

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° S4313 (clinical batch)

96/180



Test for Mycoplasma by culture method

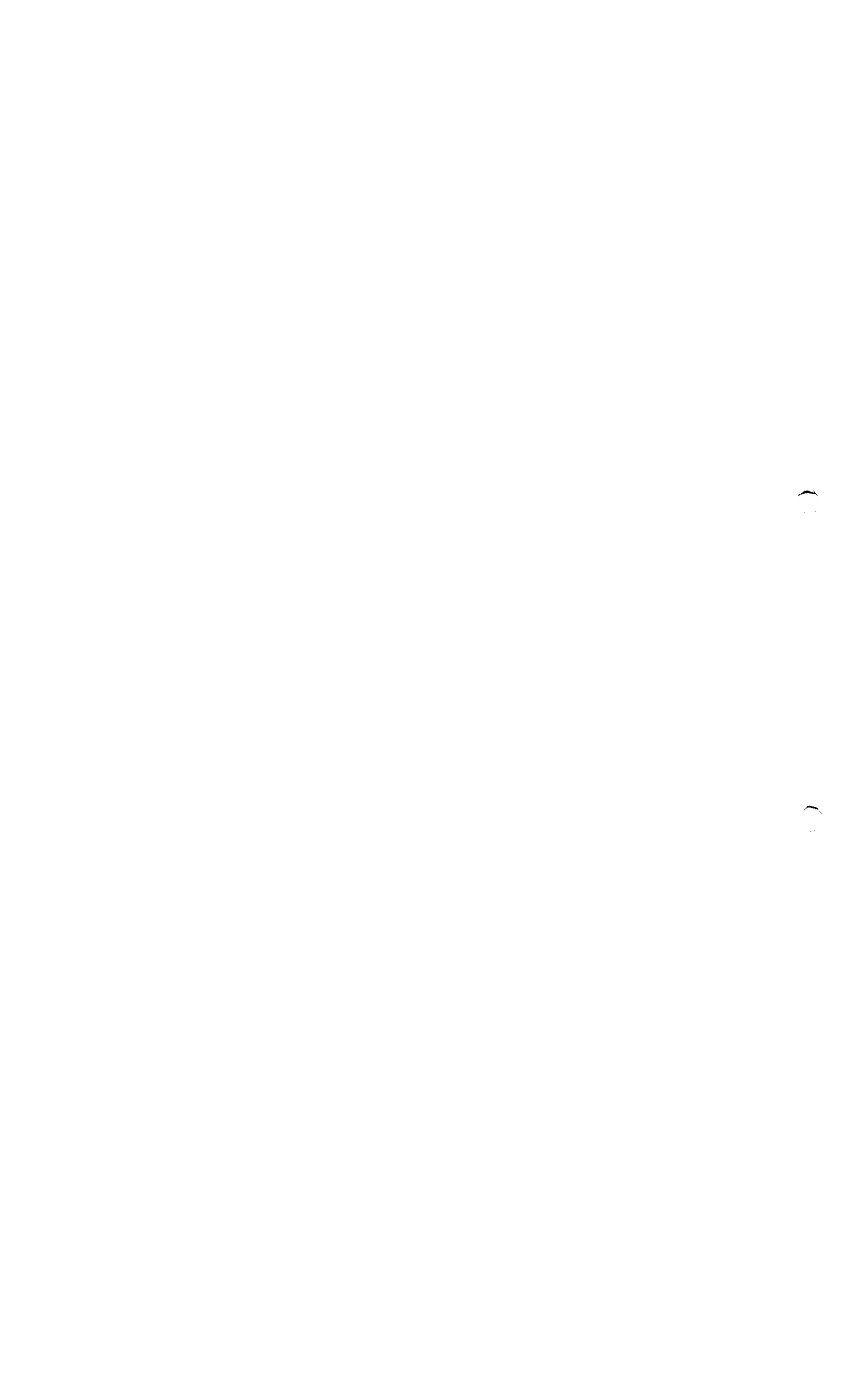
Method	: Agar culture
Volume tested	: 34 ml
Media used	: Hayflick solid Hayflick semi-solid B.O.B. solid B.O.B. semi-solid
Temperature of incubation	: 36 ± 1° C
Positive controls	: <i>M. pneumoniae</i> and <i>M. orale</i> and <i>A. laidlawii</i>

<i>Primary test</i>	
Test on	: 20.10.08
Test off	: 03.11.08
<i>Subculture at 3rd day</i>	
Test on	: 23.10.08
Test off	: 06.11.08
<i>Subculture at 7th day</i>	
Test on	: 27.10.08
Test off	: 12.11.08
<i>Subculture at 14th day</i>	
Test on	: 03.11.08
Test off	: 17.11.08
<i>Subculture at 21th day</i>	
Test on	: 10.11.08
Test off	: 17.11.08
Result	: No growth of Mycoplasma

Test for Mycoplasma by epifluorescence in cell culture

Method	: DNA fluorescence staining
Cell used	: Vero, 152 nd passage
Volume tested	: 1 ml
Temperature of incubation	: 36 ± 1° C
Positive control	: <i>M. hyorhinis</i> and <i>M. orale</i>
Test on	: 20.05.08
Test off	: 27.05.08
Result	: No Mycoplasma detected by epifluorescence in cell culture

