

**Producing an
inactivated polio vaccine:
comparability of the vaccine derived
from two cell substrates**

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(presented at Biologic 2001)



Composition of one dose of inactivated polio vaccine (IPV*)

	Type 1	Type 2
Type 3		
Protein (μg)	1.3	0.9
RNA (μg)	0.6	0.4

*IPV is derived from formaldehyde treated poliovirus suspensions



Presentation outline

- ◆ Structure of the virus
- ◆ Critical steps of the licensed process
- ◆ Comparison of the licensed process and the Vero process
- ◆ Analysis crucial intermediates
- ◆ Data from fase I and II trials



Comparison of the two production processes

Change	'Old' process	'New' process
Cell substrate for virus cultivation	Tertiary monkey kidney cells	Vero cells
Second column step	DEAE Sephadex	DEAE Sepharose
Scale	2 x 350L	150L; 2x 350 L

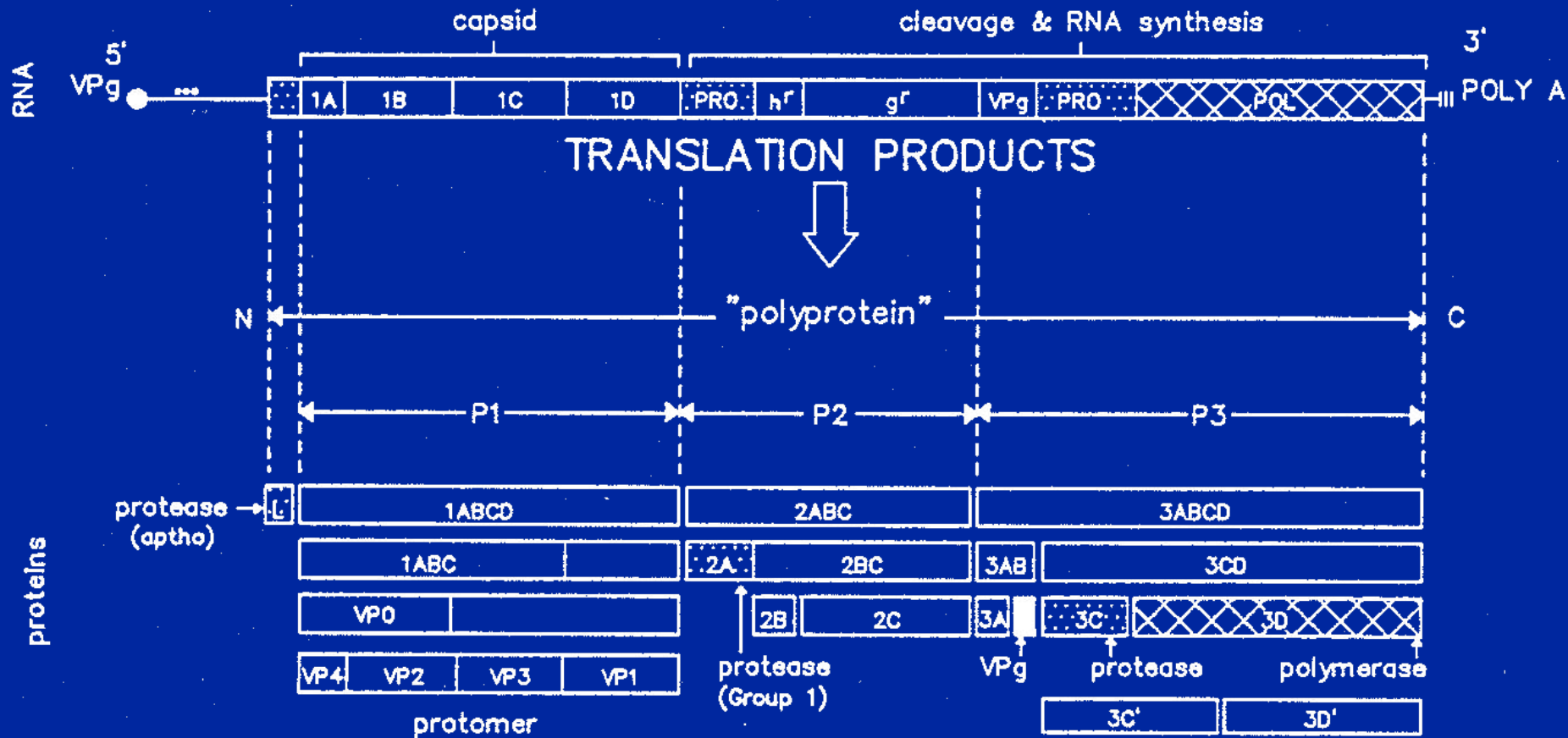


Type 1 variation; distinction of changes

- ◆ Changes with no impact on quality criteria
- ◆ Changes with impact on in-process controls without impact on drug substance and/or drug product specifications
- ◆ Changes with impact on quality criteria and no anticipated consequences on safety/efficacy
- ◆ Changes with impact on quality criteria and anticipated consequences on safety/efficacy



