

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) 128/229

SINGLE HARVEST - Type : 3
Lot Number : FA290835

CELL:

Working Cell Bank : FA106611
Date of thawing ampoule of WCB : 25.10.07
Thawing ampoule amount : One ampoule

VIRUS:

Virus Working Seed lot : FA109086
Date of inoculation : 19.11.07
Date of virus harvest : 22.11.07

CRUDE HARVEST:

Storage : from 23.11.07 to 26.11.07
Volume : 1 524 L
Storage temperature : +5°C ± 3°C

SINGLE HARVEST:

Date of single harvest : 26.11.07

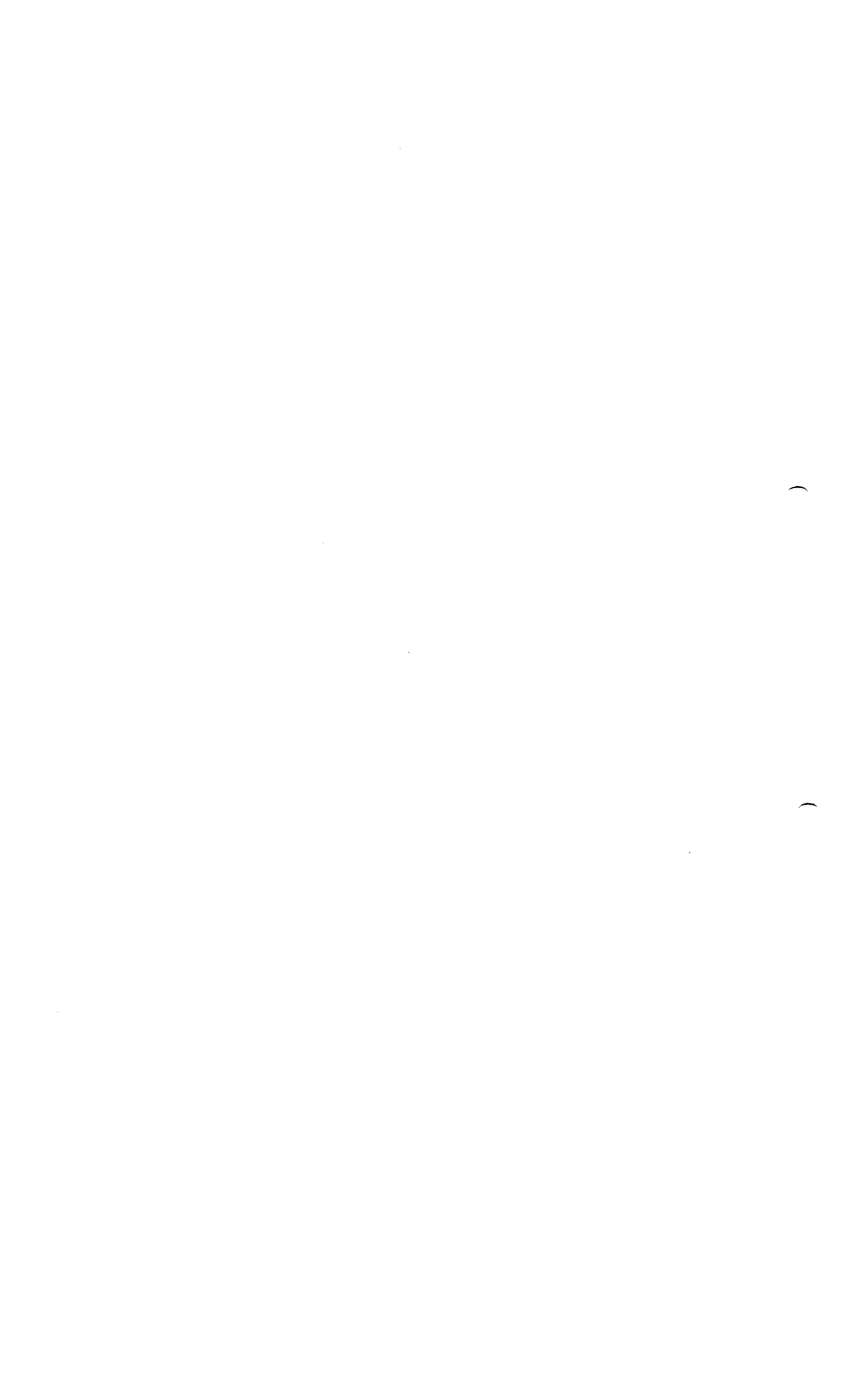
CONCENTRATION AND PURIFICATION OF THE SINGLE HARVEST

DEAE Spherodex large column : Ion exchange chromatography on 27.11.07
CL-6-B Sepharose column : Gel filtration on 28.11.07
DEAE Sphérodex small column : Ion exchange chromatography on 28.11.07

CONCENTRATED PURIFIED VIRAL SUSPENSION :

Manufacture date : 28.11.07
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, 142nd passage
Number of cells tested : 320*10⁶ cells
Test on : 19.11.07
Test off : 03.12.07
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 : 03.12.07
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 : 10.06.08
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	: Absence of cytopathic effect

