



REPORT OF A CONSULTATION ON TRANSFER OF TECHNOLOGY<sup>1</sup>  
FOR PRODUCTION OF RABIES VACCINE

Geneva, 24 April 1987

The meeting was opened by Dr Scott Halstead, Associate Director, Health Sciences Division of the Rockefeller Foundation. The list of participants is attached as Annex 1. Dr Halstead noted the recent untimely deaths of Dr F. Assaad, WHO, and Dr A.L. Van Wezel, RIVM, whose enthusiasm for development of inexpensive cell culture rabies vaccines, and transfer of this technology to developing countries, was crucial for implementing this new project.

Dr Halstead reviewed the progress made to date on selection of the cell system and vaccine virus strains to be employed, and the countries in which vaccine production would be initiated. VECOL of Colombia had been selected as the first technology transfer vaccine production site, with technical development of a Vero-based vaccine and transfer of the microcarrier Vero cell culture system by the Rijksinstituut voor de Volksgezondheid (RIVM). Drs Van Steenis and Tiesjema are responsible for the rabies vaccine development and the technology transfer project, respectively.

Dr Van Steenis reviewed RIVM's progress with the Michigan State Health Department vaccine strain (Kissling virus). The results are presented in Annex 2. The usual ratio between antigen assay and by ELISA and the NIH potency test titers for vaccine immunogenicity does not seem to hold for the virus grown in Vero cells. Preliminary antigen analysis showed the Vero-Kissling potency (compared to ELISA antigen titers) to be 20-fold less than the reference standard dog kidney vaccines. In addition, the glycoprotein appears partly lost from the Vero-Kissling vaccines during the sepharose affinity column purification step, using current techniques for dog kidney cell vaccine. These results need to be confirmed.

Other vaccine virus strains, including the L. Pasteur/Paris Vero cell strain, are being tested and compared to the Kissling strain. Antigen yields from the L. Pasteur/Paris Vero cell strain appear to be satisfactory, and the ELISA antigen titers and NIH potency test relationships were good. The L. Pasteur/Paris strain induces a high-titered neutralizing antibody response in mice that is better than the Kissling strain. The L. Pasteur/Paris Vero cell strain is available for use by the

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<sup>1</sup> Convened by the Rockefeller Foundation in collaboration with the Veterinary Public Health unit, Division of Communicable Diseases, WHO, Geneva

\* Titles of annexes concerning Pasteur Vero cell amended+ Annex 4 amended.

project. The potency tests with the L. Pasteur/Paris Vero cell strain are under way. If potency is good, 2-3 more lots would have to be produced and tested before the system could be deployed to VECOL. Dr Halstead suggested that vaccine for veterinary application be produced in microcarrier BHK cells preparatory to the Vero system, in order to gain experience. BHK cells are of particular interest because rabies virus replicates quickly to high titer in them.

Dr Reculard reviewed the rabies vaccine strains that have been derived from the original Louis Pasteur fixed rabbit brain passaged virus (Annex 3). He has been working to improve the antigenicity of Vero passaged virus (Annex 4). Bovine serum albumin replaces fetal calf serum. The temperature of incubation is 32°C for cells for viral antigen production. Good correlation is observed between glycoprotein antigen measured by ELISA, and the NIH potency test. Antigen units measured by mouse LD<sub>50</sub> and NIH potency tests were extremely high in two pilot lots of the L. Pasteur/Paris vaccine derived from rabbit brain passage 2061 produced in Vero cells (Vero passages 19 and 20).

The report of a WHO Study Group on "Acceptability of Cell Substrates for Production of Biologicals" (Annex 5) was discussed by Dr Gratchev. This group recommended the development of production systems that would permit cell culture vaccine systems to replace nervous tissue vaccines. UNDP is participating in this effort. Dr Gratchev raised the question of the need to incorporate different rabies virus strains in vaccines now that studies with monoclonal antibody reveal antigenic variability among various isolates of rabies virus.

Dr Halstead indicated that an agreement was needed concerning profits from vaccine sales by VECOL outside Colombia. One approach might be for the Rockefeller Foundation, or its representative, to receive a per dose royalty of perhaps 25 US cents per dose of human vaccine and 5 US cents per dose for veterinary vaccine. Such funds would revert to a rabies vaccine technology transfer fund to further extend development of vaccine production centres and to continuously upgrade their training and technology. Royalty negotiations should be accomplished as soon as possible.

