

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)



89/229

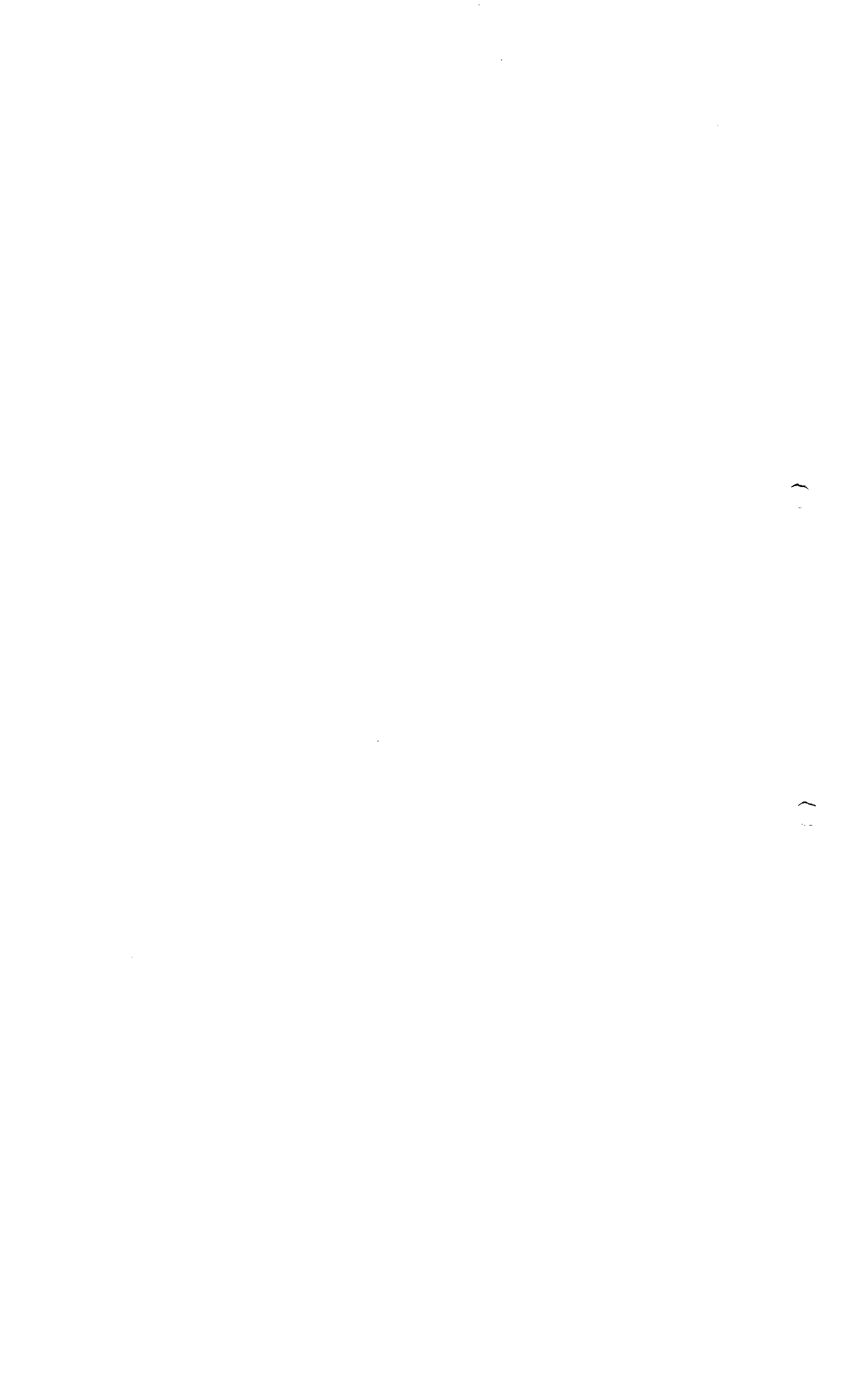
**Test for Mycoplasma by culture method**