	
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 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
 influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch) 97/180

CONTROLS ON SINGLE VIRUS HARVEST:

Polio virus identification


Method : Cytopathogenic effect neutralization
 Cell strain used : Hep 2
 Antiserum : MAHONEY type 1
 Test on : 10.09.08
 Test off : 17.09.08
 Result : Positive


Polio virus concentration

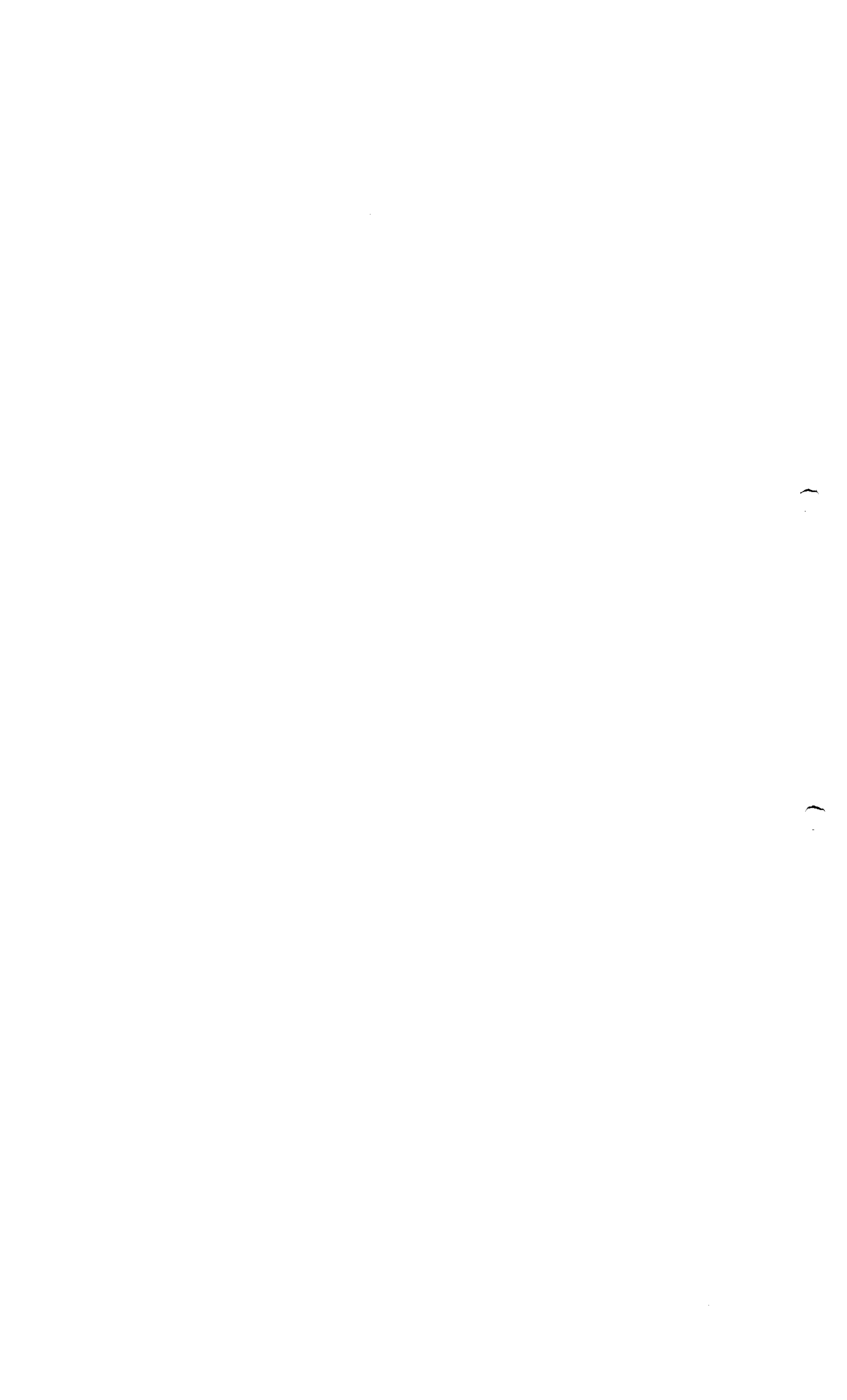
Method : Cytopathogenic effect on sensitive cells
 Cell strain used : Hep 2
 Test on : 10.09.08
 Test off : 17.09.08
 Virus concentration : 108.47 CCID₅₀/ml
 Result : Conforms

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	: 05.02.08	
Test off	: 19.02.08	
Result	: No microbial growth	


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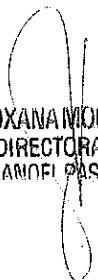