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ANNEX 1

LIST OF PARTICIPANTS

Dr George Baer, Centers for Disease Control, Lawrenceville Rabies Facility, P.O.Box 363,  
Lawrenceville, GA 30246, USA

Dr J.G. Debbie, Scientist, Veterinary Public Health unit, Division of Communicable  
Diseases, WHO, Geneva

Dr Scott Halstead, Associate Director, Health Sciences, The Rockefeller Foundation,  
1133 Ave of the Americas, New York, N.Y. 10036, USA

Dr V. Gratchev, Biologicals unit, Division of Diagnostic, Therapeutic and Rehabilitative  
Technology, WHO, Geneva

Dr Pierre Reculard, 7 rue Gustave Lambert, 92380 Garches, France

\* Dr Geoffrey Schild, Head, Division of Viral Products, National Institute for Biological  
Standards and Control, Holly Hill, Hampstead, London NW3 6RB, UK

Dr Franz Van Steenis, Head, Quality Control Biologicals, Rijksinstituut voor de  
Volksgezondheid (RIVM), P.O.Box 1, 3720 BA Bilthoven, Netherlands

Dr Thomas Yuill, Associate Dean for Research and Graduate Training, School of Veterinary  
Medicine, University of Wisconsin, 2015 Linden Drive, Madison, Wisconsin 53706, USA

\* Invited, but did not attend.

ANTIGEN DETERMINATION (ELISA), USING DIFFERENT ANTIBODIES, AND NIH POTENCY OF KISSLING AND PASTEUR 2061 VERO CELL

Vaccine	ELISA							NIH
	$\alpha$ -total polycl.	$\alpha$ -glyco (ERA) polycl.	$\alpha$ -glyco (PM) monoc1.1-11D <sub>4</sub> M <sub>1</sub>	$\alpha$ -glyco (PM) monoc1.1-10B <sub>g</sub> M <sub>1</sub>	$\alpha$ -glyco (PM) monoc1.16-8M <sub>1</sub>	$\alpha$ -nucleo (PM) monoc1.3D <sub>4</sub> M <sub>1</sub>		
DKCV (45A)	-	4.9	3.7	5.3	3.2	5.3	6.1	
V. Kissling conc.	140	45-100	>69	>97	2.2	275	6.8	
" pur.	60	3.5-8	4	4.2	0.5	>37	0.5	
" lyoph.	18	1.3	2.0	2.5	0.3	>24	-	
" conc.	100	50-120	-	180	-	240	6.7-9	
" lyoph.	12	3.2	3.2	4.2	0.5	>24	0.4	
Pasteur Sur. conc.	0.35	0.2-0.8	0.3	-	-	0.55	0.2	
" conc.	35	-	-	-	-	-	-	
" pur.	12 ?	110	>97	>97	2.3	>15	-	

PRODUCTION PROCESS OF RABIES VACCINE (KISSLING-STRAIN) IN  
VERO CELL CULTURES

Cell cultivation

The cell cultivation is performed in microcarrier cultures (3 litre followed by 40 litre) with Cytodex 3 in Eagle's MEM supplemented with non-essential aminoacids, pyruvate, 7.5% bovine serum and 2.5% fetal calf serum.

In continuous perfusion cell systems cell densities of  $2-4 \times 10^6$  cells/ml are obtained.

Virus cultivation

For the growth of the virus medium 199 + 0.0125% BSA is used. The harvest schedule for the Kissling-strain is  $\pm 4 + 3 + 4 + 3 + 3$  days.

Clarification

Clarification is performed by Pall filtration 1.2  $\mu$ m NNP.

Concentration

The clarified virus suspension is concentrated  $\pm 100x$  with Amicon Hollow fiber filters with a cut off of  $10^6$  mW.

Purification

The virus concentrate is purified by ion-exchange chromatography on DEAE-Sepharose Cl-6B fast flow with PBS + 0.2 M NaCl as elution buffer.

Inactivation

The purified virus suspension is inactivated with BPL 1:4000. After incubation for  $\pm 18$  hours at  $4^{\circ}$  C the residual BPL is hydrolysed during a 2 hours' incubation period at  $37^{\circ}$  C.

Freeze drying

The BPL-inactivated virus suspension is 1:1 diluted with PBS + 10% Lactose and freeze dried in 1 ml ampoules.

