

**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**


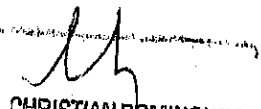
Method : ELISA  
Reference standard : 28.1.99  
Date : 05.12.07  
Result : 6 443 D Antigen U/ml

**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


  
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 DIRECTORA TÉCNICA      SUPERVISOR  
 SANOFI PASTEUR S.A.      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 Spheredex 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

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**CONTROLS ON CELLS:**

**Observation**



Method : Studied through a microscope  
 Cells : Vero, 142<sup>nd</sup> passage  
 Number of cells tested : 320\*10<sup>6</sup> 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



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

*Human diploid cells*

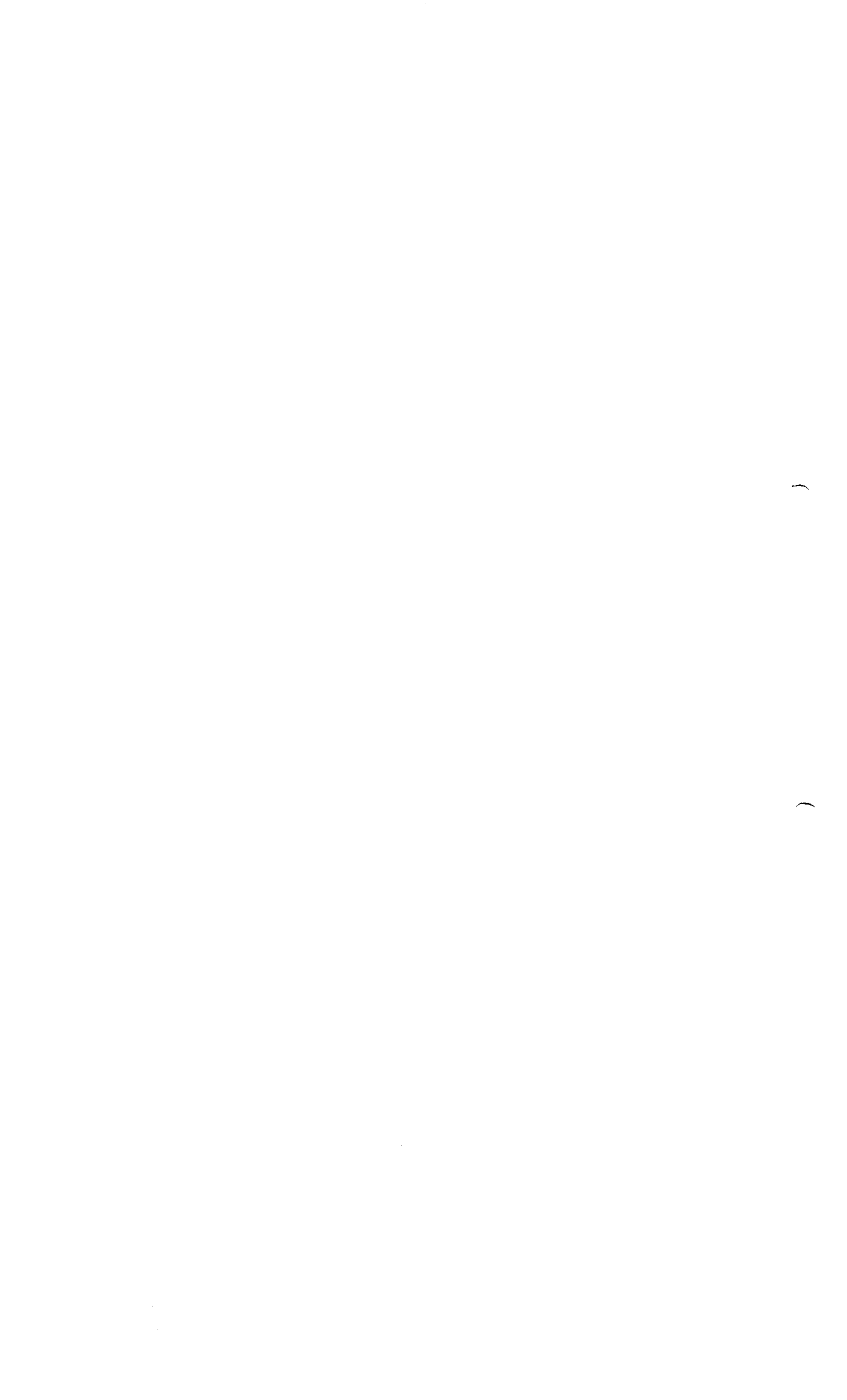
Method	: Studied through a microscope
Type of cells	: MRC5
Volume tested	: 10 ml
Temperature of incubation	: 36 ± 1° C
Test on	: 20.02.08
Test off	: 19.03.08
Result	: <b>Absence of cytopathic effect</b>