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	: <i>M. pneumoniae</i> and <i>M. orale</i> and <i>A. laidlawii</i>
<i>Primary test</i>	
Test on	: 28.10.08
Test off	: 12.11.08
<i>Subculture at 3<sup>rd</sup> day</i>	
Test on	: 31.10.08
Test off	: 14.11.08
<i>Subculture at 7<sup>th</sup> day</i>	
Test on	: 04.11.08
Test off	: 18.11.08
<i>Subculture at 14<sup>th</sup> day</i>	
Test on	: 12.11.08
Test off	: 26.11.08
<i>Subculture at 21<sup>th</sup> day</i>	
Test on	: 18.11.08
Test off	: 25.11.08
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	: 4 ml
Temperature of incubation	: 36 ± 1° C
Positive control	: <i>M. hyorhinis</i> and <i>M. orale</i>
Test on	: 20.05.08
Test off	: 27.05.08
Result	: No Mycoplasma detected by epifluorescence in cell culture

 ROXANA MONTEMILONE DIRECTORA TÉCNICA SANOFI PASTEUR S.A.	 CHRISTIAN DOMINGUEZ GERENTE SANOFI PASTEUR S.A.
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 influenzae type b conjugate vaccine (adsorbed) lot n° S4312 (clinical batch) 90/229  
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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 : 23.07.08  
 Test off : 30.07.08  
 Result : Positive

