3.2.S.2.5. Validación o evaluación del proceso

Tabla 1: Consistencia en la producción de mezclas monovalentes de la VPI fabricadas con células Vero a una escala de 150 litros (escala piloto)

Número de lote	**Tipo 1**		**Tipo 2**		**Tipo 3**	
	PVU 92-10*	**PVU 96-01**	**PVU 92-07***	**PVU 96-02**	**PVU 92-08***	**PVU 96-03**
N.º de BCTF	BCTF 2	BCTF 2	BCTF 2	BCTF 2	BCTF 2	BCTF 2
Cultivos celulares						
Código de cultivo primario	UVC 92-21	UVC 96-01	UVC 92-15	UVC 96-03	UVC 92-17	UVC 96-05
Código de cultivo secundario	UVC 92-22	UVC 96-02	UVC 92-16	UVC 96-04	UVC 92-18	UVC 96-06
Código de cultivo terciario	S/D	S/D	S/D	S/D	S/D	S/D
Duplicaciones celulares	7,42	8,4	7,95	8,2	7,64	7,5
Prueba de ausencia de micoplasmas	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de ausencia de agentes extraños	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de identidad de células Vero	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de esterilidad	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada
Cosecha del virus						
Código de cultivo del virus	UVP 92-10	UVP 96-01	UVP 92-07	UVP 96-02	UVP 92-08	UVP 96-03
Volumen de cosecha del virus (L)	150	152	150	146	150	144
Tipo de virus	1	1	2	2	3	3
Titulación del virus (log $CCID_{50}$/ml)	7,62	9,65	7,55	8,44	8,14	7,84
Antígeno D (UD/ml)	79	169	21,6	47	76,4	94
Prueba de ausencia de micoplasmas	aprobada	aprobada	S/D	aprobada	aprobada	aprobada

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3.2.S.2.5. Validación o evaluación del proceso

Número de lote	Tipo 1	Tipo 2	Tipo 3
	PVU 92-10* PVU 96-01	PVU 92-07* PVU 96-02	PVU 92-08* PVU 96-03
Prueba de ausencia de agentes extraños	aprobada aprobada	aprobada aprobada	aprobada aprobada
Prueba de esterilidad	aprobada aprobada	aprobada aprobada	aprobada aprobada

S/D: Sin determinar.

* Estas mezclas se han utilizado para la producción del lote clínico E94-3-3.

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3.2.S.2.5. Validación o evaluación del proceso

Tabla 2: Consistencia en la producción de 6 mezclas monovalentes de tipo 1 fabricadas con células Vero a una escala de producción de 700 litros

Número de lote	PVU 97-101	PVU 98-102	PVU 01-103	PV 04-118	PV 04-119	PV 04-120
N.º de BCTF	BCTF 2	BCTF 2	BCTF 2	BCTF 5	BCTF 5	BCTF 5
Cultivos celulares						
Código de cultivo primario	97 UVC 001	98 UVC 013	01 UVC 019	04VC094	04 VC 097	04 VC 100
Código de cultivo secundario	97 UVC 002	98 UVC 014	01 UVC 020	04VC095	04 VC 098	04 VC 101
Código de cultivo terciario	97 UVC 003	98 UVC 015	01 UVC 021	04VC096	04 VC 099	04 VC 102
Duplicaciones celulares	10,5	10,3	10,11/10,.03[1]	10,5/9,8[1]	10,8/11,0[1]	10,7/10,5[1]
Prueba de ausencia de micoplasmas	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de ausencia de agentes extraños	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de identidad de células Vero	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de esterilidad	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada
Cosecha del virus						
Código de cultivo del virus	97 UVP 004	98 UVP 015	01 UVP 021	04VP096	04VP099	04VP102
Volumen de cosecha del virus (L)	740	760	720	754	754	760
Tipo de virus	1	1	1	1	1	1
Titulación del virus (log $CCID_{50}$/ml)	8,76	7,93	9,11/8,97[1]	8,85/8,69[1]	8,45/8,74[1]	8,80/8,75[1]
Antígeno D (UD/ml)	73	96	77/77[1]	58	61	72
Prueba de ausencia de micoplasmas	aprobada	aprobada	aprobada	no evaluado[2]	no evaluado[2]	no evaluado[2]

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3.2.S.2.5. Validación o evaluación del proceso

Número de lote	PVU 97-101	PVU 98-102	PVU 01-103	PV 04-118	PV 04-119	PV 04-120
Prueba de ausencia de agentes extraños	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de esterilidad	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada

[1] Dos biorreactores.

[2] De acuerdo con los requisitos de la Farmacopea Europea, la prueba de detección de micoplasmas se debe realizar en la cosecha única. En su lugar, las autoridades holandesas han aceptado realizar la prueba en el cultivo celular.

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3.2.S.2.5. Validación o evaluación del proceso

Tabla 3: Consistencia en la producción de 5 mezclas monovalentes de la VPI tipo 2 fabricadas con células Vero a una escala de producción de 700 litros

Número de lote	PVU 97-201	PVU 01-202	PV 04-209	PV 04-211	PV 04-212
N.° de BCTF	BCTF 2	BCTF 2	BCTF 5	BCTF 5	BCTF 5
Cultivos celulares					
Código de cultivo primario	97 UVC 005	01 UVC 022a	04VC109	04 VC 115	04 VC 127
Código de cultivo secundario	97 UVC 006	01 UVC 023	04VC110	04 VC 116	04 VC 128
Código de cultivo terciario	97 UVC 007	01 UVC 024	04VC111	04 VC 117	04 VC 129
Duplicaciones celulares	10,2	10,19/10,21[1]	11,5/11,0[1]	11,8/11,8[1]	11,7/11,6[1]
Prueba de ausencia de micoplasmas	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de ausencia de agentes extraños	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de identidad de células Vero	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de esterilidad	aprobada	aprobada	aprobada	aprobada	aprobada
Cosecha del virus					
Código de cultivo del virus	97 UVP 008	01 UVP 024	04VP111	04VP117	04VP129
Volumen de cosecha del virus (L)	760	708	740	754	754
Tipo de virus	2	2	2	2	2
Titulación del virus (log $CCID_{50}$/ml)	8,7	8,79/8,52[1]	8,30/8,38[1]	8,73/8,11[1]	8,63/8,30[1]
Antígeno D (UD/ml)	30	19/21[1]	21	14	17
Prueba de ausencia de micoplasmas	aprobada	aprobada	no evaluado[2]	no evaluado[2]	no evaluado[2]
Prueba de ausencia de agentes extraños	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de esterilidad	aprobada	aprobada	aprobada	aprobada	aprobada

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[1] Dos biorreactores.