PRODUCTION OF KISSLING RABIES VIRUS ON VEROCELLS86 UR 570            MEDIUM 199 + SUPPLEMENTSVIRUS CULTIVATION

Harvest No.	Volume l	Cells/ml x 10 <sup>3</sup>	Cultivation days	log <sub>10</sub> LD50/ml	ELISA Antigen U/ml
1	30	1200	4	7.69	1.6
2	30	1100	9	5.4	1.1
3	30	1000	14	4.1	1.2

PRODUCTION OF KISSLING RABIES VIRUS ON VEROCELLS86 UR 570            MEDIUM 199 + SUPPLEMENTSPROCESSING

Step	Volume	ELISA Antigen U/ml	Recovery %	NIH potency	Residual serum Alb. µg/ml	Bovine proteins γ-glob. (pg/ml)	Cellular DNA (pg/ml)
Bulk harvest	90 l	1.4	100	-	-	-	-
Clarification	100 l	1.4	111	-	983	20.16	20.000
Concentration	1 l	107/90	≤85	9	>123	323	20.000
<u>DEAE-Sepharose</u>							
6B-C1 F.F. <sup>1)</sup>	90 ml	31	±70	-	7.68	<0.63	1.000
<u>Purified Product</u>							
+ BPL	80 ml	29.4	94.4	2.0	-	-	-
<u>Purified Product</u>							
BPL + Lactose <sup>2)</sup>	156 ml	14.7	100	0.4	-	-	-
<u>Freeze dried</u>							
Product <sup>3)</sup>	150 doses	12.4	84.4	0.8	-	-	-

<sup>1)</sup> Purification on small scale. Pooled fractions 19 + 23/12 1986 + 9/1 1987.

<sup>2)</sup> 1:2 dilution.

<sup>3)</sup> Equipment failure during freeze-drying process.

PRODUCTION OF KISSLING - RABIESVIRUS ON VEROCELLS

R089 MEDIUM 199 + BSA 0.0125%

VIRUS CULTIVATION

Harvest No.	Harvest volume l	Cells/ml x 10 <sup>3</sup>	Cultivation days	Log10 LD50/ml	ELISA antigen U/ml
1	38	2610	3	7.9	0.16
2	39	1970	6	7.7	0.87
3	37	1660	9	7.1	1.13
4	37	1418	13	4.4	1.93
5	36	815	16	≤2.0	1.13

PRODUCTION OF KISSLING - RABIESVIVRUS ON VEROCELLS

R089 MEDIUM 199 + BSA 0.0125%

PROCESSING

Step	Volume l	ELISA antigen U/ml	Recovery %	NIH potency	Residual Serum Alb. µg/ml	Bovine proteins γ-glob. <0.32	Res. DNA pg/ml
Bulk harvest	187	0.87	100	-			i.p.
Clarification	190	0.79	92	-			i.p.
Concentration	1.1	140	94.6	6.8	15728	161.6	i.p.
Purification	0.4->0.7	59.6	68.5	0.5 (4.7)	<0.96	<0.32	i.p.

i.p. = in process.

n.d. = not done.

ANTIGEN CONTENT (ELISA) AND IMMUNOGENICITY IN THE NIH  
 POTENCY TEST OF VERO CELL-KISSLING VACCINE AS COMPARED  
 TO SOME OTHER RABIES VACCINES AND EFFECT OF ALPO<sub>4</sub> ON  
 IMMUNOGENICITY

Vaccine	ELISA <sup>1)</sup> (EU)	NIH <sup>2)</sup> (IU)
DKCV (RIVM)	10.6	17
VCV (Mérieux)	2.7	2.7
HDCV (Mérieux)	6.5	7.8
Vero-Kissling (concentrated bulk 570.2.1A)	128	9
Vero-Kissling (purified bulk R89-4.1)	8	0.5
Vero-Kissling (purified bulk 89-4.1, AlPO <sub>4</sub> 50/50)	4 (2.8)	4.7

1) polyclonal anti-glycoprotein serum against ERA virus

2) challenge virus CVS26

PRODUCTION OF PASTEUR 2061 RABIES VIRUS ON VERO-CELLS

R091 MEDIUM 199 + BSA 0.0125%

VIRUS CULTIVATION

No.	Harvest volume l	Cells/ml x 10 <sup>3</sup>	Cultivation days	Log10 LD50/ml	ELISA antigen U/ml
1	37	3950	4	6.4	0.053
2	40	3320	3	7.0	0.54
3	40	1845	4	7.4	0.78
4	38	485	4	i.p.	0.49

i.p. = in process.

