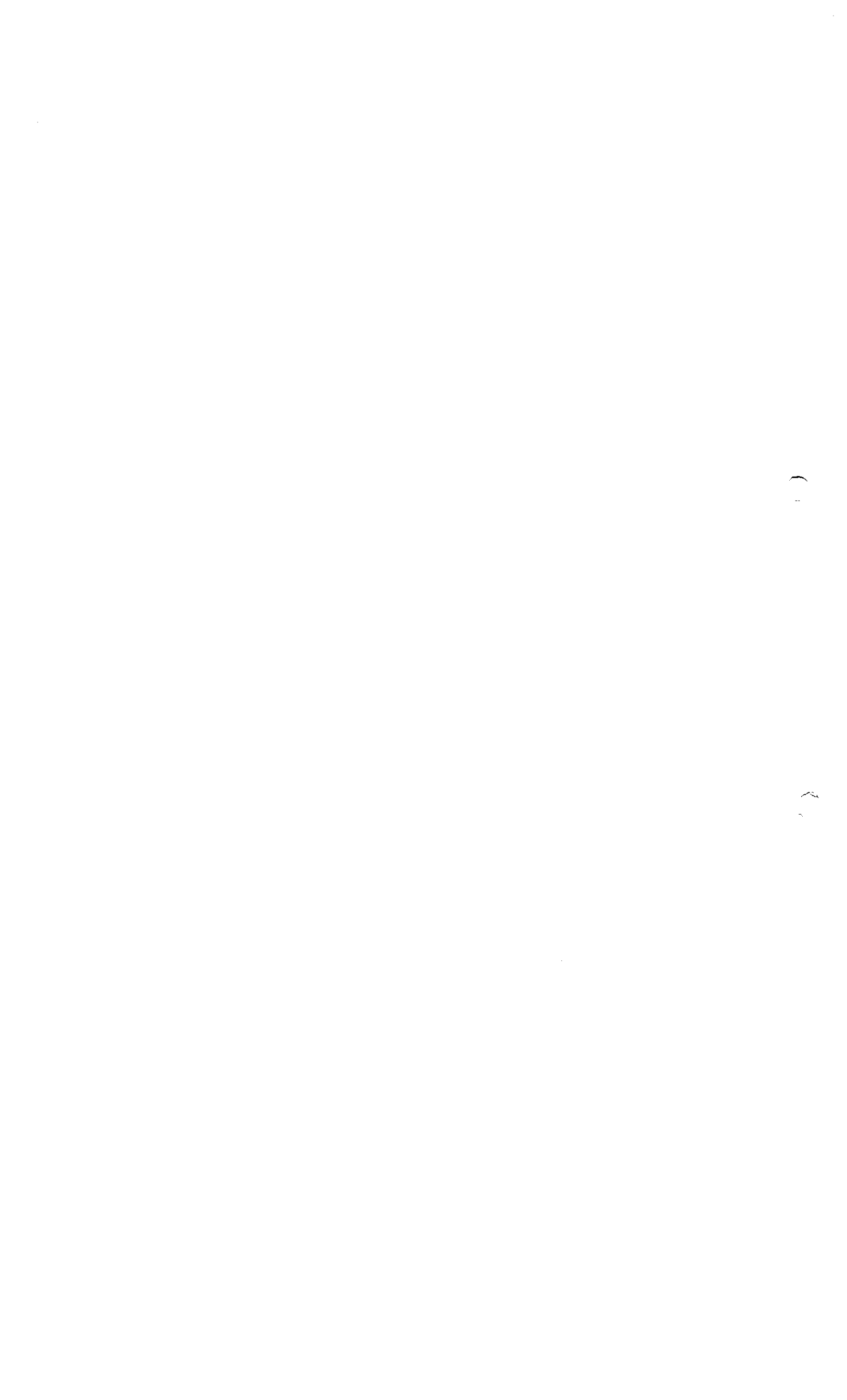
**Test for Mycoplasma by culture method**

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	: 22.04.08
Test off	: 06.05.08
<i>Subculture at 3<sup>rd</sup> day</i>	
Test on	: 25.04.08
Test off	: 13.05.08
<i>Subculture at 7<sup>th</sup> day</i>	
Test on	: 29.04.08
Test off	: 13.05.08
<i>Subculture at 14<sup>th</sup> day</i>	
Test on	: 06.05.08
Test off	: 20.05.08
<i>Subculture at 21<sup>th</sup> day</i>	
Test on	: 13.05.08
Test off	: 20.05.08
Result	: No growth of Mycoplasma

**Test for Mycoplasma by epifluorescence in cell culture**

Method	: DNA fluorescence staining
Cell used	: Vero, 152 <sup>nd</sup> 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

ROXANA MONTEMILONE	CHRISTIAN DOMINGUEZ
DIRECTORA TÉCNICA	MODERADO
SANOFI PASTEUR S.A.	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 2  
 Test on : 23.10.07  
 Test off : 30.10.07  
 Result : Positive