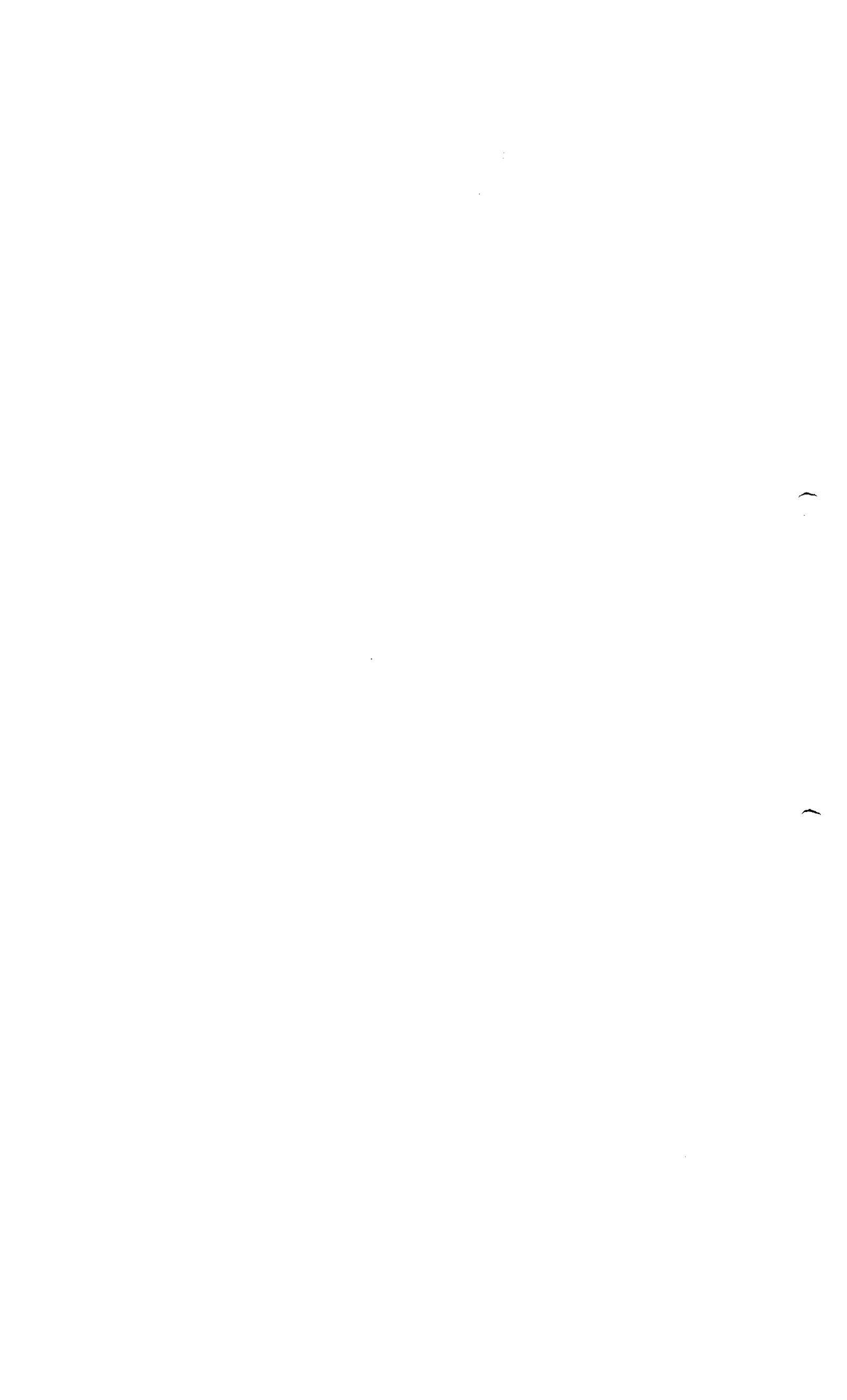
Product Quality and Disposition Department

**Diphtheria, tetanus, pertussis (acellular, component),
 hepatitis B (rDNA), poliomyelitis (inactivated) and Haemophilus
 influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch) 98/180**

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	: 20.10.08
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Test on	: 23.10.08
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<i>Subculture at 7th day</i>	
Test on	: 27.10.08
Test off	: 12.11.08
<i>Subculture at 14th day</i>	
Test on	: 03.11.08
Test off	: 17.11.08
<i>Subculture at 21th day</i>	
Test on	: 10.11.08
Test off	: 17.11.08
Result	: No growth of Mycoplasma

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Product Quality and Disposition Department

Diphtheria, tetanus, pertussis (acellular, component),
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 influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch) 99/180

CONTROLS ON CONCENTRATED PURIFIED VIRAL SUSPENSION :

Protein content

Method : Spectrophotometry
 Date : 18.02.08
 Result : 94.47 µg/ml

D Antigen content


Method : ELISA
 Reference standard : 28.1.99
 Date : 06.03.08
 Result : 8 818 D Antigen U/ml