[1] Dos biorreactores.

[2] De acuerdo con los requisitos de la Farmacopea Europea, la prueba de detección de micoplasmas se debe realizar en la cosecha única. En su lugar, las autoridades holandesas han aceptado realizar la prueba en el cultivo celular.

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3.2.S.2.5. Validación o evaluación del proceso

Tabla 4: Consistencia en la producción de 6 mezclas monovalentes de tipo 3 fabricadas con células Vero a una escala de producción de 700 litros

Número de lote	**PVU 97-301**	**PVU 98-302**	**PVU01-303**	**PVU04-308**	**PVU04-309**	**PVU04-310**
N.° de BCTF	BCTF 2	BCTF 2	BCTF 2	BCTF 5	BCTF 5	BCTF 5
Cultivos celulares						
Código de cultivo primario	97 UVC 009	98 UVC 016	01 UVC 025	04 VC 118	04 VC 121	04 VC 124
Código de cultivo secundario	97 UVC 010	98 UVC 017	01 UVC 026	04 VC 119	04 VC 122	04 VC 125
Código de cultivo terciario	97 UVC 011	98 UVC 018	01 UVC027	04VC 120	04 VC 123	04 VC 126
Duplicaciones celulares	10,8	10,5	10,06/10,28[1]	11,1/11,2[1]	11,5/11,2[1]	11,0/10,9[1]
Prueba de ausencia de micoplasmas	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de ausencia de agentes extraños	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de identidad de células Vero	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de esterilidad	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada
Cosecha del virus						
Código de cultivo del virus	97 UVP 012	98 UVP 018	01 UVP 027	04 VP 120	04 VP 123	04 VP 126
Volumen de cosecha del virus (L)	760	760	670	752	754	754
Tipo de virus	3	3	3	3	3	3
Titulación del virus (log $CCID_{50}$/ml)	8,3	7,9	8,52/8,30[1]	8,60/8,57[1]	8,69/8,52[1]	8,16/8,72[1]
Antígeno D (UD/ml)	74	93	50/56[1]	45	46	35

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3.2.S.2.5. Validación o evaluación del proceso

Número de lote	PVU 97-301	PVU 98-302	PVU01-303	PVU04-308	PVU04-309	PVU04-310
Prueba de ausencia de micoplasmas	aprobada	aprobada	aprobada	no evaluado[2]	no evaluado[2]	no evaluado[2]
Prueba de ausencia de agentes extraños	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada
Prueba de esterilidad	aprobada	aprobada	aprobada	aprobada	aprobada	aprobada

[1] Dos biorreactores.

[2] De acuerdo con los requisitos de la Farmacopea Europea, la prueba de detección de micoplasmas se debe realizar en la cosecha única. En su lugar, las autoridades holandesas han aceptado realizar la prueba en el cultivo celular.

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3.2.S.2.5. Validación o evaluación del proceso

3 Validación retrospectiva del proceso de inactivación

El informe se incluye en los apéndices de IPVV.3.2.S.2.5.

IPVV .3.2.S.2.5. inactivation curves.01.pdf

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3.2.S.2.5. Validación o evaluación del proceso

4 Validación del proceso de purificación

El informe se incluye en los apéndices de IPVV.3.2.S.2.5.

IPVV.3.2.S.2.5.validation use DEAE sepharose colums.01.pdf

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Rapport RAP-21709
Version: 1

VOR OK/QCV: Comparison of the inactivation curves of poliovirus produced by Vero and MKC

General information

Old code	N/A
Expiry period	25 years
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Key words	Inactivation, curve, poliovirus, Vero, MKC

Authorization
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This document was compiled by P. Koedam, in the capacity of *staff member QCV*

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Version	Date	Amended
1		New document

Document references.

Number:	Title:
ANA-10101	Measurement of poliovirus titre

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1 Introduction

Vero cells and monkey kidney cells are used in the production of poliovirus. During both processes an inactivation stage with formaldehyde takes place in order to inactivate the living poliovirus. This process is followed over a period of time after initiation of the inactivation process (t=0, t=24, t=48, t=72, t=96 and t=120 hours), whereby the virus sample taken at t=120 hours must not contain living poliovirus. This report describes a retrospective comparison of the inactivation curve of the three separate poliovirus types produced by MKC and Vero cells.

1.1 Requirements of study

The inactivation curves of poliovirus produced by Vero and MKC must have the same shape, confirming that the decrease in virus titer is the same in these two cell types.

2 Administrative information

2.1 Identification

2.1.1 Contractor

S. Schuler

2.1.2 Permission (animal) Ethics committee

N/A

2.1.3 Duties and Authorizations

P. Koedam, technician, practical implementation, author of report
F. van Nimwegen, reviewer
S. Schuler, assessor, authorizer
T. Zandvliet, assessor, authorizer

2.1.4 Required capacity (staff & resources)

N/A

2.1.5 Time period

Start date of Study (from):	08Oct2007
End date of Study (before):	22Oct2007
End date of Report (at latest) :	12Nov2007

2.1.6 Details with regard to Assessment, Verification and Validation of Study Design

N/A

2.2 Reference for Method of Study

This document reports the results of a small-scale study. This is formalised beforehand via a so-called "StartFormulier VOR" [StartForm VOR]. This is signed and authorized by the Head of Department to ensure correct operational procedure. See Appendix §9.3.

2.3 Deviations from Method of Study

N/A

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3 Background information

3.1 Principle of the study / method

The inactivation curve consists in principle of 6 virus titrations (6 different time points, table 1). Determination of the virus titer by titration relies on the principle of end-point dilution. Poliovirus-sensitive cells are added to the different dilutions of the poliovirus. The cell cultures are analysed for the presence of CPE after 7 days of incubation at 36°C. The virus titer is determined according to the Reed & Muench method that determines the CCID50-value. The analysis is carried out according to the European Pharmacopoeia 0214.

Table 1: Dilution scheme inactivation curve

	Dilution	Dilution
Time point	96-well plate	6-well plate
T = 0 hours	$10^{-4}/10^{-11}$	N/A
T = 24 hours	$10^{-3}/10^{-10}$	N/A
T = 48 hours	$10^{-4}/10^{-6}$	undiluted/10^{-3}
T = 72 hours	$10^{-4}/10^{-6}$	undiluted /10^{-3}
T = 96 hours	$10^{-3}/10^{-6}$	undiluted /10^{-2}
T = 120 hours	$10^{-3}/10^{-6}$	undiluted /10^{-2}

3.2 Safety and precautions

The usual precautions and stipulated safety requirements must be adhered to.
Since the study includes the use of virally infected materials, all disposable materials must be discarded in the closed blue containers (WIVA-drums) and all glassware discarded in the stainless steel containers.

