

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) 99/229

**CONTROLS ON CONCENTRATED PURIFIED VIRAL SUSPENSION:**

**Protein content**

Method : Spectrophotometry  
 Date : 23.05.07  
 Result : 101.51 µg/ml

**D Antigen content**



Method : ELISA  
 Reference standard : 28.1.99  
 Date : 14.05.07  
 Result : 2 716 D Antigen U/ml

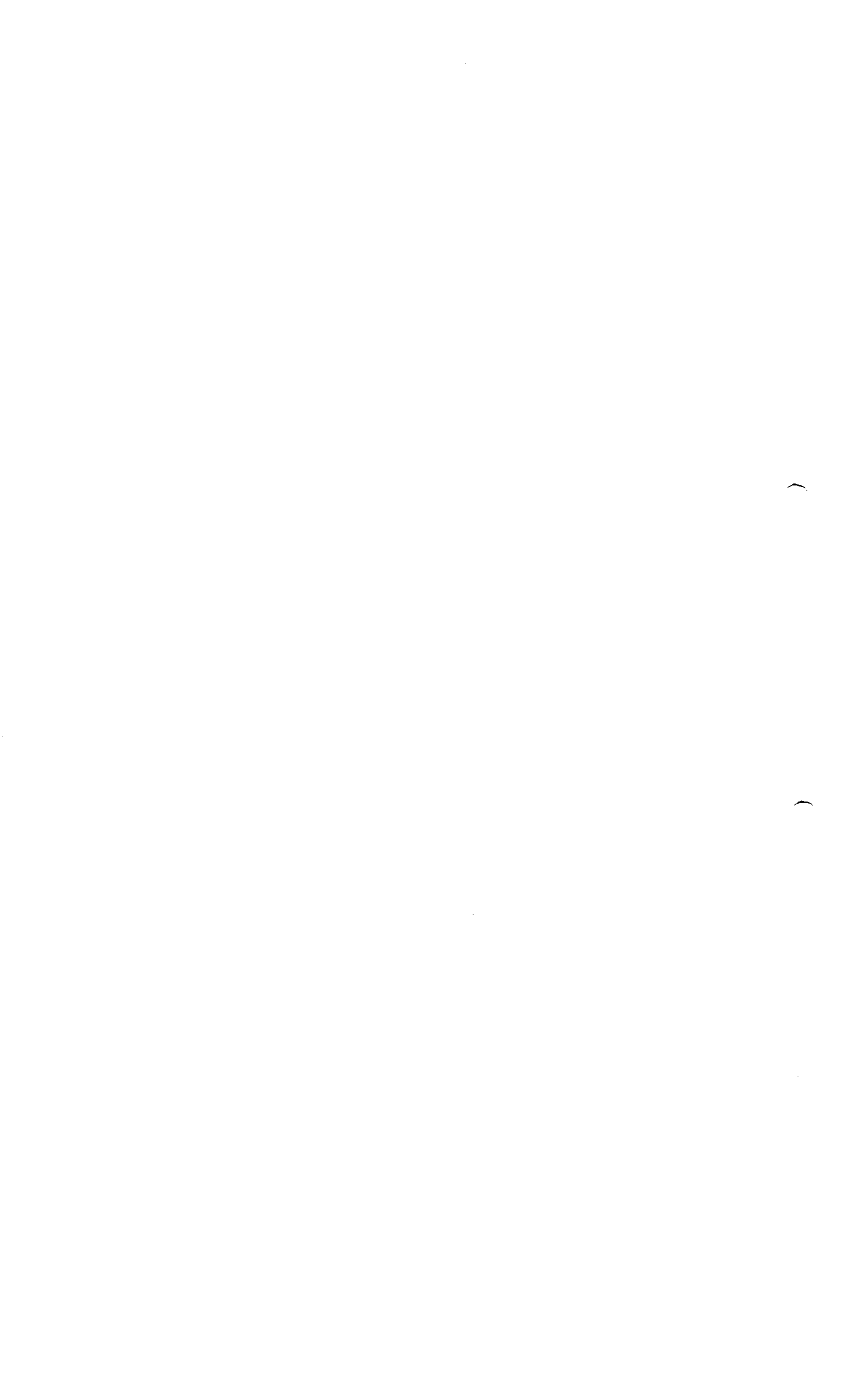
**Bacterial and fungal sterility**

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	:	14.05.07	
Test off	:	29.05.07	
Result	:	No microbial growth	