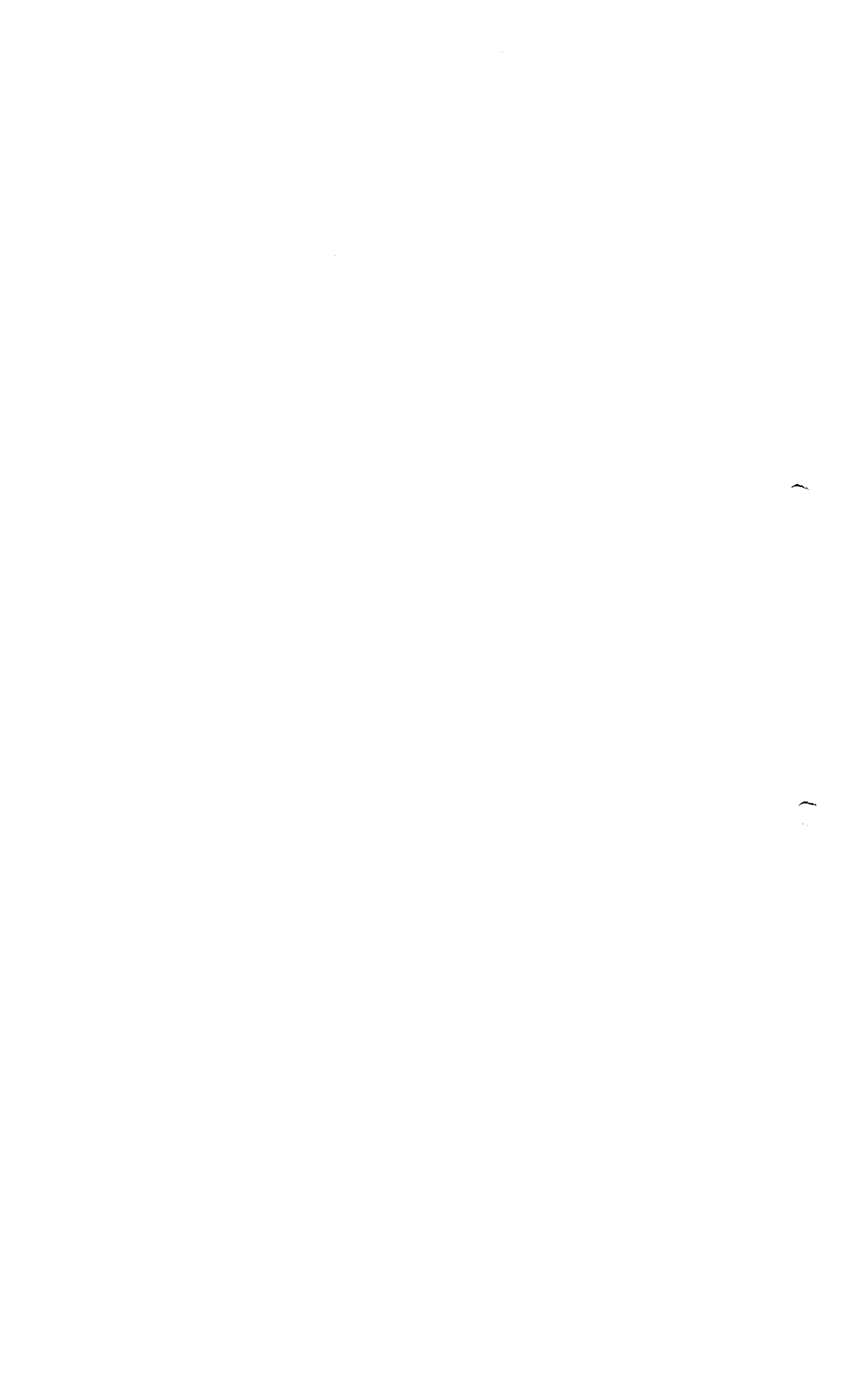
**Polio virus concentration**

Method : Cytopathogenic effect on sensitive cells  
 Cell strain used : Hep 2  
 Test on : 23.07.08  
 Test off : 30.07.08  
 Virus concentration :  $10^{9.07}$  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	:	30.01.08	
Test off	:	13.02.08	
Result	:	No microbial growth	


  
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 DIRECTORA TÉCNICA      ENGERADO  
 SANOFI PASTEUR S.A.      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	: 28.10.08
Test off	: 12.11.08
<i>Subculture at 3<sup>rd</sup> day</i>	
Test on	: 31.10.08
Test off	: 14.11.08
<i>Subculture at 7<sup>th</sup> day</i>	
Test on	: 04.11.08
Test off	: 18.11.08
<i>Subculture at 14<sup>th</sup> day</i>	
Test on	: 12.11.08
Test off	: 26.11.08
<i>Subculture at 21<sup>th</sup> day</i>	
Test on	: 18.11.08
Test off	: 25.11.08
Result	: No growth of Mycoplasma