PRODUCTION OF PASTEUR 2061 RABIES VIRUS ON VEROCELLS

R091 MEDIUM 199 + BSA 0.0125%

PROCESSING

Step	Volume l	ELISA antigen U/ml	Recovery %	NIH potency	Residual Serum Alb. µg/ml	Bovine proteins γ-glob. pg/ml	Res. DNA pg/ml
Bulk harvest	154	0.37	100	n.d.			i.p.
Clarification	155	0.30	81.6	n.d.	<30	<315	i.p.
Concentration	1.4	34.3	84.3	i.p.	245	10.1	i.p.
Purification 0.4->0.75		12.1	55.7	i.p.	<0.06	<0.63	i.p.
Lyophilization	-	i.p.		i.p.	n.d.	n.d.	i.p.

i.p. = in process.

n.d. = not done.

PRODUCTION OF PASTEUR 2061 RABIES VIRUS ON VERO-CELLS

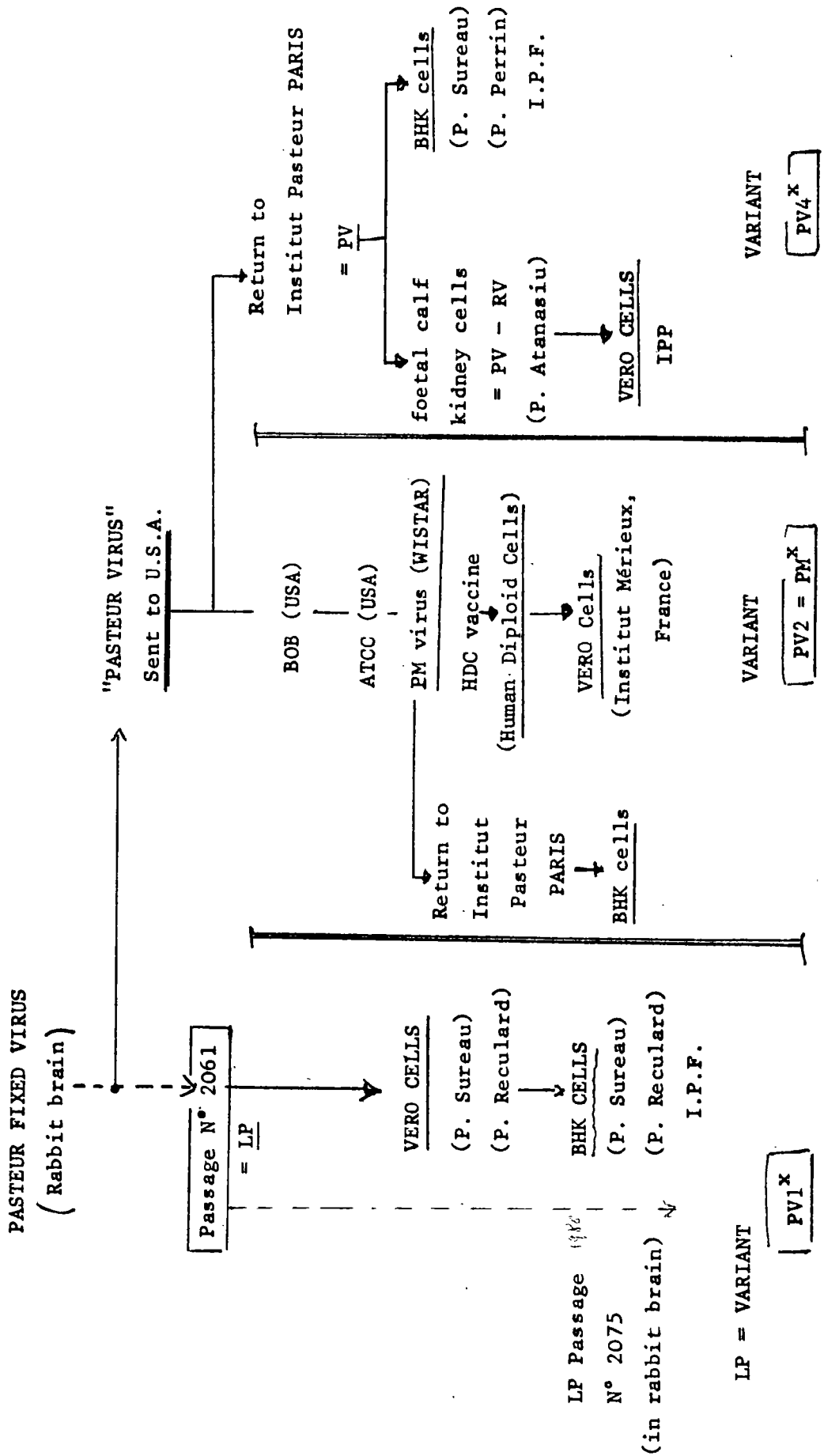
R092 MEDIUM 199 + BSA 0.0125% (3 LITER)

VIRUS CULTIVATION

No.	Harvest volume l	Cells/ml x 10 <sup>3</sup>	Cultivation days	Log10 LD50/ml	ELISA antigen U/ml
1	2.9	3500	5	i.p.	0.11
2	2.9	3063	4	i.p.	1.35
3	2.7	1225	5	i.p.	1.49
4	3.0	380	5	i.p.	0.92

i.p. = in process.