**Purity**

Result : 0.0374 µg of protein / D Antigen U

 ROXANA MONTEMILONE DIRECTORA TÉCNICA SANOFI PASTEUR S.A.	 CHRISTIAN DOMÍNGUEZ SUPERADO SANOFI PASTEUR S.A.
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**SINGLE HARVEST - Type : 2**  
**Lot Number : FA274124**

**CELL:**

Working Cell Bank : **FA106611**  
 Date of thawing ampoule of WCB : **12.04.07**  
 Thawing ampoule amount : **One ampoule**

**VIRUS:**

Virus Working Seed lot : **FA091251**  
 Date of inoculation : **07.05.07**  
 Date of virus harvest : **09.05.07**

**CRUDE HARVEST:**

Storage : from **10.05.07** to **14.05.07**  
 Volume : **1 486 L**  
 Storage temperature : **+5°C ± 3°C**

**SINGLE HARVEST:**



Date of single harvest : **14.05.07**

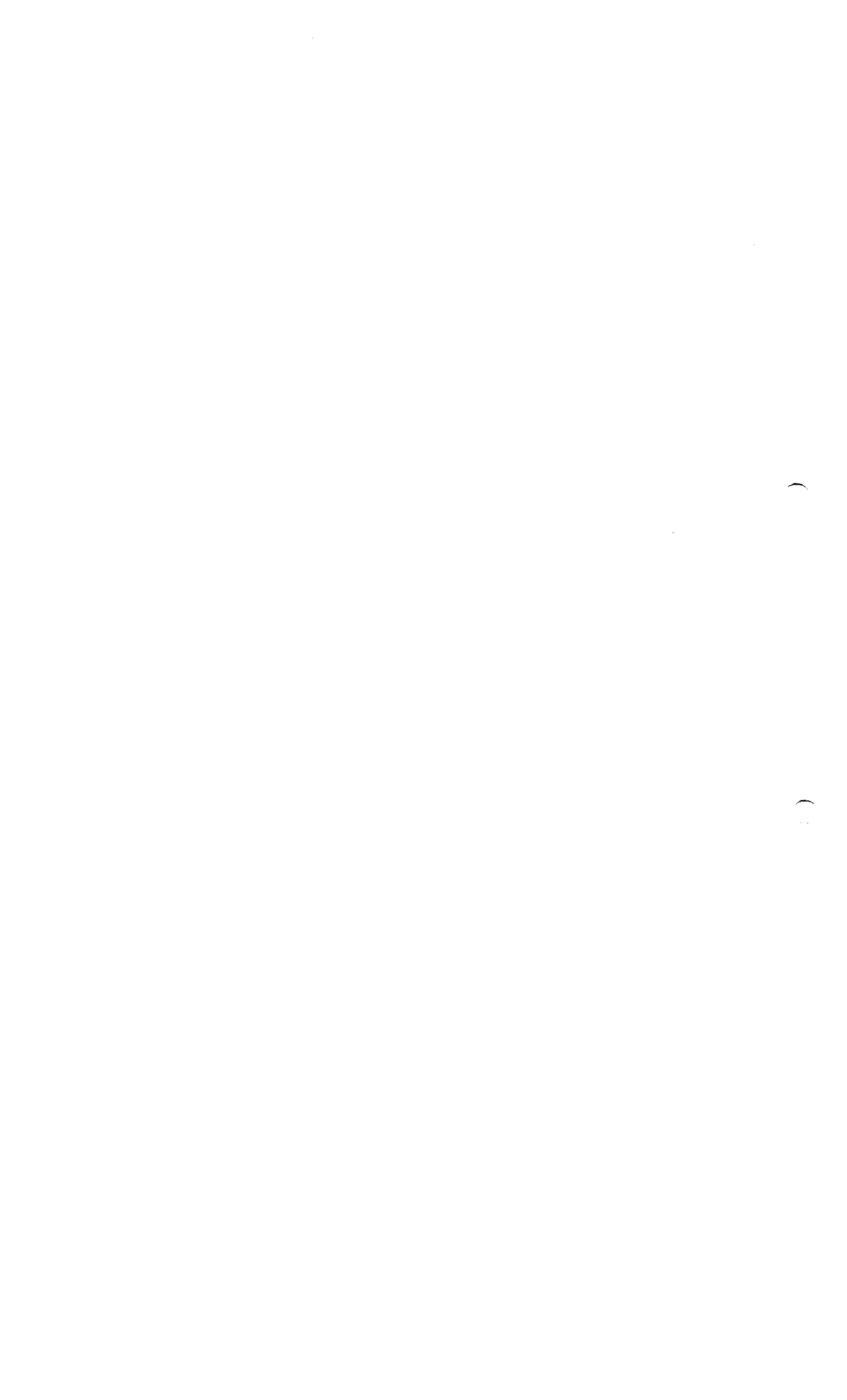
**CONCENTRATION AND PURIFICATION OF THE SINGLE HARVEST**

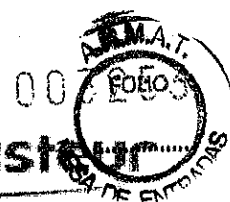
DEAE Spherodex large column : Ion exchange chromatography on **15.05.07**  
 CL-6-B Sepharose column : Gel filtration on **16.05.07**  
 DEAE Sphérodex small column : Ion exchange chromatography on **16.05.07**

**CONCENTRATED PURIFIED VIRAL SUSPENSION :**

Manufacture date : **16.05.07**  
 Volume : **19 400 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 : 07.05.07  
Test off : 22.05.07  
Result : Absence of cytopathic effect

Test for haemadsorbing viruses

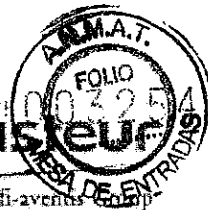
Method : visual inspection  
Number of vials tested : 8  
Type of red blood cells : Guinea-pig  
Incubation : 4 vials, 30 minutes at 5°C ± 3°C  
: 4 vials, 30 minutes at 20-25°C  