Bacterial and fungal sterility

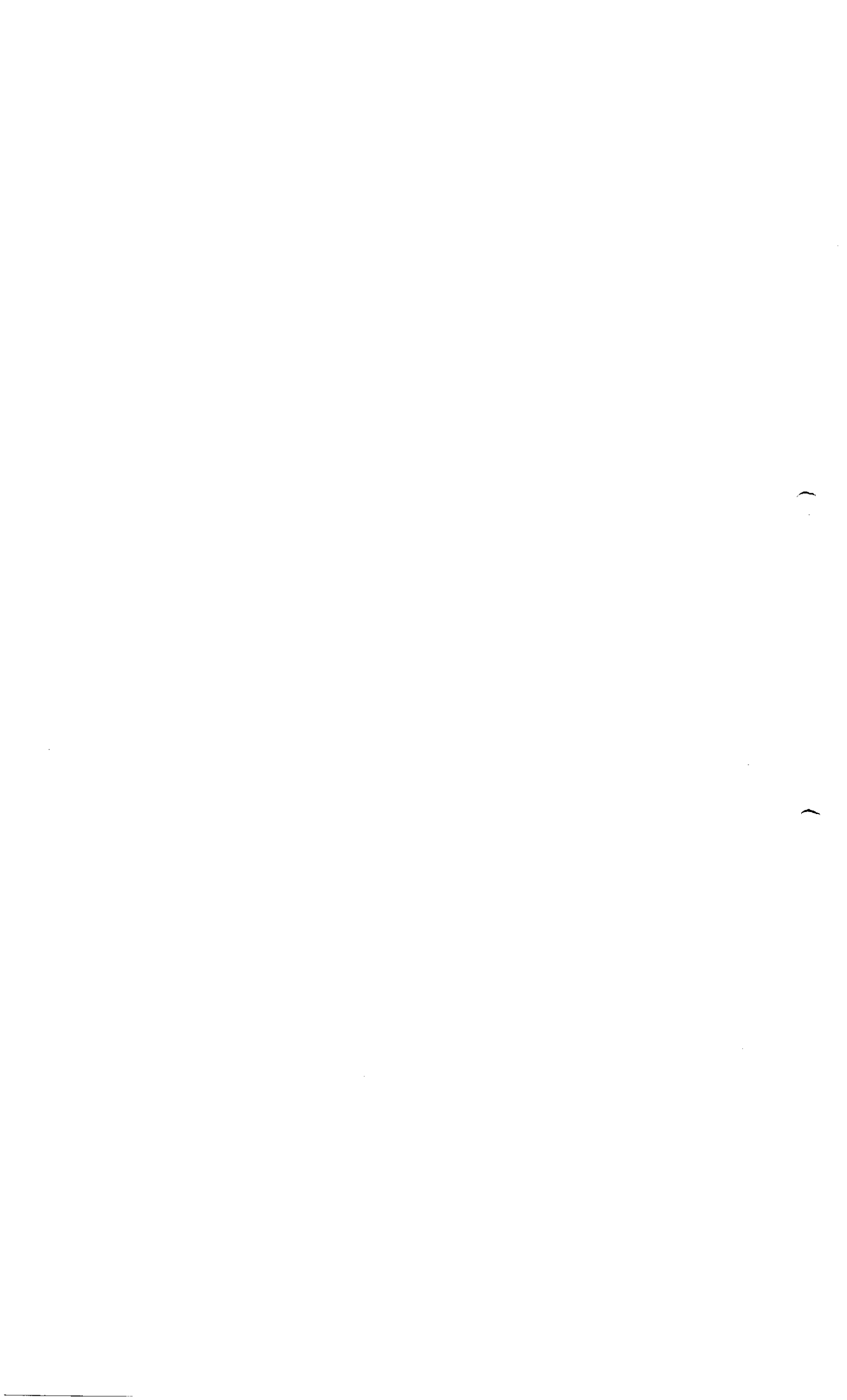
Method used	:	Membrane filtration
Media	:	Thioglycollate medium Soybean Casein Digest medium
Temperatures	:	30-35° C 20-25° C
Volume tested	:	20 ml
Inoculum per Steritest	:	10 ml 10 ml
Volume of medium per Steritest	:	100 ml 100 ml
Test on	:	11.02.08
Test off	:	25.02.08
Result	:	No microbial growth