RABIES STRAINS ORIGINATED FROM LOUIS PASTEUR STRAIN



<sup>x</sup> Antigenic differences recognized by using monoclonal antibodies.

ANALYTICAL DATA ON RABIES VIRUS - LOUIS PASTEUR STRAIN ADAPTED TO VERO CELLS (INSTITUT PASTEUR - PARIS)

N° of passage & date	time after infection (in days)	cytopathic effect	Immunofluorescence % of cells +	Hemagglutination (Dilution 50%)	Glycoprotein ng/ml	LD50 in mice (per ml)	POTENCY	
							ELISA I.U./ml	NIH IU/ml
2061 - 18	6	+	10%	1/8	3600	10 <sup>5,7</sup>	1,2	
06/02/87	10	++	50%	1/32	4200	10 <sup>5,2</sup>	1,4	
	13	++	80%	1/32	4600	10 <sup>5</sup>	1,5	
	17	+++	80%	1/16	3900	ND	1,3	
	19	cells detached		1/4	540	ND	0,15	
2061 - 19	7	++	30%	1/8	1200	10 <sup>6,6</sup>	0,4	
05/03/87	11	+++	50%	1/64	5600	10 <sup>6,2</sup>	1,8	0,8
	14	++++	90%	1/16	5280	ND	1,7	
2061 - 20	5	±	25%	1/2	1280	ND	0,4	
13/03/87	7	+	50%	1/8	4300	10 <sup>6,2</sup>	1,4	
	10	++	80%	1/64	17.000	10 <sup>5,5</sup>	5,9	2,1
	12	+++	80%	1/64	10.800	ND	3,6	
	15	++++	90%	1/64	5.550	ND	1,8	
2061-21	5	+	10%	0	860		0,3	
03/04/87	7	++	20%	1/4	8400		2,8	
	10	+++	80%	1/64	14.400	ND	5,0	
	14	++++	80%	1/128	11.200		3,8	

\* Calculated values from ELISA results

ANALYTICAL DATA ON RABIES VIRUS - LOUIS PASTEUR STRAIN ADAPTED TO VERO CELLS (INSTITUT PASTEUR - PARIS)

N° of passage & date	time after infection (in days)	cytopathic effect	Immunofluorescence % of cells +	Hemagglutination (Dilution 50%)	Glycoprotein ng/ml	LD50 in mice (per ml)	POTENCY		
							ELISA I.U./ml	NIH IU/ml	
2061 - 18	6	+	10%	1/8	3600	10 <sup>5,7</sup>	1,2		
06/02/87	10	++	50%	1/32	4200	10 <sup>5,2</sup>	1,4		
	13	++	80%	1/32	4600	10 <sup>5</sup>	1,5		
	17	+++	80%	1/16	3900	ND	1,3		
	19	cells detached		1/4	540	ND	0,15		
<hr/>									
2061 - 19	7	++	30%	1/8	1200	10 <sup>6,6</sup>	0,4		
05/03/87	11	+++	50%	1/64	5600	10 <sup>6,2</sup>	1,8	0,8	
	14	++++	90%	1/16	5280	ND	1,7		
<hr/>									
2061 - 20	5	±	25%	1/2	1280	ND	0,4		
13/03/87	7	+	50%	1/8	4300	10 <sup>6,2</sup>	1,4		
	10	++	80%	1/64	17.000	10 <sup>5,5</sup>	5,9	2,1	
	12	+++	80%	1/64	10.800	ND	3,6		
	15	++++	90%	1/64	5.550	ND	1,8		
<hr/>									
2061-21	5	+	10%	0	860		0,3		
03/04/87	7	++	20%	1/4	8600		2,8		
	10	+++	80%	1/64	14.400	ND	5,0		
	14	++++	80%	1/128	11.200		3,8		

VPH/87.70  
ANNEX 4  
Revision

\* Calculated values from ELISA results