Date : 22.05.07  
Result : Absence of hemadsorption

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	: 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	: 26.06.07
Test off	: 24.07.07
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	: 05.06.07
Test off	: 19.06.07
Result	: Absence of cytopathic effect

	
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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>
Primary test	
Test on	: 28.08.07
Test off	: 11.09.07
Subculture at 3 <sup>rd</sup> day	
Test on	: 31.08.07
Test off	: 14.09.07
Subculture at 7 <sup>th</sup> day	
Test on	: 04.09.07
Test off	: 18.09.07
Subculture at 14 <sup>th</sup> day	
Test on	: 11.09.07
Test off	: 25.09.07
Subculture at 21 <sup>th</sup> 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, 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	: 20.09.07
Test off	: 27.09.07
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 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<sup>9.07</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	:	16.05.07	
Test off	:	30.05.07	
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	: 28.08.07
Test off	: 11.09.07
<i>Subculture at 3<sup>rd</sup> day</i>	
Test on	: 31.08.07
Test off	: 14.09.07
<i>Subculture at 7<sup>th</sup> day</i>	
Test on	: 04.09.07
Test off	: 18.09.07
<i>Subculture at 14<sup>th</sup> day</i>	
Test on	: 11.09.07
Test off	: 25.09.07
<i>Subculture at 21<sup>th</sup> day</i>	
Test on	: 18.09.07
Test off	: 25.09.07
Result	: No growth of Mycoplasma

  
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