*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	: 15.01.08
Test off	: 29.01.08
Result	: <b>Absence of cytopathic effect</b>

  
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 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus  
 influenzae type b conjugate vaccine (adsorbed) lot n° S4312 (clinical batch) 138/229**  
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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 3<sup>rd</sup> day</i>	
Test on	: 27.11.08
Test off	: 11.12.08
<i>Subculture at 7<sup>th</sup> day</i>	
Test on	: 01.12.08
Test off	: 15.12.08
<i>Subculture at 14<sup>th</sup> day</i>	
Test on	: 08.12.08
Test off	: 22.12.08
<i>Subculture at 21<sup>th</sup> 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 <sup>th</sup> passage
Volume tested	: 1 ml
Temperature of incubation	: 36 ± 1° C
Positive control	: <i>M. hyorhinae</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

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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**

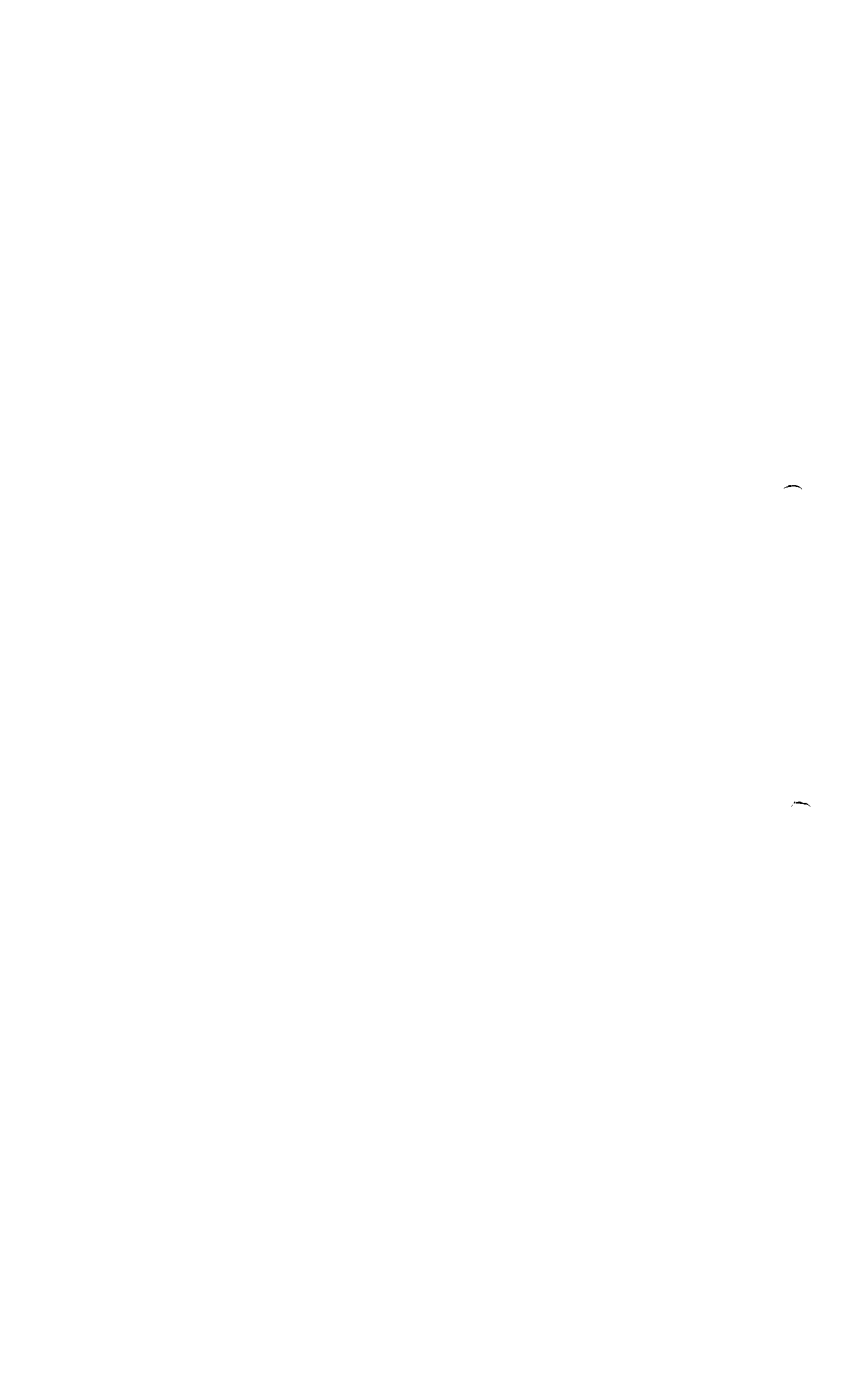
Method : Cytopathogenic effect on sensitive cells  
 Cell strain used : Hep 2  
 Test on : 21.07.08  
 Test off : 29.07.08  
 Virus concentration :  $10^{8.40}$  CCID<sub>50</sub>/ml  
 Result : Conforms