 ROXANA MONTEMILONE DIRECTORA TÉCNICA SANOFI PASTEUR S.A.	 CHRISTIAN DOMINGUEZ SUPERVISOR SANOFI PASTEUR S.A.
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hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus  
influenzae type b conjugate vaccine (adsorbed) lot n° S4312 (clinical batch) 92/229  
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**CONTROLS ON CONCENTRATED PURIFIED VIRAL SUSPENSION :**

**Protein content**

Method : Spectrophotometry  
Date : 12.02.08  
Result : 96.75 µg/ml

**D Antigen content**

Method : ELISA  
Reference standard : 28.1.99  
Date : 04.02.08  
Result : 10 327 D Antigen U/ml

**Bacterial and fungal sterility**

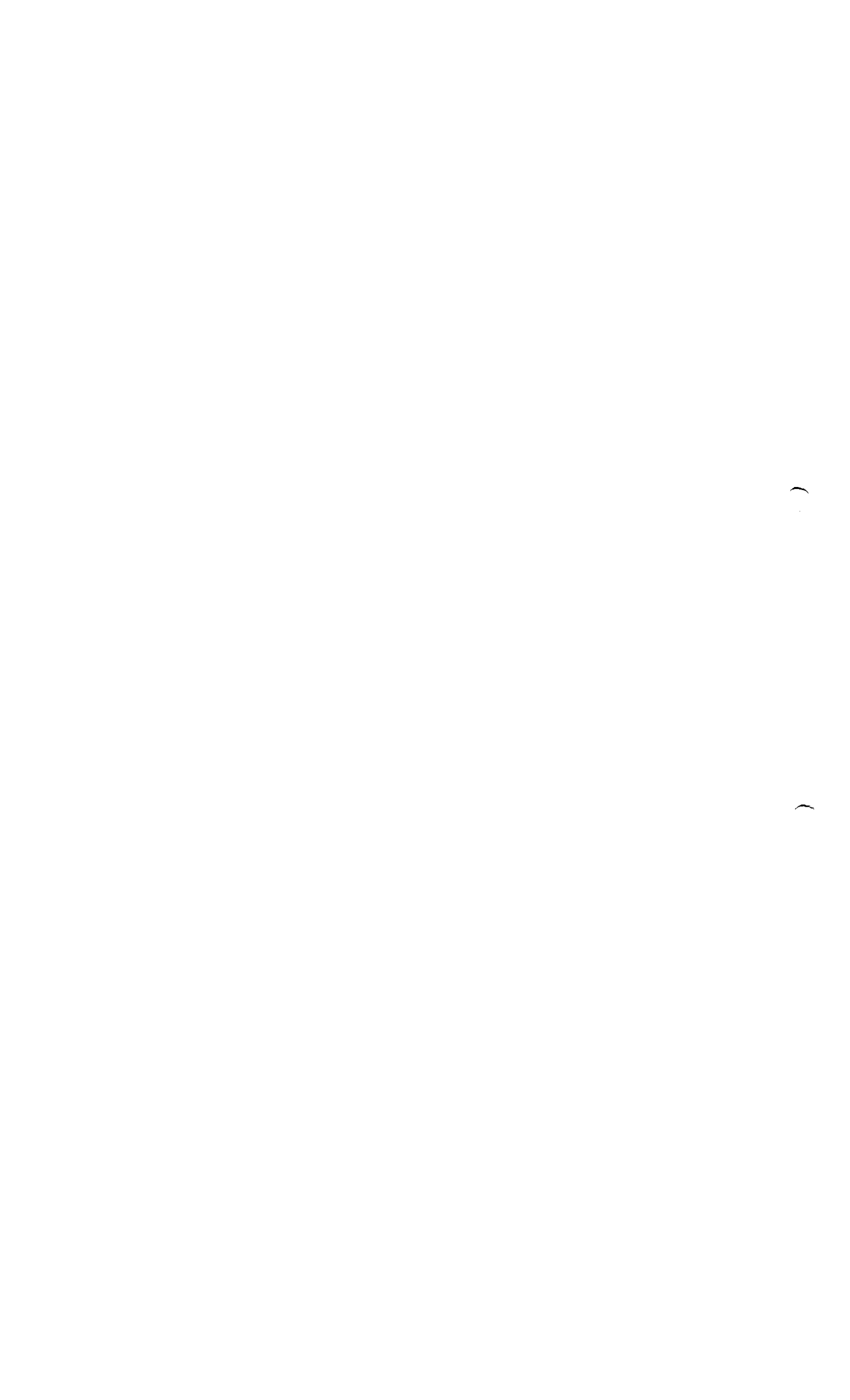
Method used	: Membrane filtration	
Media	: Thioglycollate medium	: Soybean Casein Digest medium
Temperatures	: 30-35° C	: 20-25° C
Volume tested	: 20 ml	
Inoculum per Steritest	: 10 ml	: 10 ml
Volume of medium per Steritest	: 100 ml	: 100 ml
Test on	: 04.02.08	
Test off	: 18.02.08	
Result	: No microbial growth	