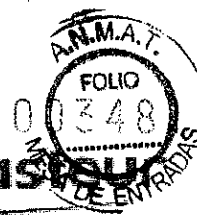
Purity

Result : 0.011 µg of protein / D Antigen U


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100/180

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

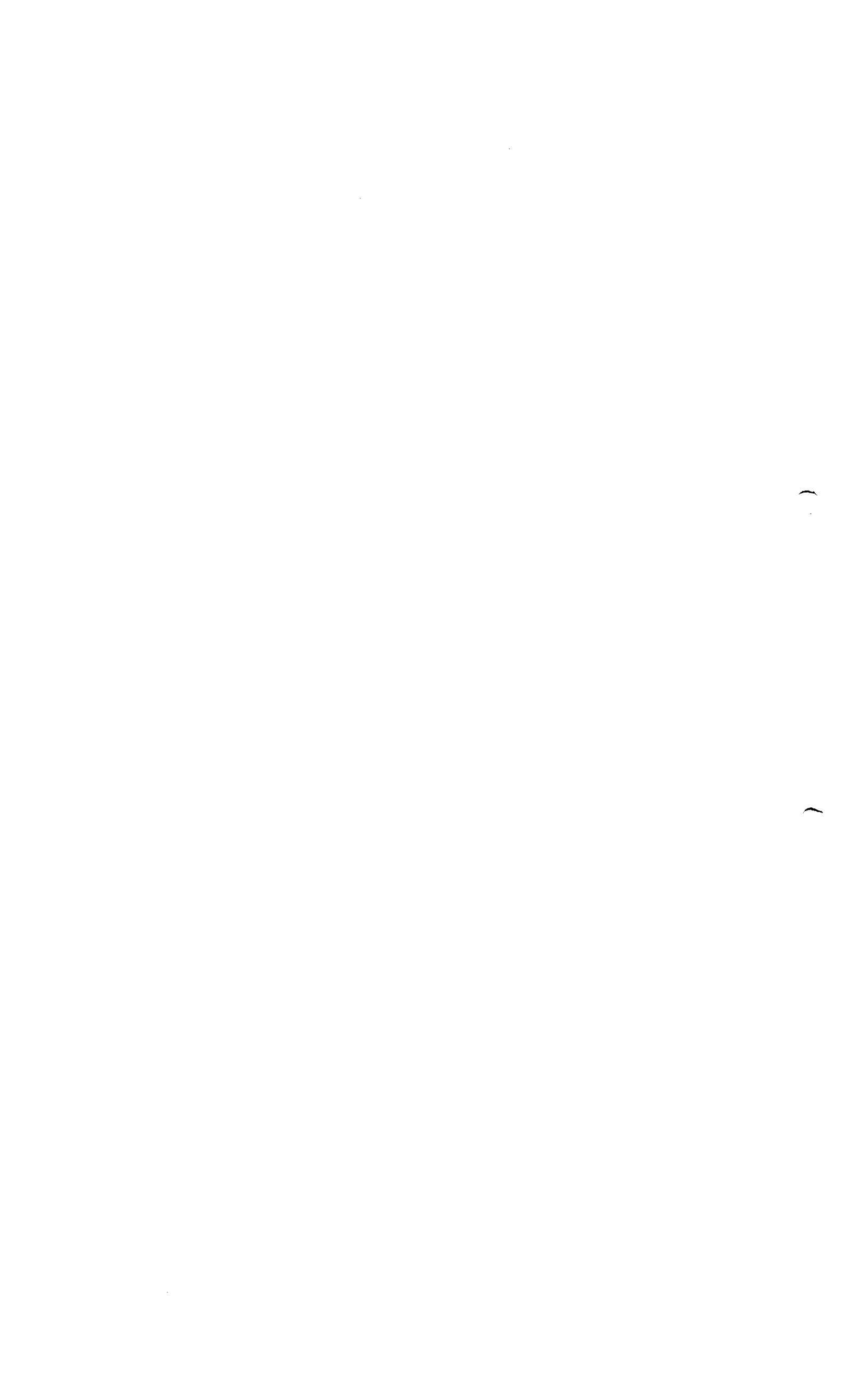
CONCENTRATION AND PURIFICATION OF THE SINGLE HARVEST

DEAE Spherodex 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

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 influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch) 101/180

CONTROLS ON CELLS:

Observation



Method : Studied through a microscope
 Cells : Vero, 142nd passage
 Number of cells tested : 320*10⁶ cells
 Test on : 08.10.07
 Test off : 22.10.07
 Result : Absence of cytopathic effect