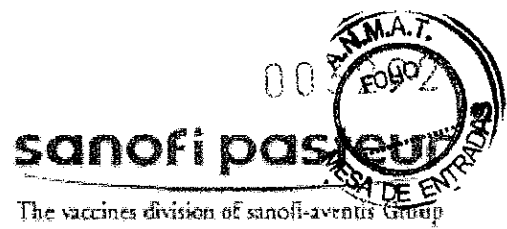
**Bacterial and fungal sterility**

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	:	19.12.07	
Test off	:	03.01.08	
Result	:	No microbial growth	


  
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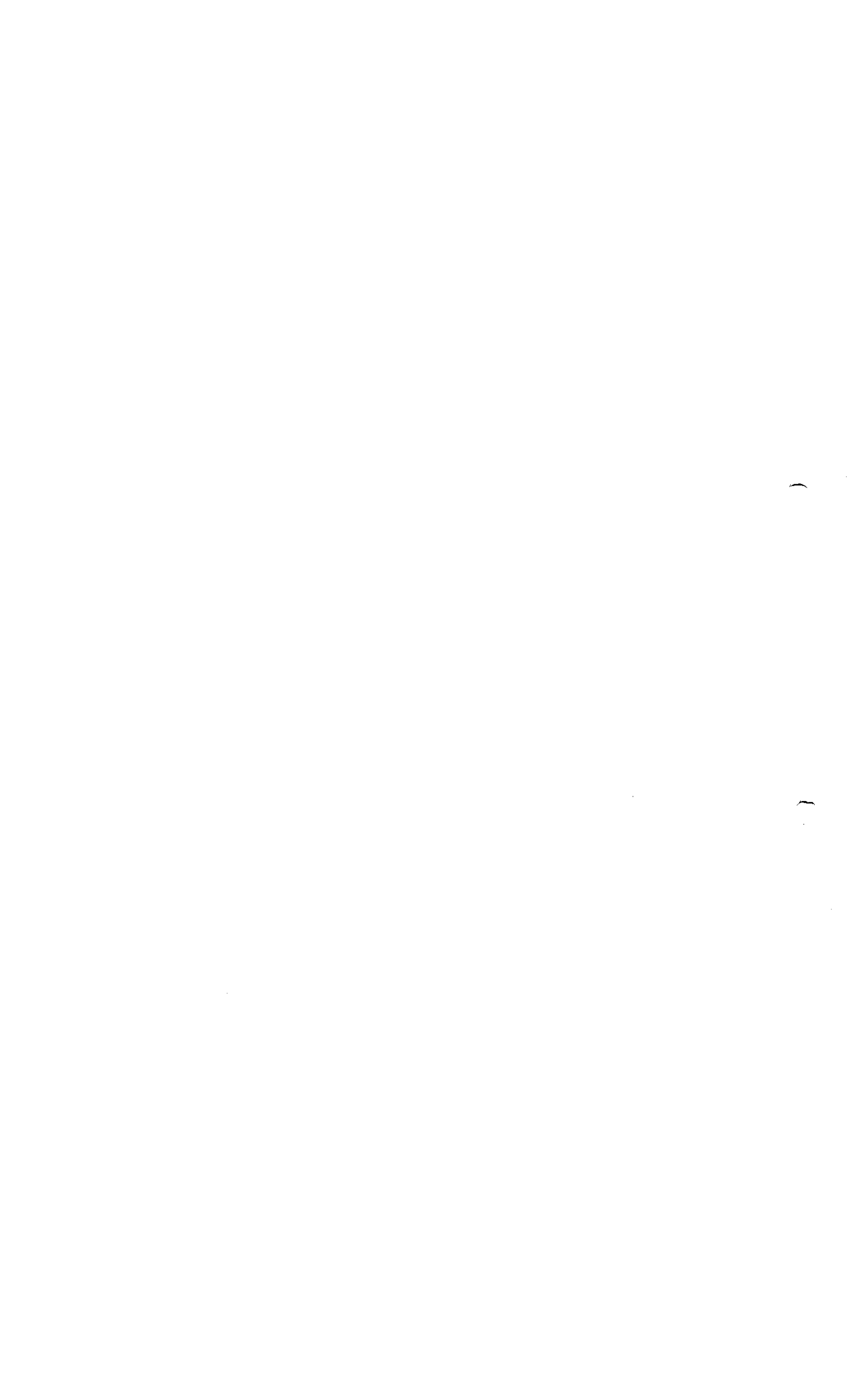
Diphtheria, tetanus, pertussis (acellular, component), hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus influenzae type b conjugate vaccine (adsorbed) lot n° S4312 (clinical batch) 140/229