**Purity**

Result : 0.009 µg of protein / D Antigen U

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SINGLE HARVEST - Type : 2  
Lot Number : FA272631

**CELL:**

Working Cell Bank : FA106611  
Date of thawing ampoule of WCB : 05.04.07  
Thawing ampoule amount : One ampoule

**VIRUS:**

Virus Working Seed lot : FA091251  
Date of inoculation : 30.04.07  
Date of virus harvest : 03.05.07

**CRUDE HARVEST:**

Storage : from 04.05.07 to 07.05.07  
Volume : 1 529 L  
Storage temperature : +5°C ± 3°C

**SINGLE HARVEST:**

Date of single harvest : 07.05.07

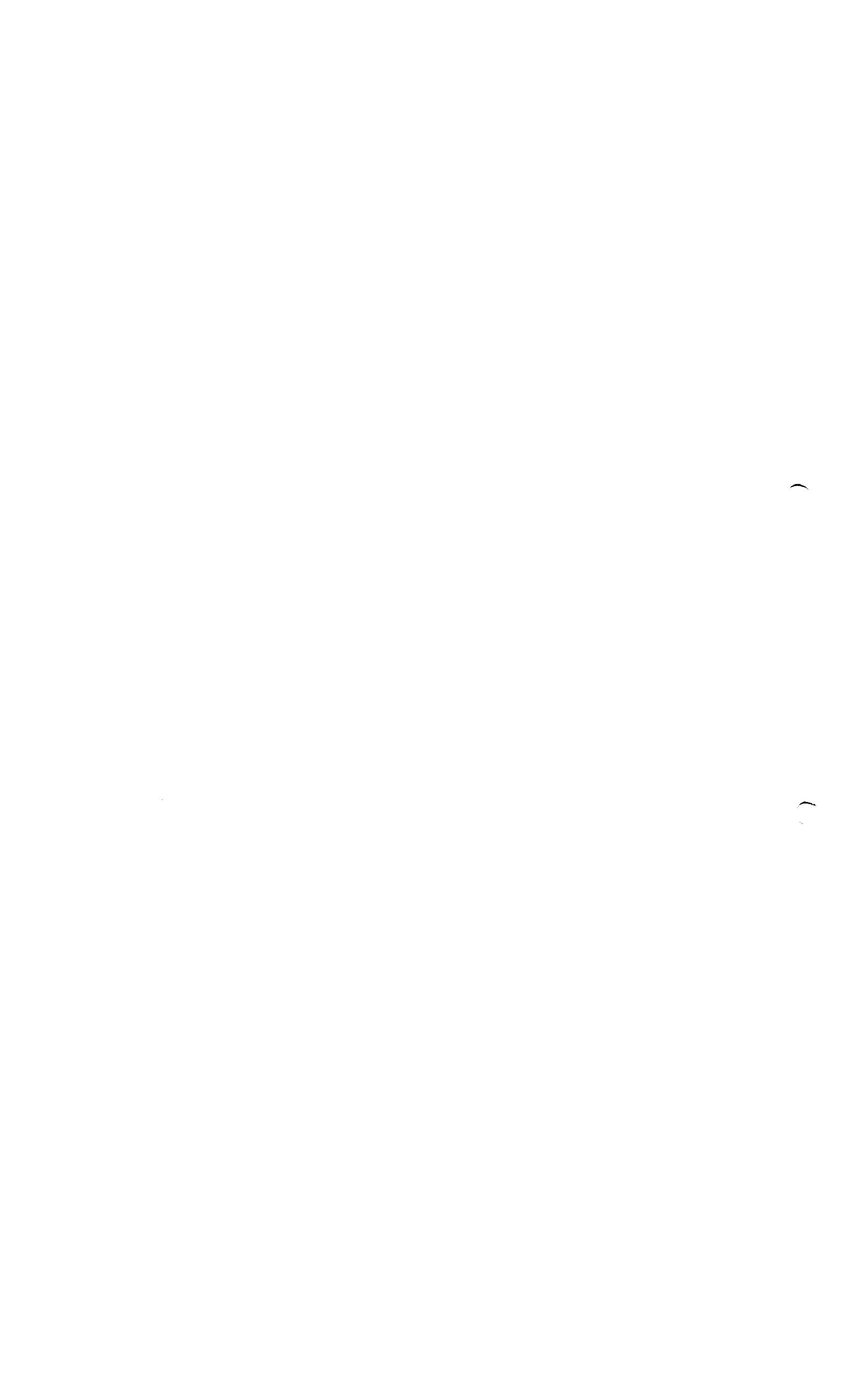
**CONCENTRATION AND PURIFICATION OF THE SINGLE HARVEST**

DEAE SpheroDex large column : Ion exchange chromatography on 09.05.07  
CL-6-B Sepharose column : Gel filtration on 10.05.07  
DEAE SphéroDex small column : Ion exchange chromatography on 10.05.07

**CONCENTRATED PURIFIED VIRAL SUSPENSION :**

Manufacture date : 10.05.07  
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 : 30.04.07  
 Test off : 15.05.07  
 Result : **Absence of cytopathic effect**