3.3 List of abbreviations

CPE : Cytopathic effect
CCID50 : Cell Culture Infective Dose 50%
MKC : Monkey Kidney Cells

3.4 References, incl. Rules & Legislation

ANA-10101 Measurement of poliovirus titer

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4 Approach

4.1 Materials

4.1.1 Test and reference materials

See ANA-10101

4.1.2 (Bio)chemicals, media, biological materials, etc.

See ANA-10101

4.1.3 Materials & Apparatus

See ANA-10101

4.2 Implementation

4.2.1 Reagents and solutions

See ANA-10101

4.2.2 Method

This method investigates retrospectively whether the shape of the inactivation curves of poliovirus produced by Vero and MKC cells is the same, whereby no living poliovirus may be present after 120 hours.
The virus samples used for the inactivation curve are taken at 6 different time points during the inactivation process and tested at the dilutions shown in table 1.
For polio types 1, 2 and 3 produced by Vero cells in the period 2003-2007, there are respectively 27, 13 and 16 curves used. For polio types 1, 2 and 3 produced by MKC cells in the period 1998-2004, there are respectively 9, 8 and 10 curves used.
The results are shown in appendix §9.1.

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5 Results

Tables 2 to 4 show the average virus titers from the results of §9.1 for the three separate poliovirus types. The results are shown in CCID50/ml.
Since calculation of the results not only generates whole numbers but also values such as 0.8<CCID50<1.3 and <0.8, this has been adjusted in the table in the appendix. The value 0.8<CCID50<1.3 is shown in the table as 1.30 and the value <0.8 is shown as 0.00. This adjustment is necessary in order to calculate the average titer as shown in tables 2 to 4.
A clear reduction in poliovirus strength is noticeable for all three polio types. The virus titer of polio produced by Vero cells is higher at the start of inactivation (0 hours) than when MKC cells are used. This has no effect on the shape of the curve however.

Table 2: average virus titer for type 1 in CCID50/ml

	Type 1					
	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Av. MKC	8.47	6.80	3.78	1.47	0.00	0.00
Av. Vero	9.52	6.54	4.13	1.42	0.05	0.00

Table 3: average virus titer for type 2 in CCID50/ml

	Type 2					
	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Av. MKC	9.33	6.86	4.30	1.72	0.16	0.00
Av. Vero	9.85	6.75	4.08	1.39	0.20	0.00

Table 4: average virus titer for type 3 in CCID50/ml

	Type 3					
	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Av. MKC	8.29	6.53	3.69	0.65	0.00	0.00
Av. Vero	9.48	6.64	4.18	1.29	0.00	0.00

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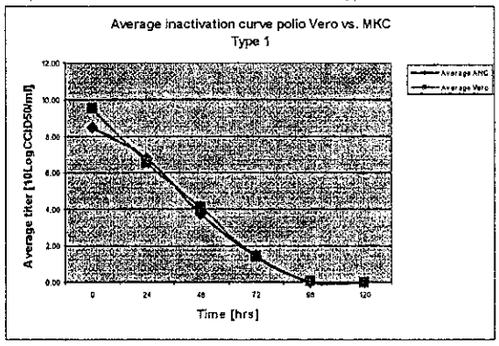
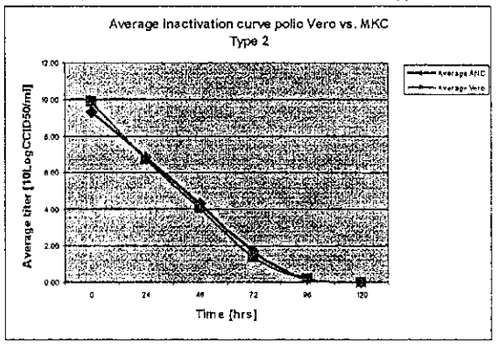
Graphs 1 to 3 plot the results of tables 2 to 4 for the three separate virus types.
The horizontal axis shows the inactivation time in hours. The vertical axis shows the average virus titer (in CCID50/ml). The curves clearly have a linear decrease and a near identical shape.

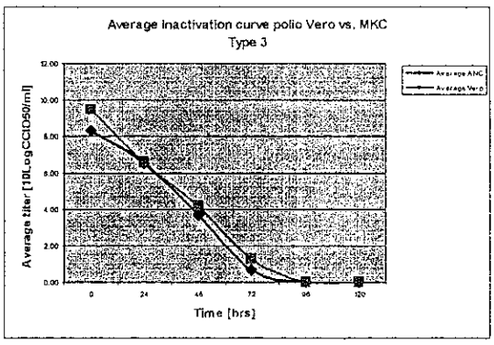
Graph 1: Av. Inact. Curve of Vero vs. MKC type 1



Graph 2: Av. Inact. Curve of Vero vs. MKC type 2



Graph 3: Av. Inact. Curve of Vero vs. MKC type 3



6 Review of objectives

The inactivation curve of poliovirus produced by both MKC and Vero cells shows the same linear decrease in virus titer. The requirements of the study as described in §1.1 have therefore been met. Further investigation is not necessary.

7 Guidelines for further use (incl. validation)

N/A

8 Conclusion

Based on the likeness of the two inactivation curves, it can be concluded that there are no visible differences in the decrease in virus titer during the inactivation process of polio produced by Vero and MKC cells.

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9 Appendices

9.1 Results inactivation curve of polio produced by Vero and MKC.

Tables 5 to 7 show the results of the virus titers of polio produced by Vero, tables 8 to 10 show the results of polio produced by MKC.
Since calculation of the results not only generates whole numbers but also values such as 0.8<CCID50<1.3 and <0.8, this has been adjusted in the table.
The value 0.8<CCID50<1.3 is shown in the table as 1.30 and the value <0.8 is shown as 0.00. This adjustment is necessary in order to calculate the average titer and for an accurate graphical representation of the results. The results are shown in CCID50/ml.