**Polio virus concentration**

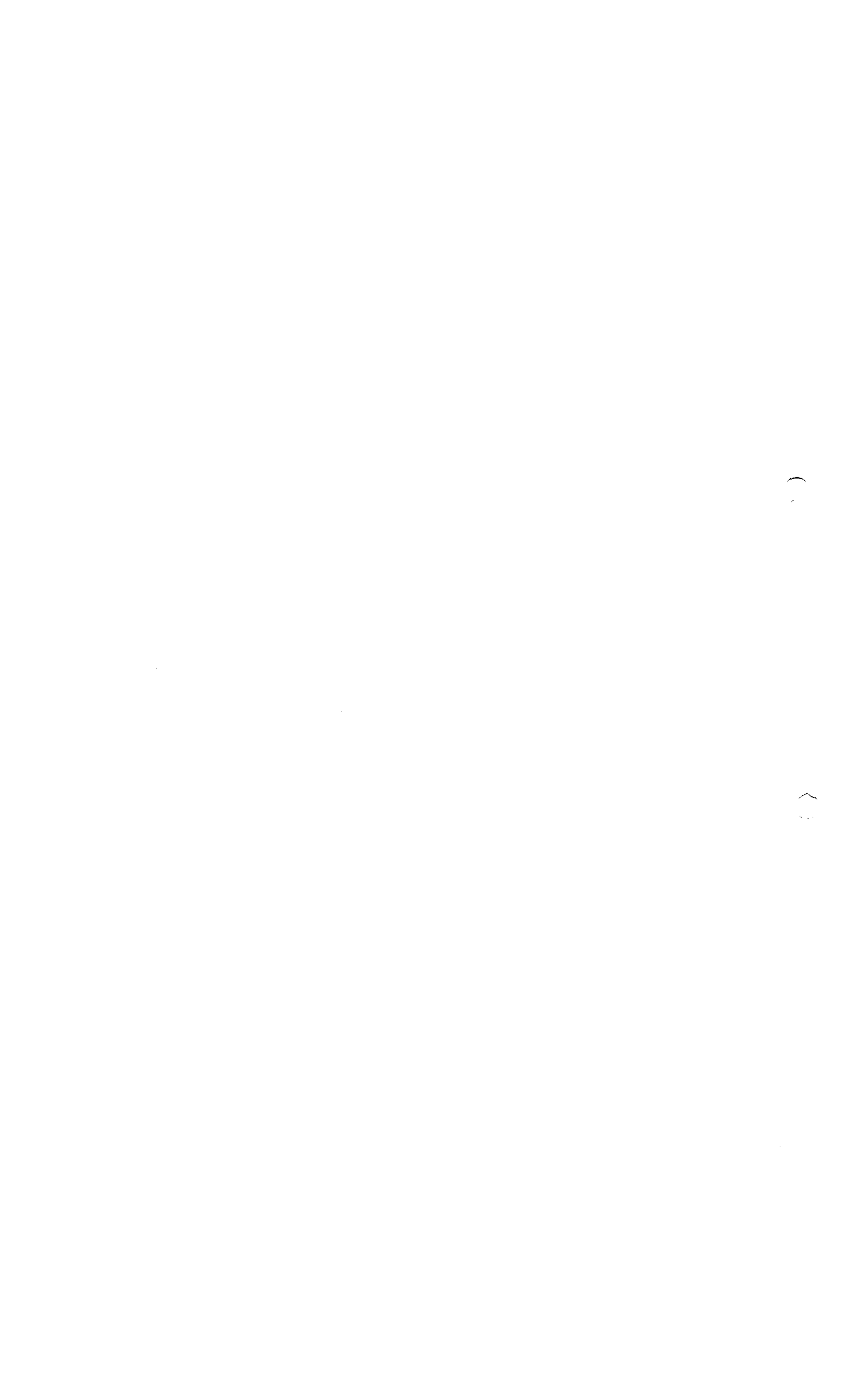
Method : Cytopathogenic effect on sensitive cells  
 Cell strain used : Hep 2  
 Test on : 23.10.07  
 Test off : 30.10.07  
 Virus concentration : 10<sup>9.03</sup> CCID<sub>50</sub>/ml  
 Result : Conforms