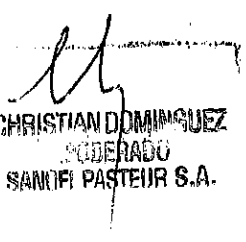
**Test for haemadsorbing viruses**

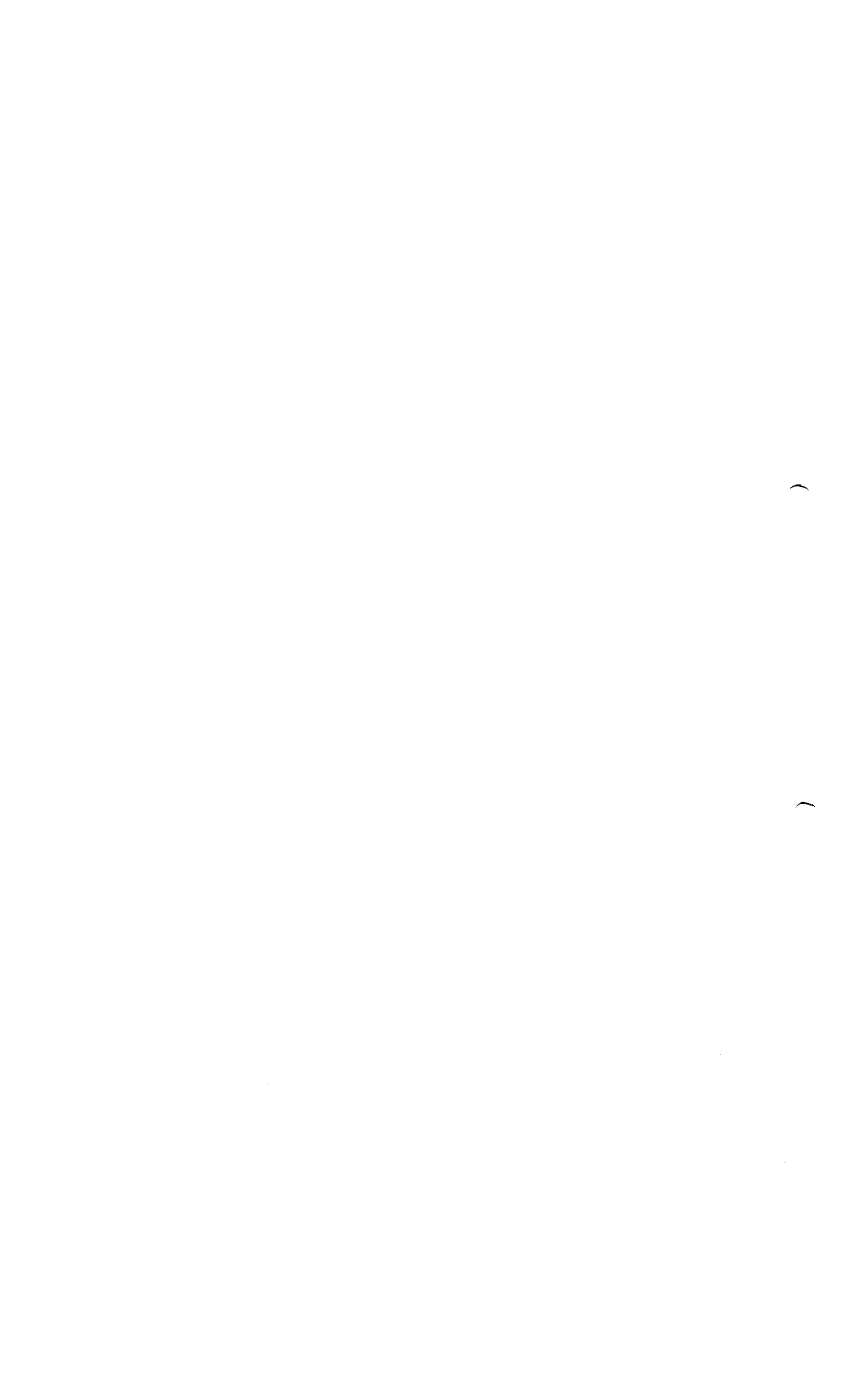
Method : visual inspection  
 Number of vials tested : 8  
 Type of red blood cells : Guinea-pig  
 Incubation : 4 vials, 30 minutes at 5°C ± 3°C  
                   : 4 vials, 30 minutes at 20-25°C  
 Date : 15.05.07  
 Result : **Absence of hemadsorption**

**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 : 16.07.07  
 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	: 15.06.07
Test off	: 29.06.07
Result	: <b>Absence of cytopathic effect</b>