Table 5: Results inact. curve polio type 1 Vero

	Vero Type 1					
Sample name	0	24	48	72	96	120
PV03-104	9.72	6.83	3.80	1.45	0.00	0.00
PV03-105	9.75	6.18	2.80	1.30	0.00	0.00
PV03-106	9.55	6.30	4.05	1.62	0.00	0.00
PV03-107	9.47	6.66	4.16	2.45	0.00	0.00
PV03-108	9.23	6.89	4.60	1.76	0.00	0.00
PV03-109	9.13	6.23	3.99	1.30	0.00	0.00
PV03-110	9.75	6.55	4.30	1.55	0.00	0.00
PV03-111	9.75	7.00	4.55	1.30	0.00	0.00
PV03-112	9.60	6.63	4.55	1.30	0.00	0.00
PV03-113	9.45	6.55	4.55	1.90	0.00	0.00
PV03-114	9.30	6.16	4.55	1.80	0.00	0.00
PV03-115	9.75	6.23	3.80	2.30	0.00	0.00
PV04-116	9.84	6.86	3.97	1.30	0.00	0.00
PV04-117	9.52	6.23	3.45	1.30	0.00	0.00
PV04-118	9.80	6.11	4.18	0.00	0.00	0.00
PV04-119	10.05	6.85	3.90	1.45	1.30	0.00
PV04-120	9.38	6.69	4.05	1.30	0.00	0.00
PV04-121	9.85	5.97	3.70	0.00	0.00	0.00
PV04-122	9.80	6.23	3.80	1.30	0.00	0.00
PV04-123	9.30	6.60	3.90	0.00	0.00	0.00
PV05-125	9.23	6.65	3.65	1.30	0.00	0.00
PV06-126	9.74	6.90	4.84	1.62	0.00	0.00
PV06-127	9.05	6.60	4.60	1.55	0.00	0.00
PV06-128	9.23	6.70	4.75	1.30	0.00	0.00
PV06-129	9.84	7.18	4.60	2.80	0.00	0.00
PV07-131	9.85	6.30	4.30	1.90	0.00	0.00
PV07-132	9.11	6.75	4.05	1.30	0.00	0.00

Table 6: Results inact. curve polio type 2 Vero

	Vero Type 2					
Sample name	0	24	48	72	96	120
PV03-203	10.00	6.30	3.70	1.30	0.00	0.00
PV03-204	9.90	6.30	3.05	0.00	0.00	0.00
PV03-205	9.90	7.00	3.80	1.30	0.00	0.00
PV03-206	9.80	6.52	3.30	1.45	0.00	0.00
PV04-207	8.98	5.97	3.55	1.30	0.00	0.00
PV04-209	10.03	7.23	4.38	1.45	0.00	0.00
PV04-210	9.80	6.90	4.05	0.00	0.00	0.00
PV04-211	9.69	6.30	3.70	1.30	0.00	0.00
PV04-212	9.85	6.90	3.90	1.30	0.00	0.00
PV06-213	10.18	7.18	5.05	2.70	1.30	0.00
PV06-214	10.16	7.23	5.03	1.97	0.00	0.00
PV07-215	9.95	6.79	4.70	2.07	0.00	0.00
PV07-217	9.86	7.16	4.80	1.97	1.30	0.00

Table 7: Results inact. curve polio type 3 Vero

	Vero Type 3					
Sample name	0	24	48	72	96	120
PV03-304	8.92	6.55	3.45	0.00	0.00	0.00
PV03-305	9.83	5.95	3.55	1.30	0.00	0.00
PV03-306	9.74	6.90	4.90	1.30	0.00	0.00
PV03-307	9.45	6.60	4.05	1.30	0.00	0.00
PV03-309	9.69	6.75	4.11	1.30	0.00	0.00
PV04-310	9.45	6.63	3.90	1.30	0.00	0.00
PV05-311	9.74	6.15	4.18	1.80	0.00	0.00
PV06-313	9.74	6.05	4.05	1.55	0.00	0.00
PV06-314	9.69	6.66	4.38	1.30	0.00	0.00
PV06-315	9.53	6.92	4.55	1.30	0.00	0.00
PV06-317	9.73	6.85	4.05	1.30	0.00	0.00
PV07-321	9.80	6.97	4.55	1.30	0.00	0.00
PV07-322	9.23	6.85	4.38	1.62	0.00	0.00
PV07-323	9.55	6.97	4.60	1.30	0.00	0.00
PV07-324	8.97	6.80	4.30	1.30	0.00	0.00
PV07-325	8.95	6.55	3.80	1.30	0.00	0.00

Table 8: Results inact. curve polio type 1 MKC

	MKC Type 1					
Sample name	0	24	48	72	96	120
PU98-1292	8.55	5.78	4.05	1.30	0.00	0.00
PU98-1293	8.30	6.18	4.52	2.90	0.00	0.00
PU98-1294	8.05	8.05	3.80	1.55	0.00	0.00
PU98-1295	7.55	6.84	2.84	1.66	0.00	0.00
PU98-1296	8.70	7.30	2.90	1.30	0.00	0.00
PU01-1322	7.80	5.05	3.12	0.00	0.00	0.00
PU02-1331	8.74	6.85	4.80	1.30	0.00	0.00
PU02-1332	8.97	6.57	3.90	1.95	0.00	0.00
PU04-1341	9.57	6.60	4.05	1.30	0.00	0.00

Table 9: Results inact. curve polio type 2 MKC

	MKC Type 2					
Sample name	0	24	48	72	96	120
PU98-269	9.30	6.84	3.90	0.80	0.00	0.00
PU97-272	9.30	7.80	3.80	1.53	0.00	0.00
PU97-274	9.80	7.16	4.97	1.47	0.00	0.00
PU97-275	9.80	6.05	4.05	2.00	0.00	0.00
PU97-276	9.55	6.30	4.16	1.70	0.00	0.00
PU01-291	8.38	5.66	3.90	1.30	0.00	0.00
PU02-294	9.63	6.76	4.70	3.47	1.30	0.00
PU02-296	8.86	6.30	4.89	1.45	0.00	0.00

Table 10: Results inact. curve polio type 3 MKC

	MKC Type 3					
Sample name	0	24	48	72	96	120
PU98-3406	8.80	7.76	3.80	1.30	0.00	0.00
PU98-3407	8.97	7.90	3.97	1.30	0.00	0.00
PU98-3409	7.70	7.70	3.90	0.00	0.00	0.00
PU98-3410	8.70	6.30	3.74	0.00	0.00	0.00
PU98-3411	7.84	6.97	4.05	1.30	0.00	0.00
PU01-3434	6.89	4.97	2.60	0.00	0.00	0.00
PU02-3440	8.52	5.80	3.75	1.30	0.00	0.00
PU02-3445	7.80	5.66	3.07	0.00	0.00	0.00
PU02-3447	8.92	6.22	3.80	0.00	0.00	0.00
PU02-3448	8.73	6.03	4.16	1.30	0.00	0.00