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 : M. pneumoniae and M. orale and A. laidlawii

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

Handwritten signatures and printed names: ROXANA MONTEILONE, DIRECTORA TÉCNICA, SANOFI PASTEUR S.A. and CHRISTIAN DOMINGUEZ, SUPERVISOR, 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

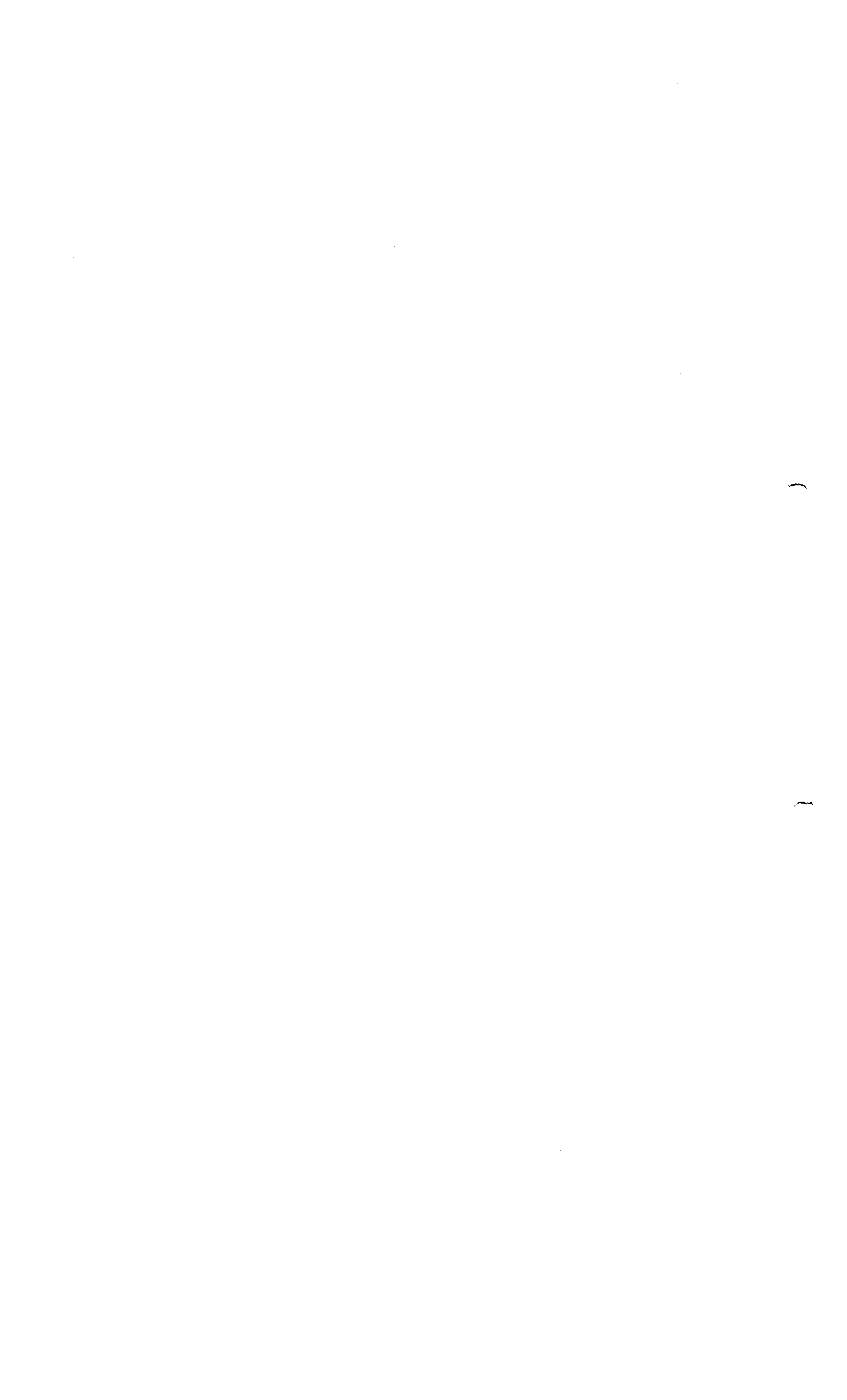
**Bacterial and fungal sterility**

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



  
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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

  
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