Organisation and expression of the poliovirus genome

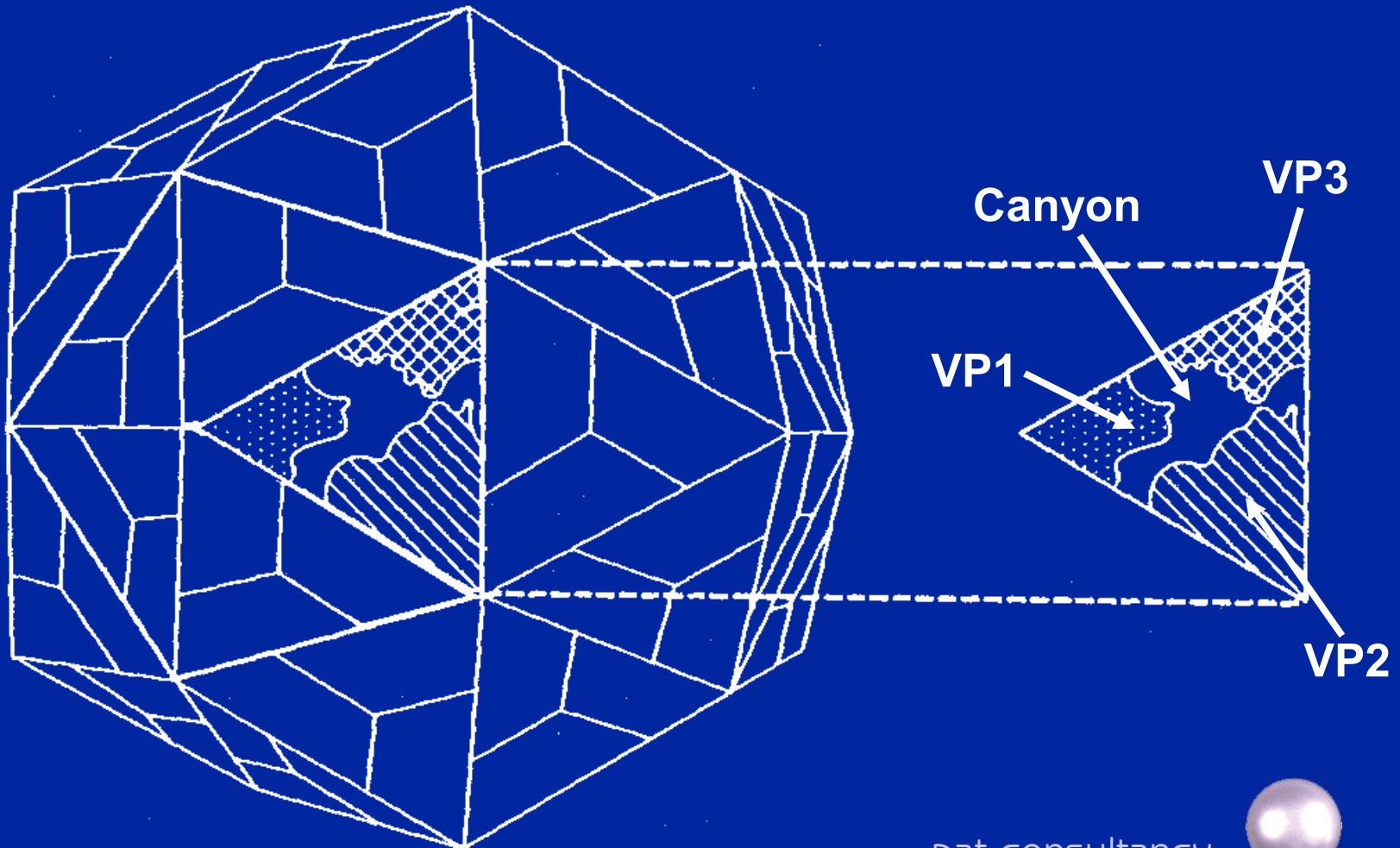


Organisation and expression of the poliovirus genome

- ◆ The RNA contains a single long reading frame encoding a long polypeptide chain
- ◆ This polypeptide chain is cleaved during translation by three virus-coded proteases
- ◆ N-terminal glycine is myristylated during post-translation by a cellular N-myristoyltransferase.
- ◆ Segments 1D (VP1), 1B (VP2) and 1C (VP3) of the P1 region are elements of the coat protein subunit



Packaging of VP1, VP2 and VP3 in the poliovirus shell



Chemistry of formaldehyde and amino groups

- ◆ Formalin reacts with free amino groups in capsid proteins and RNA resulting in reactive Schiff-bases
- ◆ The Schiff-base reacts with amino groups present in the matrix



Formalin treatment and consequences for antigenicity

- ◆ Formalin treatment of poliovirus suspensions results in a reduced antigenicity
- ◆ Differences in antigenicity are observed between suspensions from different manufactures (Ferguson et al, 1992)



Formalin treatment and consequences for immunogenicity

Immunogen	Day 0	Day 35	Day48
Virus	0	3.6	9.0
Vaccine	0	3.4	10.2

(immunogenicity is presented as neutralizing antibody titers)



Relation: Process - Product

- ◆ The output of the production process is: a product
- ◆ The critical steps of the production process define the quality of the product



Production of monovalent pools

Cell culture (three passages)

Virus culture

Clarification

Concentration

Gel filtration

Anion exchange chromatography

Addition of components for inactivation

Inactivation (formaldehyde treatment)

Preparation of monovalent pool



Production of monovalent pools (*Critical steps*)

Cell culture (three passages)