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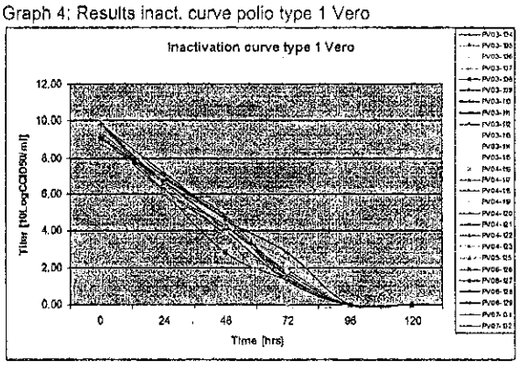
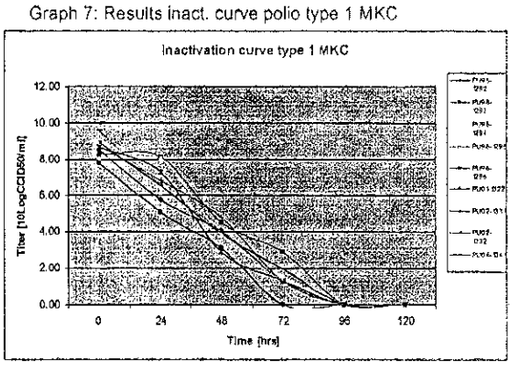
9.2 Graphical representation of the inactivation curves for tables 5 to 10 §9.1

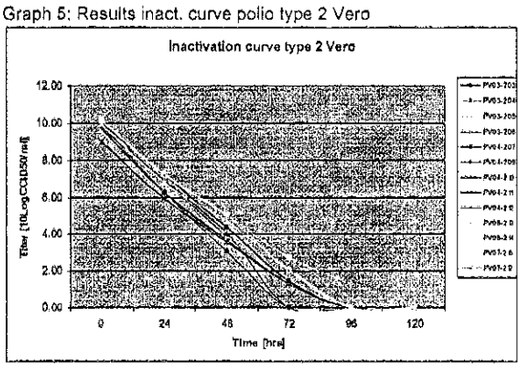
Graph 4: Results inact. curve polio type 1 Vero



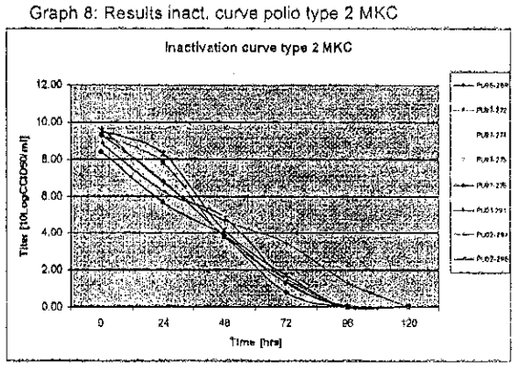
Graph 7: Results inact. curve polio type 1 MKC



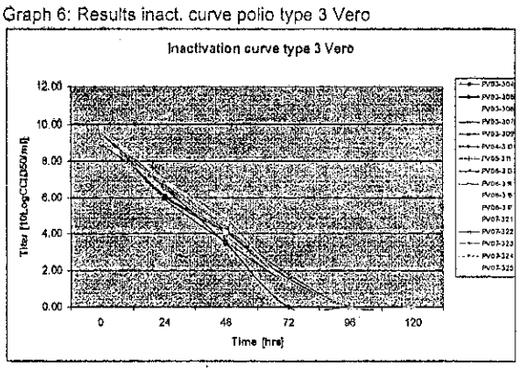
Graph 5: Results inact. curve polio type 2 Vero



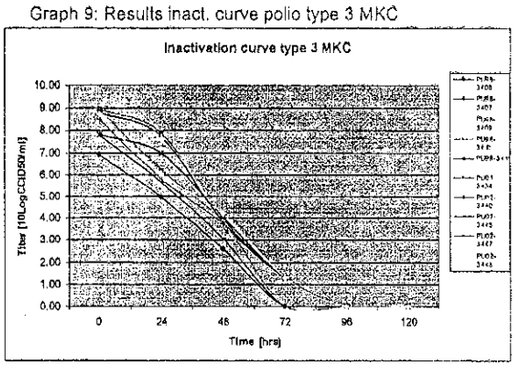
Graph 8: Results inact. curve polio type 2 MKC



Graph 6: Results inact. curve polio type 3 Vero



Graph 9: Results inact. curve polio type 3 MKC



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9.3 VOR Start Form

Formulier FOR-20173
Versie: 2

AANMELDINGS-FORMULIER Blad 2 van 2

Gegevens m.b.t. het Onderzoek :

Onderwerp / Titel : Vergelijking van de inactiveringscurve van polio geproduceerd op Vero en ANC.

'Nieuw' Onderzoek : Nee
'Aanvullend' Onderzoek: Ja

Project Titel + nr.: N.v.t.
SOP Titel + nr.: ANA-10101, Polivirusgehalte bepaling

Gegevens Aanvrager :

Naam : P. Koedam
Lab/Unit : OK/QCV
Datum : 08OKT2007

Administratieve Gegevens:

Opdrachtgever (Afd.hoofd/Proj.leider) : Saskia Schuler SS
Autorisatie (Opdrachtgever/Afd.hoofd) : Saskia Schuler Datum : 06 nov 2007

PLAN VAN AANPAK (verkort) :

Dit rapport beschrijft retrospectief de vergelijking van de inactiveringscurve van de drie afzonderlijke poliotypes geproduceerd op ANC en Verocellen.
Beide cellen worden gebruikt voor de productie van polio, maar nog niet eerder was gekeken of de inactivering die plaatsvindt overeen komt.

Afbakening :

Afhankelijk van de resultaten van dit onderzoek zal worden bepaald of een vervolgonderzoek noodzakelijk is.

Aanvullende Opmerkingen : N.v.t.

Bijlagen / Referenties : N.v.t.