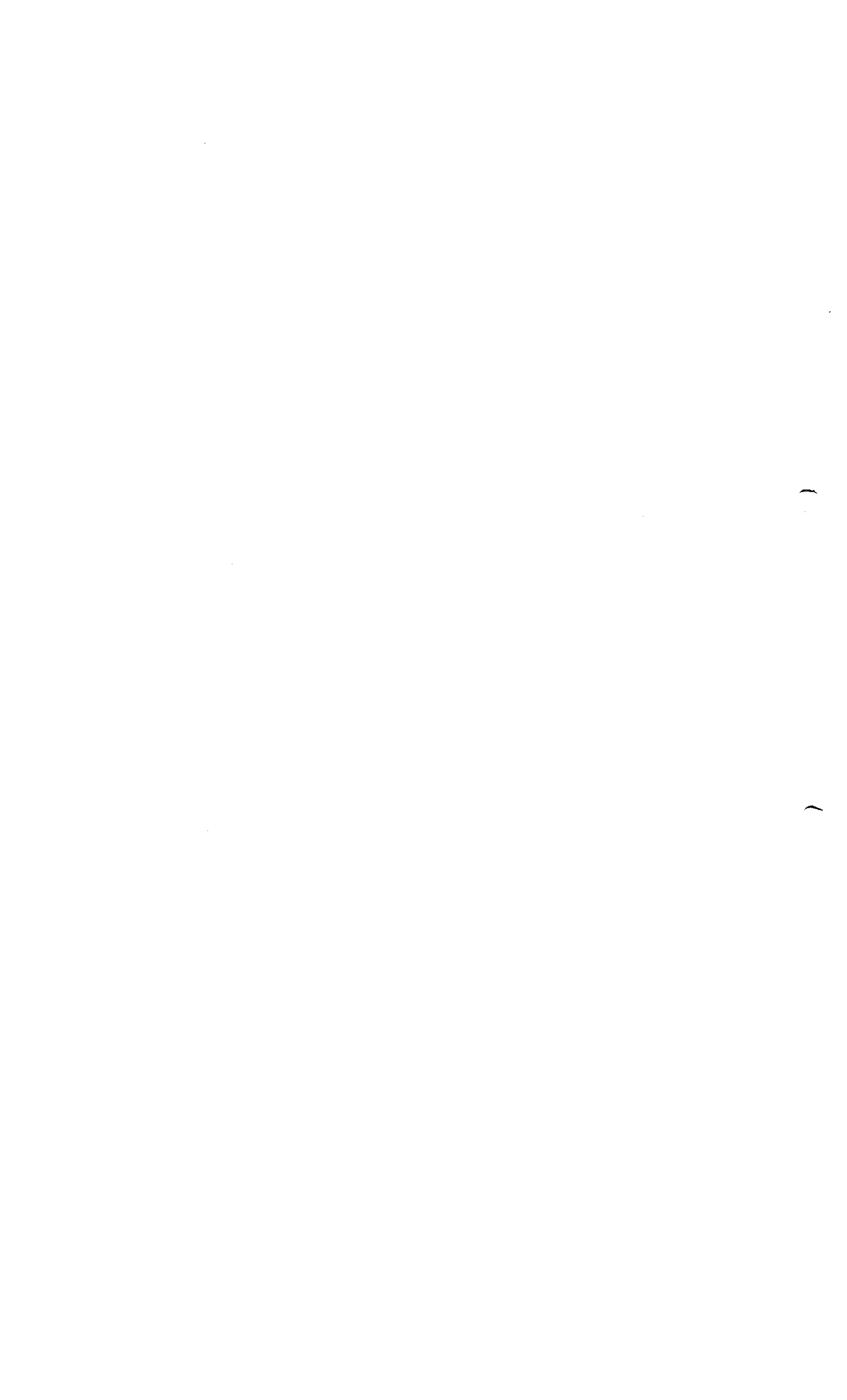
Test for haemadsorbing viruses

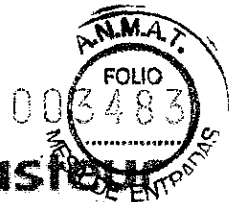
Method : visual inspection
 Number of vials tested : 8
 Type of red blood cells : Guinea-pig
 Incubation : 4 vials, 30 minutes at 5°C ± 3°C
 : 4 vials, 30 minutes at 20-25°C
 Date : 22.10.07
 Result : Absence of hemadsorption

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



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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 : **Absence of cytopathic effect**


Human diploid cells

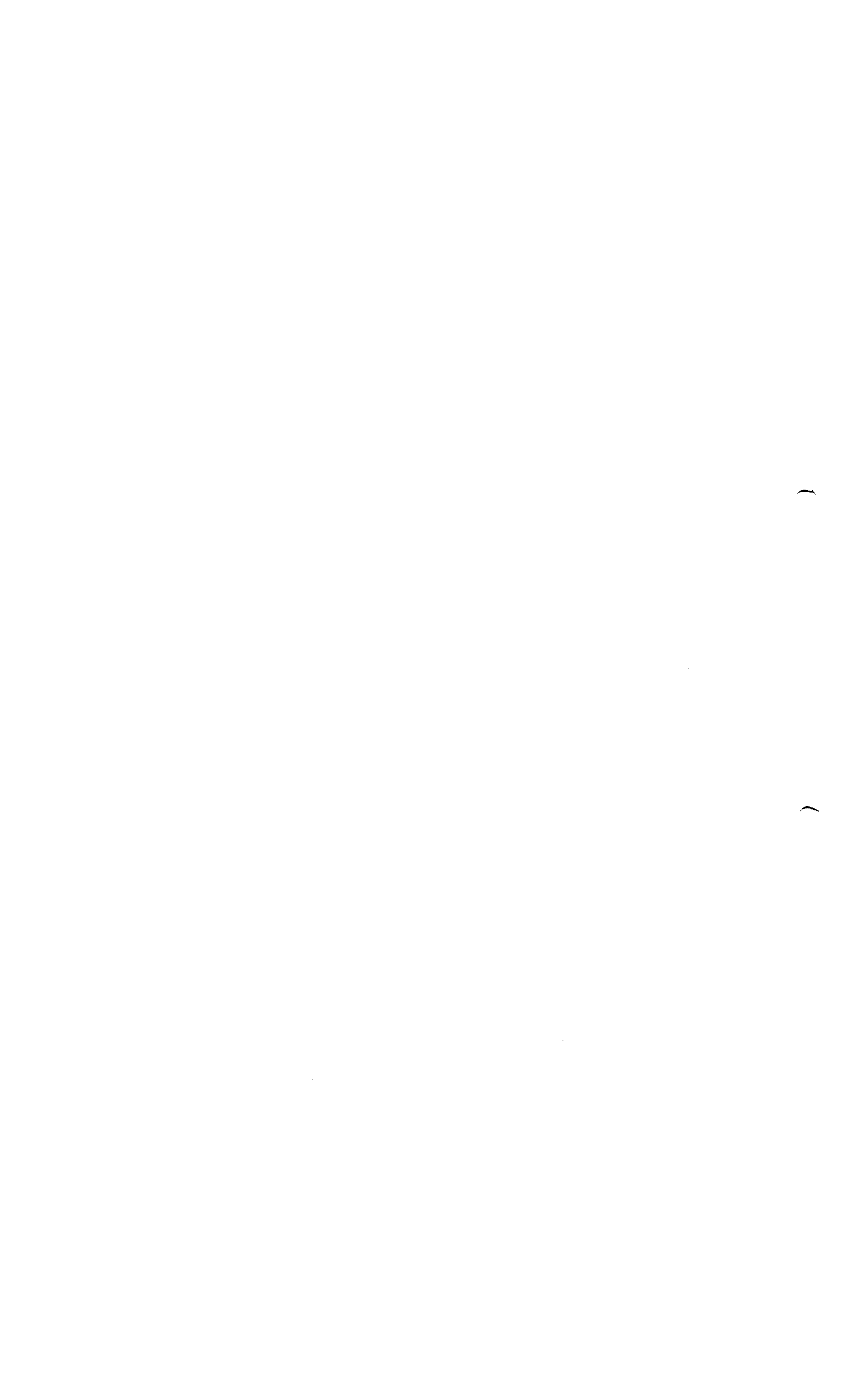
Method : Studied through a microscope
Type of cells : MRC5
Volume tested : 10 ml
Temperature of incubation : 36 ± 1° C
Test on : 22.01.08
Test off : 19.02.08
Result : **Absence of cytopathic effect**