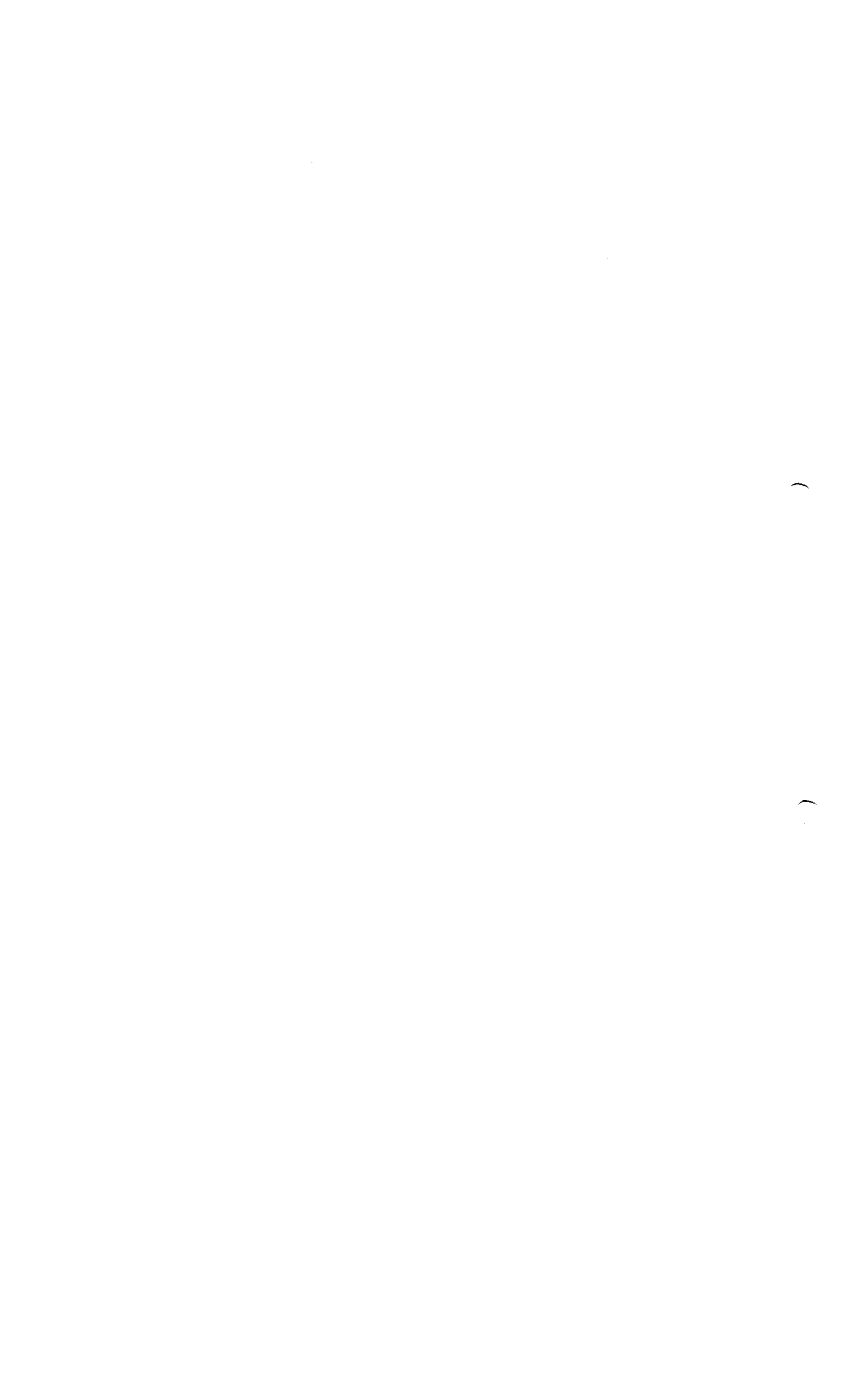
*Human diploid cells*

Method	: Studied through a microscope
Type of cells	: MRC5
Volume tested	: 10 ml
Temperature of incubation	: 36 ± 1° C
Test on	: 26.06.07
Test off	: 24.07.07
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	: 05.06.07
Test off	: 19.06.07
Result	: <b>Absence of cytopathic effect</b>