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Report
Project and project number: Registration IPV-Vero (V/000013/01/AA)
Corresponding test design: Ton Val Ion CHR IPV-Vero
Period: August to December 2000

Effect of repeated use of DEAE-Sepharose FF columns on the purification of the IPV-Vero fraction 4.1

Aim

The ion exchange chromatography for the preparation of IPV-Vero for fieldwork and registration activities is carried out using DEAE-Sepharose FF. The advantage of this material over the previously used DEAE-Sephadex A50 is that few bed volume differences occur during the purification and rinsing steps. A DEAE-Sepharose column can, therefore, be used for more than one run. This research is intended to determine whether the product quality remains constant after repeated use of the column.

Materials

- PVU 97-301-3.1, kept at –20 °C.
- DEAE-Sepharose FF (charge no. 241652, produced on 020597, expired on 200402).
- 0.04 M phosphate buffer pH 7.0 (Z. 2625c, charge 2000333, produced on 310100, expired on 300101).
- 0.1 M NaOH (Z. 6224c, charge 2000442, produced on 100300, expired on 100301).
- Distilled water (Z. 3950c, charge 2000825, produced on 150200, expired on 140202).
- 1 M NaCl (produced on 260700, expired on 250701).
- PIERCE protein determination: BCA*Protein Assay Reagent A No. 23223 and Reagent B No. 23224 and Albumin Standard No. 23209 from PIERCE.
- SDS PAGE: see SOP 14N-59.
- D-antigen: see SOP 14C-05.
- Column: HR 5/20.
- Pump: LKB 2150 HPLC Pump.
- Injector: Rheodyne 7125 with coupled loops of 1000 µl and 500 µl.
- Detector: LKB RSD (diode array) and 7 LKB 2158 Uvicord SD from run 7.
- Recorder: LKB 2210.
- Fraction collector: LKB Frac 100.
- Spectrophotometer: Pharmacia Biotech Ultrospec 1000E.

Methods

The purification was reduced to lab scale. Fraction 3.1, kept at –20 °C, was purified (see Table 1) after which a number of analyses that are indicative for the purity were carried out on the virus peak. Because of the limited availability of the IPV-Vero fraction 3.1 ten purifications were carried out: two initial purifications with PVU 97-101-3.1 to test the system (evaluation of the chromatogram only) and eight purifications with PVU 97-301-3.1. After downscaling, the ion exchange chromatography was carried out as far as possible as described in DRAFT PRODUCTION SOP MONOVALENT POLIO VACCINE ON VERO-CELLS: 11P-IPV-04. For practical reasons the sterilisation step using 0.1 M NaOH at higher flow and 5 x column volume was carried out without standing for 24 hours in NaOH.

Table 1 Comparison of purification, regeneration and disinfection conditions during production scale and during lab scale

Procedure at production scale	Procedure after downscaling
• Column BPG 200/500	• Column HR 5/20
• Surface area 314 cm^2, height 25 cm, vol. 7.8 l.	• Surface area 0.2 cm^2, height 20 cm, vol. 4 ml.
• Void volume: 2.7 l.	• Void volume: 1.3 ml
• Vol. gel: Vol. fr. 3.1 = 2.5 : 1	• Vol. gel: Vol. fr. 3.1 = 2.5 : 1
• Elution 15 cm/hour ≅ 78 ml/min.	• Elution 15 cm/hour ≅ 0.05 ml/min.
• Fraction: 4.1, 5.5 to 5.9 l.	• Fractions: 1 ml, 4.1 composed on basis of A260/280 ratio of the fractions (peak 1)
Detection at 260 and 280 nm Recorder 100 mV and 1.0 mm/min.	
Regeneration and disinfection of DEAE-Sepharose FF column	
• 12 litres 1M NaCL overnight 11.7 ml/min (2.23 cm/hour, 1.6 x Col.Vol.)	• 10 ml 1 M NaCL overnight 0.01 ml/min. (3 cm/hour, 2.5 x Col. Vol.)
• 60 litres 0.1 M NaOH 2 days 16.7 ml/min + 24 hour soaking without flow (7 x Col. Vol.).	• 21 ml 0.1 M NaOH 0.05 ml/min. by day 7 hours (5 x Col. Vol.).
• 60 litres 0.04 M phosphate buffer 33.7 – 66.7 ml/min overnight (7 x Col. Vol.).	• 51 ml 0.04 M phosphate buffer 0.05 ml/min overnight (13 x Col. Vol.).

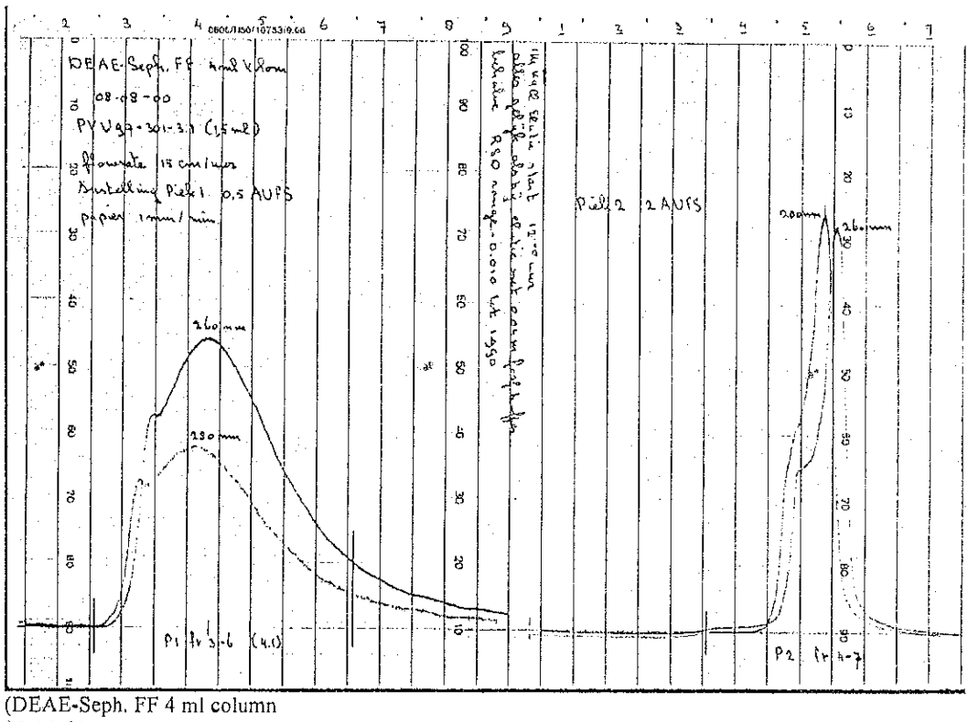
Results

Quality of starting material

After melting PVU 97-301-3.1 was clear and the protein content before and after 0.22µ filtration was the same (1011 and 1039 µg/ml respectively). The protein content on the basis of the nitrogen determination (immediately after purification and not frozen) and from the Pierce protein determination (after keeping at –20 °C and melting) was the same (1039 µg/ml and 1025 µg/ml). This material therefore seems suitable for the present experiment; fresh or non-frozen fraction 3.1 was not available.