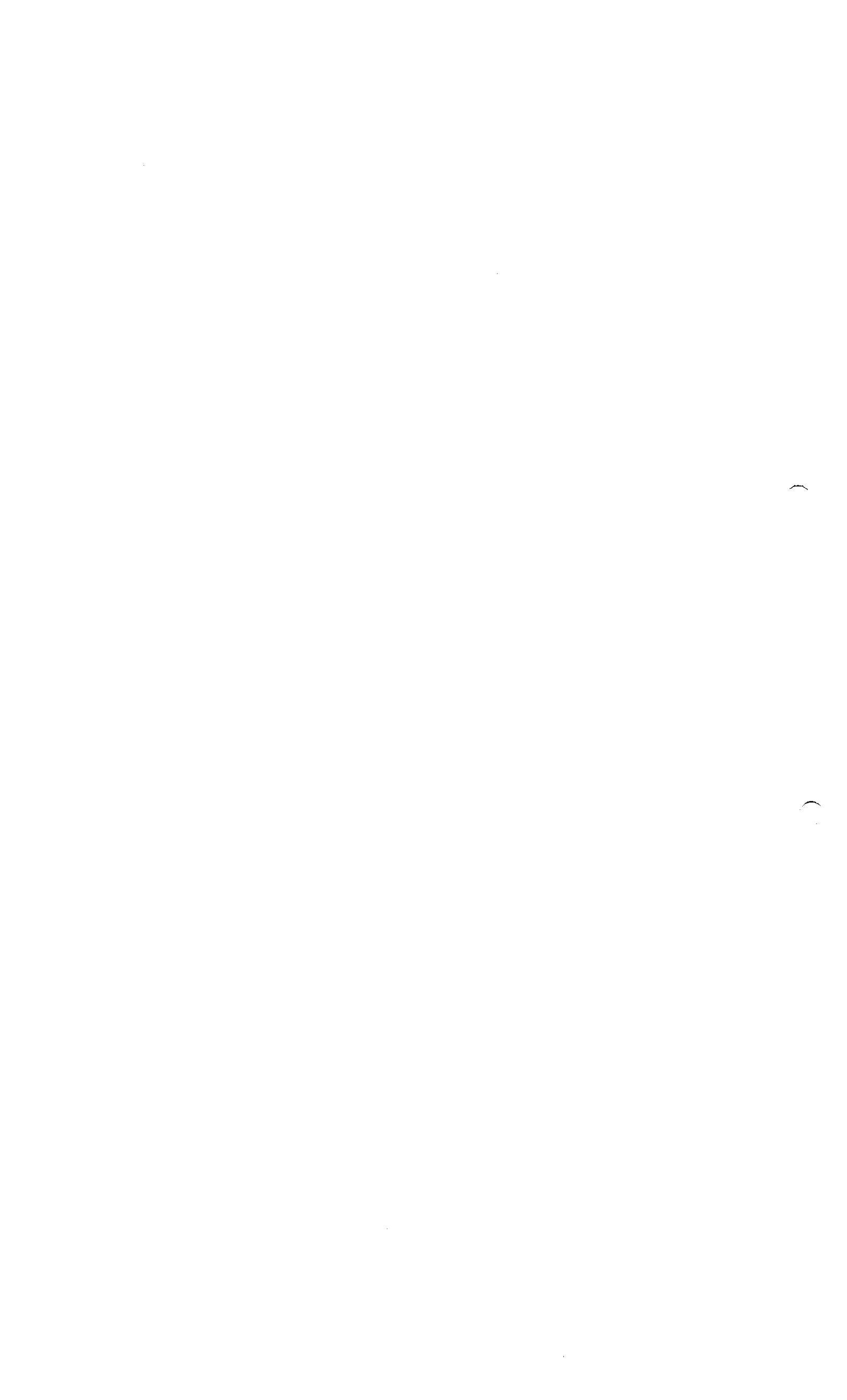
Human diploid cells

Method	: Studied through a microscope
Type of cells	: MRC5
Volume tested	: 10 ml
Temperature of incubation	: 36 ± 1° C
Test on	: 12.02.08
Test off	: 11.03.08
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

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

Primary test
Test on : 28.07.08
Test off : 11.08.08
Subculture at 3rd day
Test on : 31.07.08
Test off : 14.08.08
Subculture at 7th day
Test on : 04.08.08
Test off : 18.08.08
Subculture at 14th day
Test on : 11.08.08
Test off : 25.08.08
Subculture at 21th day
Test on : 18.08.08
Test off : 25.08.08
Result : No growth of Mycoplasma

Test for Mycoplasma by epifluorescence in cell culture

Method : DNA fluorescence staining
Cell used : Vero, 148th passage
Volume tested : 1 ml
Temperature of incubation : 36 ± 1° C
Positive control : *M. hyorhinis* and *M. orale*
Test on : 19.02.08
Test off : 26.02.08
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 : 22.02.08
 Test off : 29.02.08
 Result : Positive

Polio virus concentration

Method : Cytopathogenic effect on sensitive cells
 Cell strain used : Hep 2
 Test on : 22.02.08
 Test off : 29.02.08
 Virus concentration : 10^{8.63} 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	:	28.11.07	
Test off	:	12.12.07	
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	: 28.07.08
Test off	: 11.08.08
<i>Subculture at 3rd day</i>	
Test on	: 31.07.08
Test off	: 14.08.08
<i>Subculture at 7th day</i>	
Test on	: 04.08.08
Test off	: 18.08.08
<i>Subculture at 14th day</i>	
Test on	: 11.08.08
Test off	: 25.08.08
<i>Subculture at 21th day</i>	
Test on	: 18.08.08
Test off	: 25.08.08
Result	: No growth of Mycoplasma


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