**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 DOMINGUEZ  
 SUPERVISOR  
 SANOFI PASTEUR S.A.





sanofi pasteur

The vaccines division of sanofi-aventis Group

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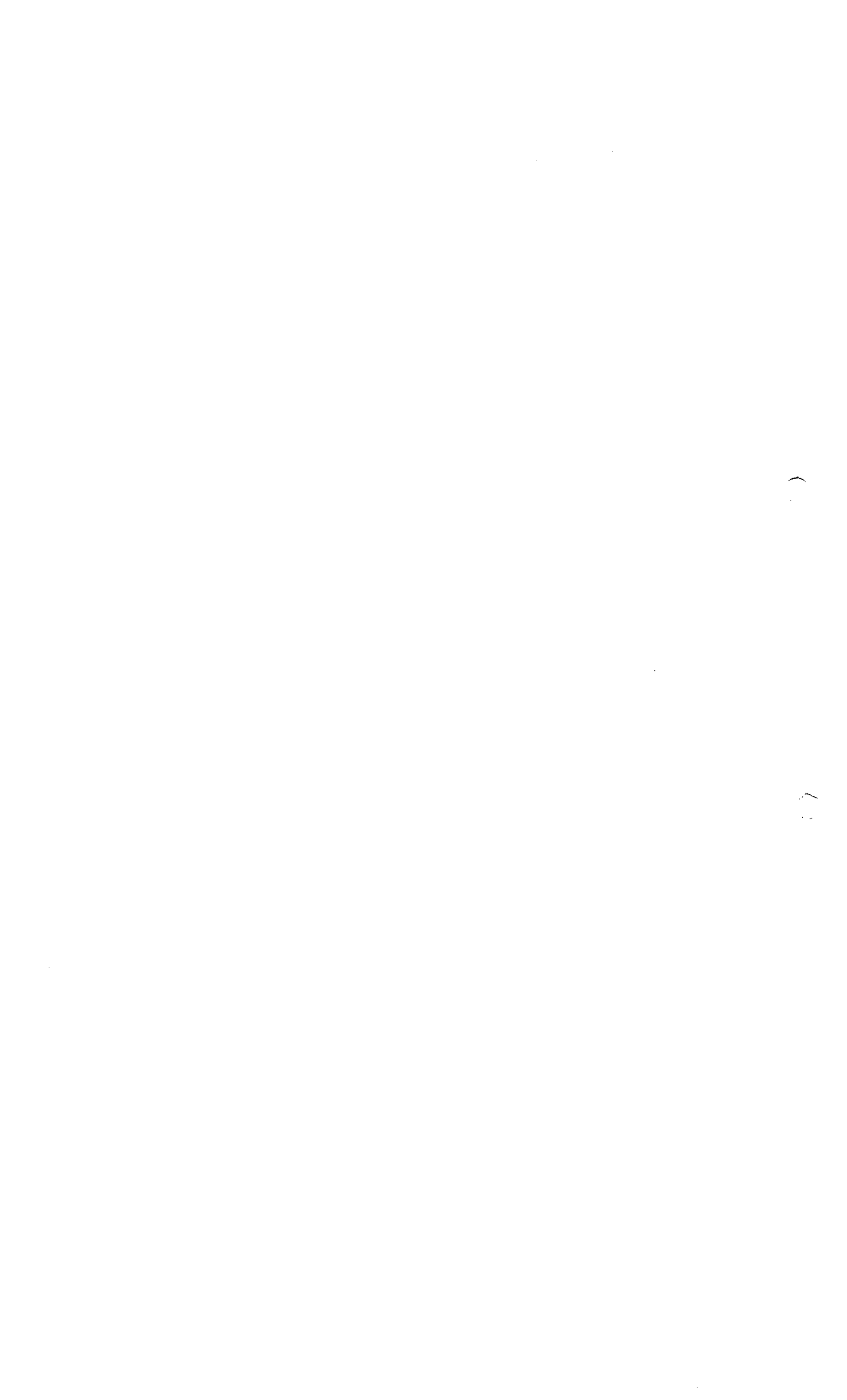
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Test for Mycoplasma by culture method

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 3<sup>rd</sup> day</i>	
Test on	: 25.04.08
Test off	: 13.05.08
<i>Subculture at 7<sup>th</sup> day</i>	
Test on	: 29.04.08
Test off	: 13.05.08
<i>Subculture at 14<sup>th</sup> day</i>	
Test on	: 06.05.08
Test off	: 20.05.08
<i>Subculture at 21<sup>th</sup> day</i>	
Test on	: 13.05.08
Test off	: 20.05.08
Result	: No growth of Mycoplasma

  
 ROXANA MONTEMILOME  
 DIRECTORA TÉCNICA  
 SANOFI PASTEUR S.A.

  
 CHRISTIAN DOMINGUEZ  
 ASISTENTE  
 SANOFI PASTEUR S.A.



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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

  
 ROXANA MONTEMILONE  
 DIRECTORA TÉCNICA  
 SANOFI PASTEUR S.A.

  
 CHRISTIAN DOMINGUEZ  
 ASUPERADO  
 SANOFI PASTEUR S.A.





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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 Sphérodex 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

ROXANA MONTEMILONE  
DIRECTORA TÉCNICA  
SANOFI PASTEUR S.A.

CHRISTIAN DOMÍNGUEZ  
ACUERDADO  
SANOFI PASTEUR S.A.