Virus culture

Clarification

Concentration

Gel filtration

Anion exchange chromatography

Addition of components for inactivation

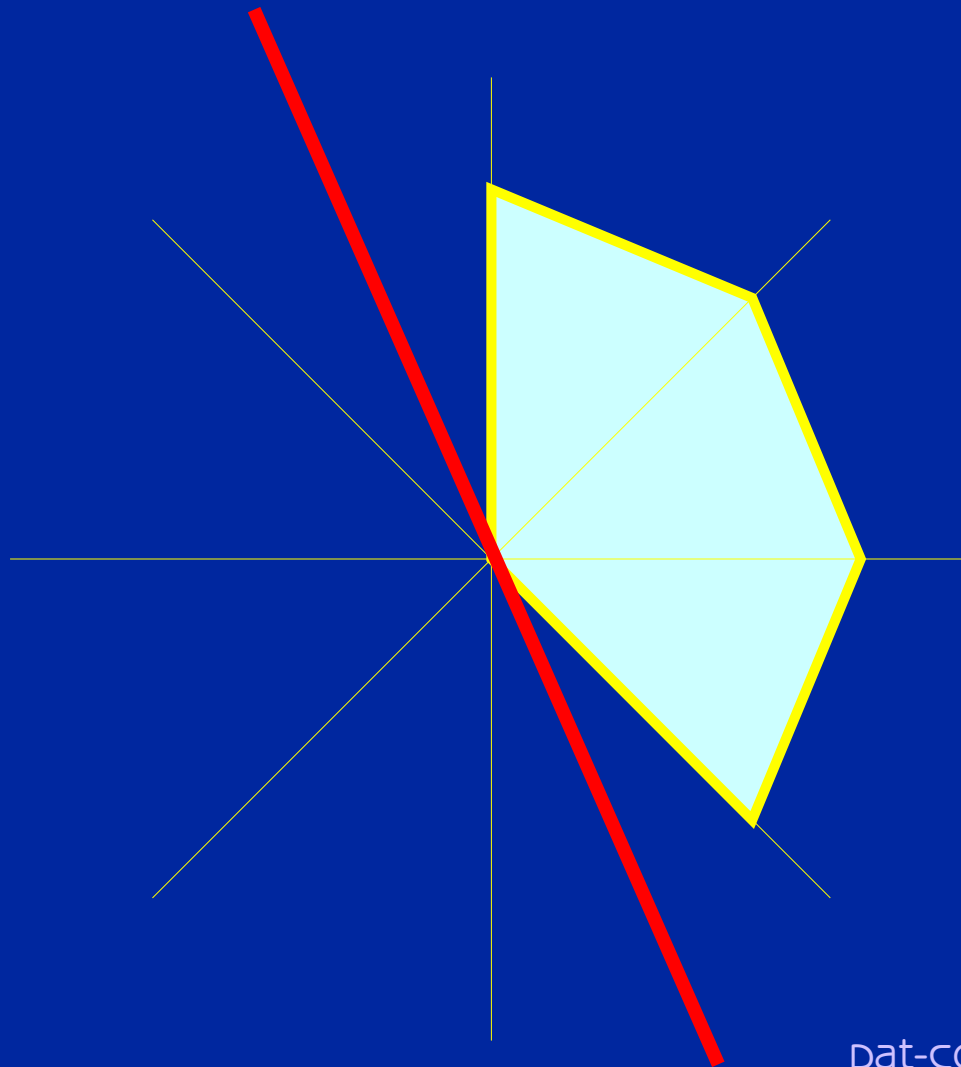
Inactivation (formaldehyde treatment)