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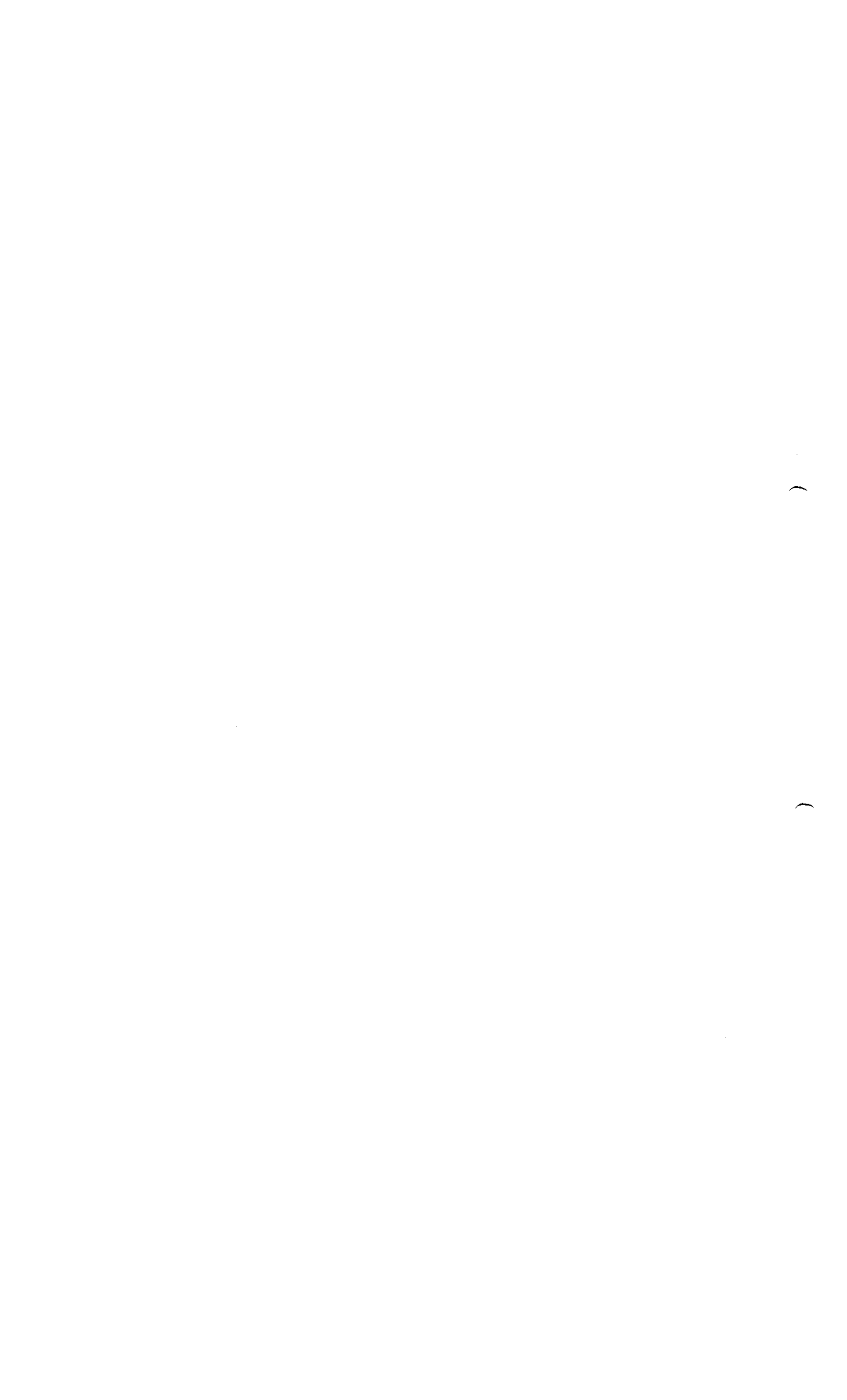
Test for Mycoplasma by culture method

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 : M. pneumoniae and M. orale and A. laidlawii
Primary test
Test on : 28.08.07
Test off : 11.09.07
Subculture at 3rd day
Test on : 31.08.07
Test off : 14.09.07
Subculture at 7th day
Test on : 04.09.07
Test off : 18.09.07
Subculture at 14th day
Test on : 11.09.07
Test off : 25.09.07
Subculture at 21th day
Test on : 18.09.07
Test off : 25.09.07
Result : No growth of Mycoplasma

Test for Mycoplasma by epifluorescence in cell culture

Method : DNA fluorescence staining
Cell used : Vero, 152nd passage
Volume tested : 1 ml
Temperature of incubation : 36 ± 1° C
Positive control : M. hyorhinis and M. orale
Test on : 20.09.07
Test off : 27.09.07
Result : No Mycoplasma detected by epifluorescence in cell culture

Handwritten signatures and stamps for Roxana Montemilone (Directora Técnica) and Christian Domínguez (Moderador) at 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 : 04.06.07  
 Test off : 11.06.07  
 Result : Positive

Polio virus concentration

Method : Cytopathogenic effect on sensitive cells  
 Cell strain used : Hep 2  
 Test on : 04.06.07  
 Test off : 11.06.07  
 Virus concentration :  $10^{8.93}$  CCID<sub>50</sub>/ml  
 Result : Conforms

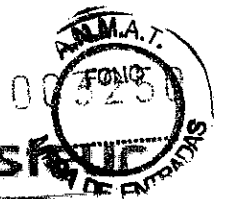
Bacterial and fungal sterility

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	:	11.05.07	
Test off	:	29.05.07	
Result	:	No microbial growth	

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 SUPERVISOR  
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Volume tested	: 34 ml
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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	: 31.08.07
Test off	: 14.09.07
<i>Subculture at 3<sup>rd</sup> day</i>	
Test on	: 03.09.07
Test off	: 17.09.07
<i>Subculture at 7<sup>th</sup> day</i>	
Test on	: 07.09.07
Test off	: 21.09.07
<i>Subculture at 14<sup>th</sup> day</i>	
Test on	: 14.09.07
Test off	: 28.09.07
<i>Subculture at 21<sup>th</sup> day</i>	
Test on	: 21.09.07
Test off	: 28.09.07
Result	: No growth of Mycoplasma

  
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