The test runs with PVU 97-101-3.1 show a chromatogram with one peak during the elution with 0.04 M phosphate buffer with a ratio of E 260 : E 280 of 1.62, which is characteristic of the purified virus, and one peak during the elution with 1 M NaCl. It was decided to use detection sensitivity of 0.5 AUFS to detect the virus peak and 2 AUFS for the peak of contaminating proteins on the basis of the height of the peaks. On the basis of the position of the peaks it was decided to collect 9 fractions of 1 ml from both peaks and to pool fractions 3 to 6 from peak 1 corresponding to fraction 4.1 from the production, and fractions 4 to 7 from peak 2 corresponding to the clearance peak 4.4 from the production (see Figures 1 and 2 below). Eight purifications using PVU 97-301-3.1 were then carried out. This material was kept at 4°C in the period between the first and last purification (± 3 weeks). The virus was filtered just before the chromatography in order to remove any precipitation that may have formed.

Figure 1 Elution pattern of the purification of PVU 97-301-3.1 using HR 5/20 column with 4 ml DEAE-Sepharose FF (downscaling).



(DEAE-Seph. FF 4 ml column
08-08-00
PVU 97-301-3.1 (1.5 ml)
flowrate 15 cm/hour
Setting: peak 1 0.5 AUFS
paper 1 mm/min.

1 M NaCl elution start 12.00 hours
everything the same as with elution using 0.04 M phosphate buffer except for RSD range - 0.010 to 1.990

peak 2 2 AUFS)

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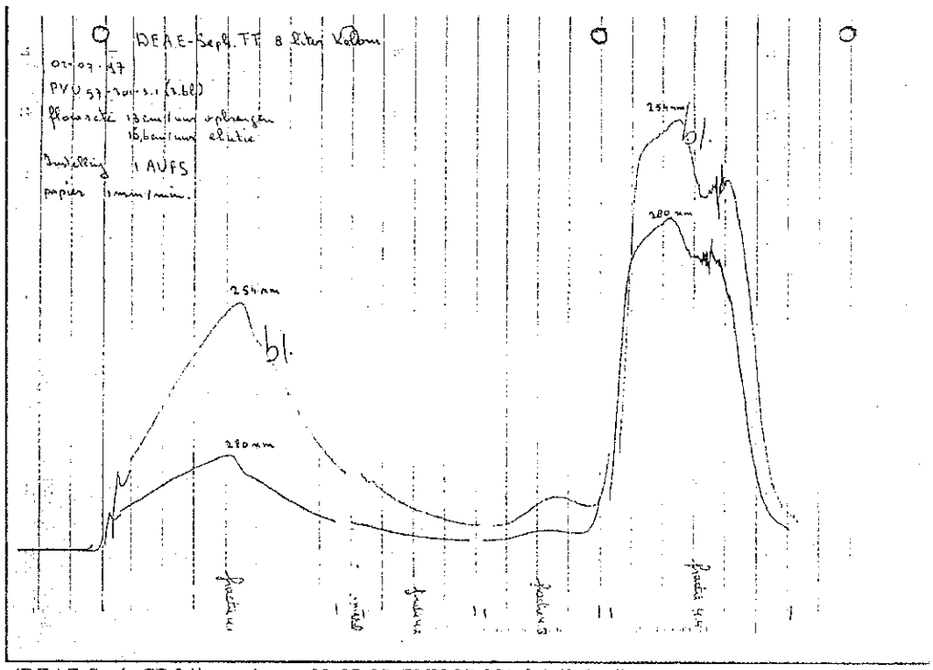
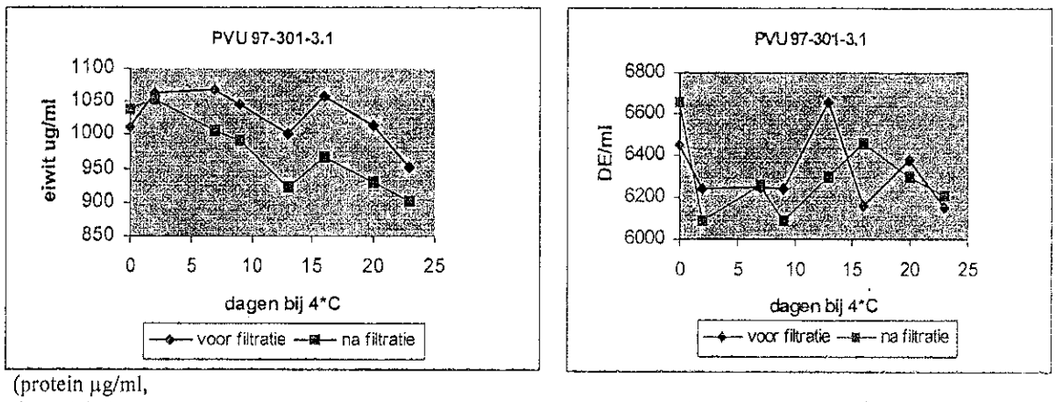
Figure 2 Elution pattern of the purification of PVU 97-301-3.1 using BPG 200/500 column with 8 litres DEAE-Sepharose FF (production scale)



(DEAE-Seph. FF 8 litre column ,02-07-97, PVU 97-301-3.1 (3.6 ml)
flowrate 13 cm/hour addition, 16.6/hour elution
Setting: 1 AUFS
paper 1 mm/min.
fractions)

The results from the Pierce protein determination and the D-antigen determination carried out on the day of the purification, or at the latest 2 days later, are shown in Figure 3. The protein content appears to drop slightly over time (10-15% in 3 weeks). As well as this the protein content is somewhat lower after filtration (maximum 10%). The D-antigen content remained constant (less than 10% variation).

Figure 3 Influence of keeping at 4 °C and filtering of the starting material



(protein µg/ml,
days at 4 °C,
before filtration, after filtration)

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Reproducibility of the purification

1. Elution patterns

Due to problems with the detector LKB RSD (diode array) which was used to take measurements at 260 nm and 280 nm elution patterns that could be compared properly were not obtained for all runs. The lamp in the detector broke during the elution with 1 M NaCl during the second run. After replacement of the lamp good but slightly deviating patterns were obtained during runs three and four. A fault in the detector occurred again during runs 5 and 6. Another detector (LKB 2158 Uvicord SD) was used during runs 7 and 8. This detector measures at only one wavelength, namely 280 nm. The available chromatograms are attached to this report as appendices. As a result of the technical problems the shapes of the chromatograms have not been included in the evaluation of the results.