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 : 30.10.07
Test off : 13.11.07
Result : **Absence of cytopathic effect**


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103/180

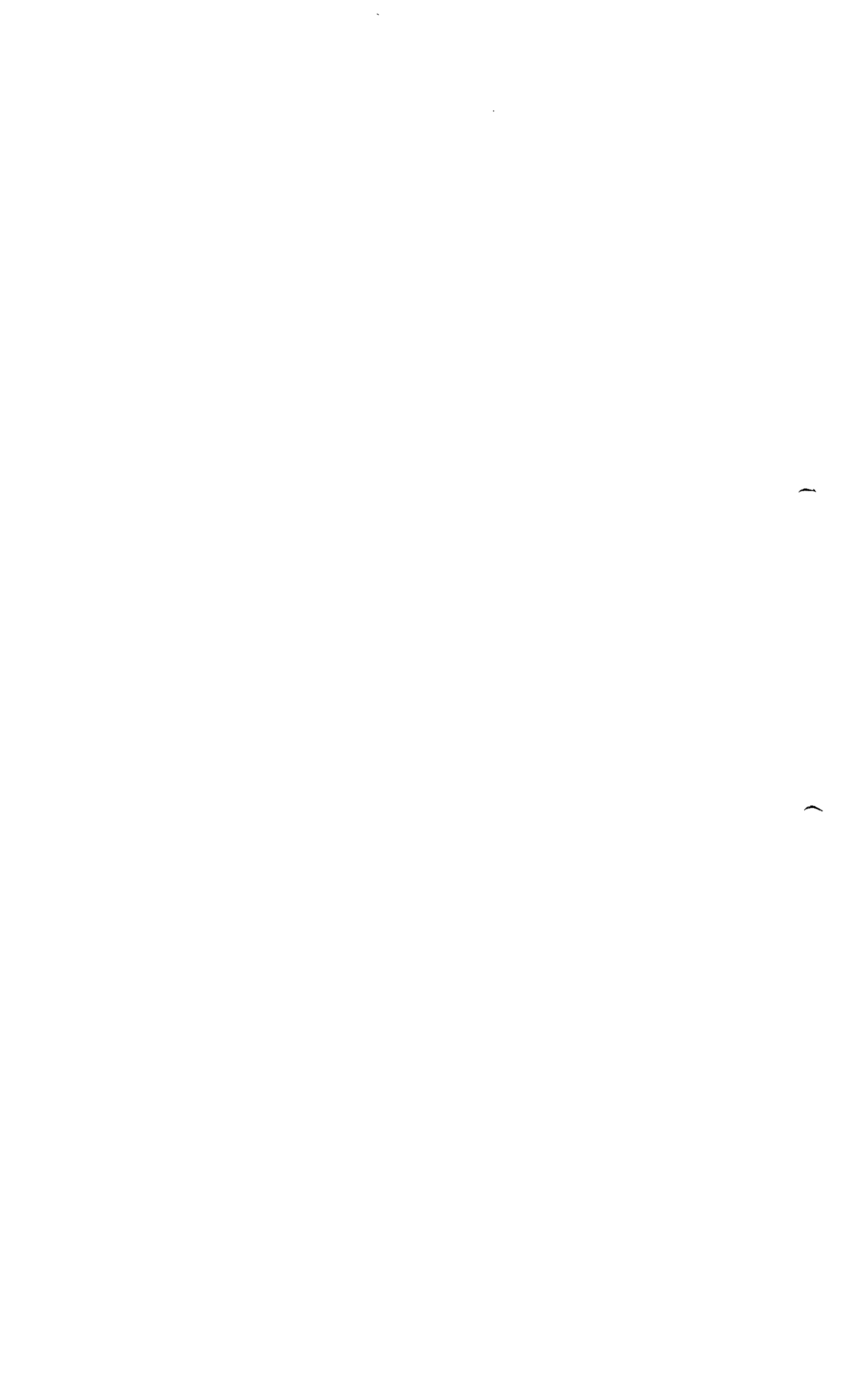
Test for Mycoplasma by culture method

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 3rd day</i>	
Test on	: 25.04.08
Test off	: 13.05.08
<i>Subculture at 7th day</i>	
Test on	: 29.04.08
Test off	: 13.05.08
<i>Subculture at 14th day</i>	
Test on	: 06.05.08
Test off	: 20.05.08
<i>Subculture at 21th 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 nd 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