Preparation of monovalent pool

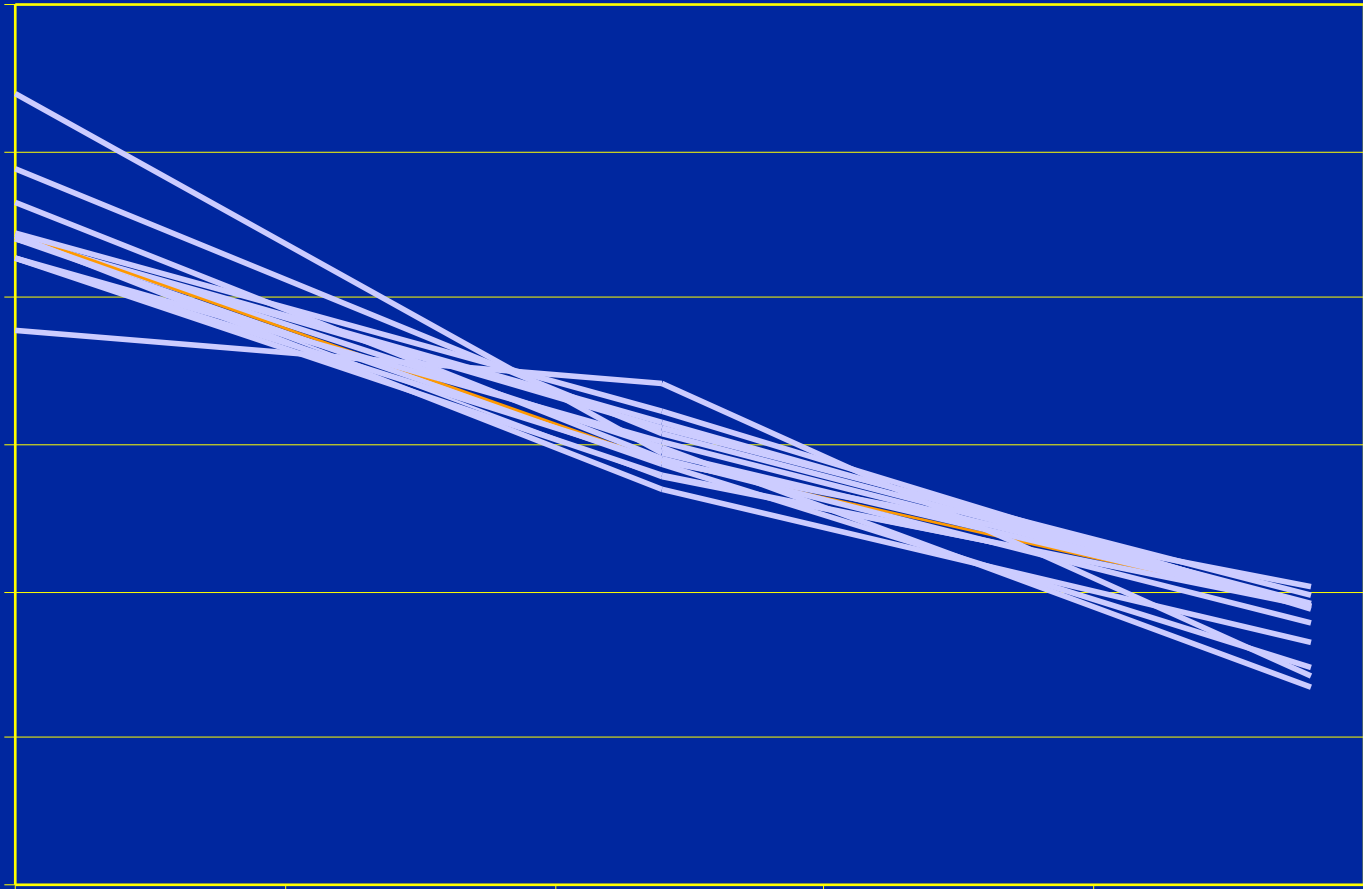


Virus cultivation

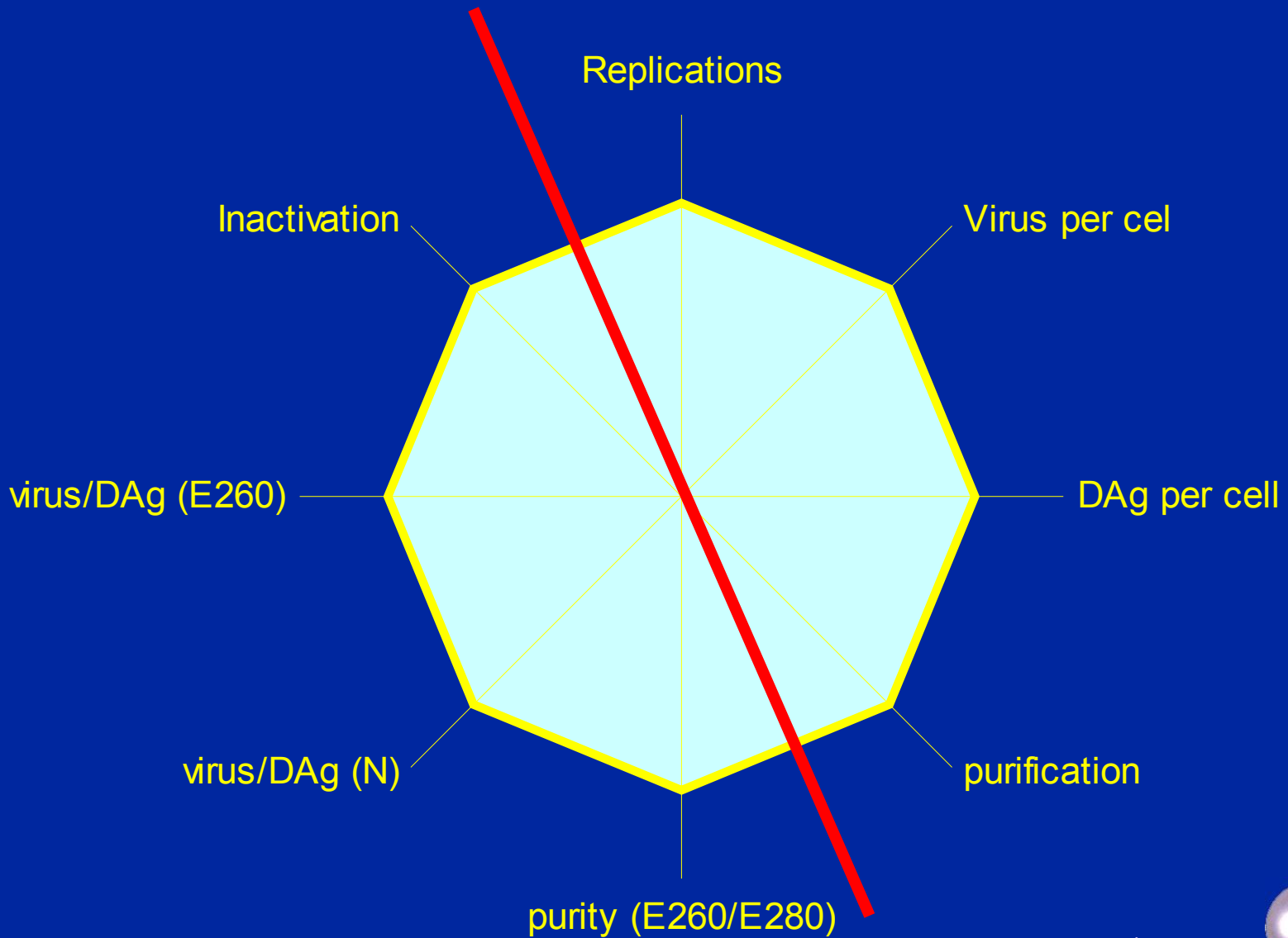
Replication, Virus yield, DAg per cell & Purification



Inactivation of the virus



'Poliogram'



Production of monovalent pools (*Comparison of the two processes*)

Cell culture (three passages)