2. Retention times

The retention times of the virus peak and the peak with contaminations (the clearance peak) are given in Table 2. This shows that there is no difference in the retention times of both peaks between the first and the last purification.

Table 2 Retention time of peak 1 of the purified virus (4.1)(elution with 0.04 M phosphate buffer) and of peak 2 of the contaminating proteins (elution with 1 M NaCl)

purification	peak of purified virus	peak of contaminating protein
	Retention time (minutes)	
run 1	66	91
run 2	66	-[1)]
run 3	74[3)]	99[2)]
run 4	64	87
run 5	-[1)]	87
run 6	-[1)]	88
run 7	66	88
run 8	64	88
average	67 ± 4[2)]	90 ± 4[3)]

[1)] Not detected (however was collected) due to technical fault
[2)] The flowrate was slightly less than 50 µl/minute for run 3.
[3)] ± SD

3. Purity of virus fraction 4.1

The purity of the virus fraction was calculated as the ratio A260/A280 and on the basis of the specific antigenicity (the quantity of D-antigen per µg protein). The A260/A280 (Table 3) is characteristic for poliovirus with its fixed protein/RNA ratio. The ratio A260/A280 remains constant, which indicates an equal degree of purity.

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Table 3 A 260, A280 and the ratio A 260/A 280 of purified virus (4.1)

Purification	A 260 (AU)	A 280 (AU)	A 260/A 280
run 1	0.273	0.175	1.56
run 2	0.253	0.158	1.60
run 3	0.260	0.164	1.59
run 4	0.244	0.154	1.58
run 5	0.220	0.135	1.63
run 6	0.239	0.152	1.57
run 7	0.217	0.133	1.63
run 8	0.230	0.142	1.62
average ± SD	0.242 ± 0.020	0.152 ± 0.014	1.60 ± 0.03

The purity expressed as specific antigenicity (DU/µg protein) appears to increase slightly with the number of purifications (Table 4). However, this is possibly caused by the decrease in the protein content of the fraction 3.1 while being kept at 4 °C (Figure 3). The specific antigenicity thus already increases somewhat in the starting material.
It can be concluded that the purity does not decrease as more purifications are carried out.

Table 4 Protein on the basis of protein content (Pierce determination) and A 260 and the ratio DU/µg protein

Purification	Protein µg/ml		DU/ml	DU/µg protein	
	Pierce	A 260		Pierce	A 260
run 1	41	37	1640	40.0	44.5
run 2	34	34	1520	44.7	44.5
run 3	36	35	1680	46.7	47.8
run 4	33	33	1500	45.5	45.5
run 5	29	30	1580	54.5	53.1
run 6	38	32	1760	46.3	54.5
run 7	29	29	1640	56.6	55.9
run 8	33	31	1630	49.4	52.4
average ± SD	34.1 ± 4.2	32.7 ± 2.6	1619 ± 85	48 ± 5.4	49.8 ± 4.7

4. Protein balance

Table 5 shows that the yield and the purity of the first and last run are the same. The recovery of the contaminating protein (the clearance peak) and the loss of D-antigen in de peak during elution with 1 M NaCl are also almost equal from the first to the last run.

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Table 5 Protein balance. Percentage recovery of material used in the virus peak and in the peak of contaminating protein.

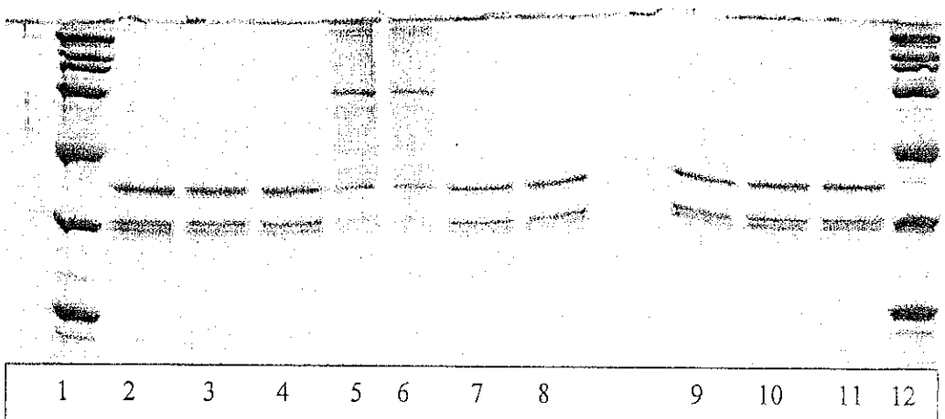
Purification	peak of purified virus % loading		peak of contaminating protein % loading		purified virus + contaminating protein (%)	
	% protein	% DU	% protein	% DU	% protein	% DU
run 1	11	66	76	15	87	81
run 2	9	67	55	16	64	83
run 3	10	72	71	17	81	89
run 4	9	66	52	16	61	82
run 5	8	67	83	12	91	79
run 6	10	73	65	11	75	84
run 7	8	69	78	13	86	82
run 8	10	70	55	10	65	80
average ± SD	9.4 ± 1.1	68.8 ± 2.7	67 ± 11.9	14 ± 2.6	76 ± 11.7	83 ± 3.1[1)]

[1)]An extra 2 ml fraction was collected after the peak of the purified virus in which an average of 2% protein and 9% D-antigen recovery was found.

5. SDS PAGE.

PVU 97-301-3.1 before and after 0.22µ filtration and the purified virus fraction (peak 1 fr 3-6) were placed on a gel to determine the protein purity (Figure 4). The purified virus fraction from runs 1 to 8 contained only the three virus proteins (lines 2-4 and 7-11). PVU 97-301-3.1 before and after 0.22µ filtration (lines 5 and 6 respectively) contains the three virus bands and a number of extra bands from the contaminating proteins.

Figure 4. SDS PAGE from purified virus and starting material



Lines 1 and 12: reference proteins, lines 2, 3, 4, 7, 8, 9, 10 and 11: purified virus fraction from runs 1 to 8, line 5: PVU 97-301-3.1 before 0.22µ filtration and line 6: PVU 97-301-3.1 after 0.22µ filtration. Coomassie colour, 12.5 % acrylamide.

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