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 influenzae type b conjugate vaccine (adsorbed) lot n° S4313 (clinical batch) 104/180

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

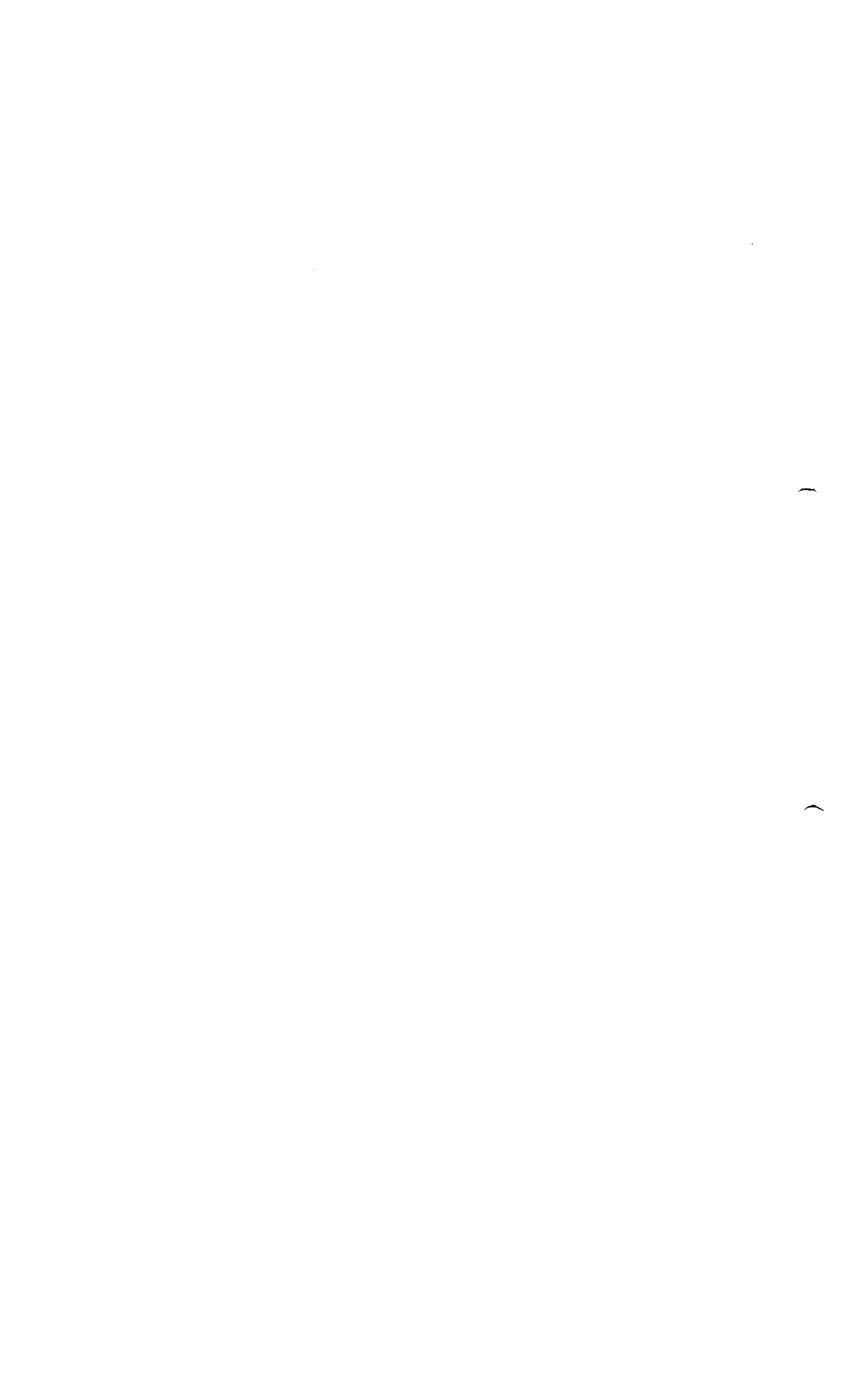
Method : Cytopathogenic effect on sensitive cells
 Cell strain used : Hep 2
 Test on : 23.10.07
 Test off : 30.10.07
 Virus concentration : $10^{9.03}$ CCID₅₀/ml
 Result : Conforms

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	:	18.10.07	
Test off	:	05.11.07	
Result	:	No microbial growth	


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Test for Mycoplasma by culture method

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

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