Monkey kidney cells versus Vero cells

Virus culture

Clarification

Concentration

Gelfiltration

Anion exchange chromatography

DEAE Sephadex versus DEAE Sepharose

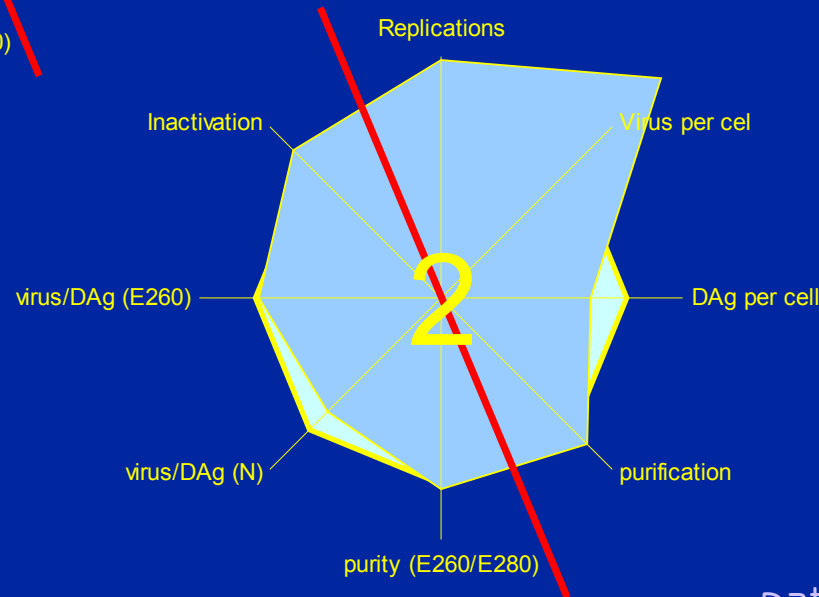
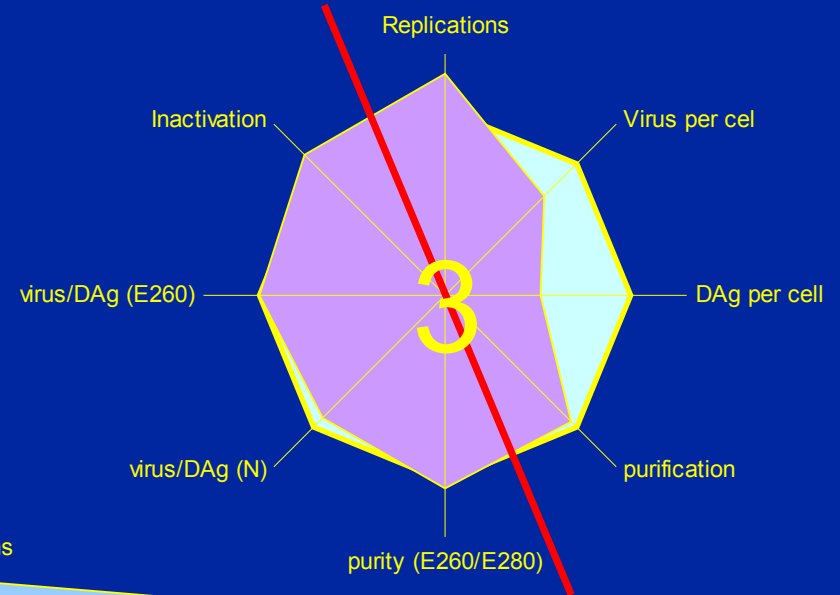
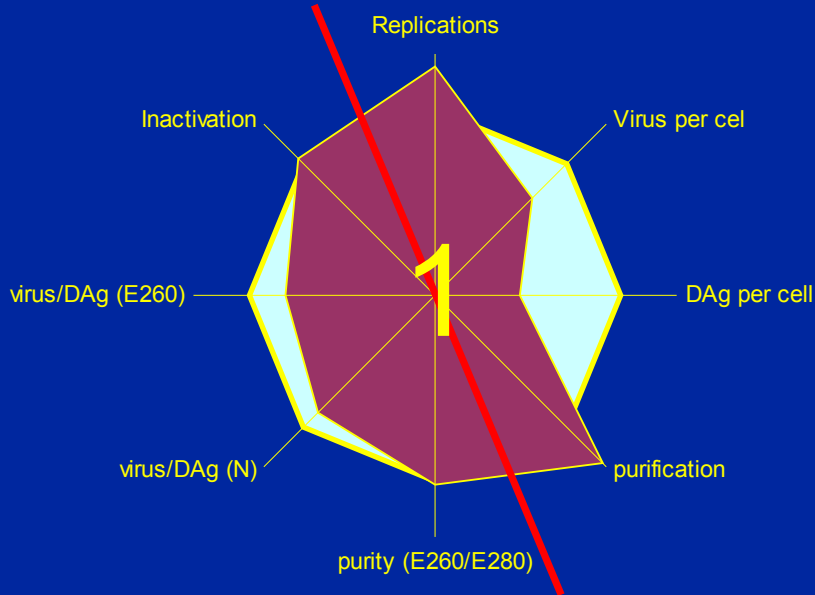
Addition of components for inactivation

Inactivation (formaldehyde treatment)

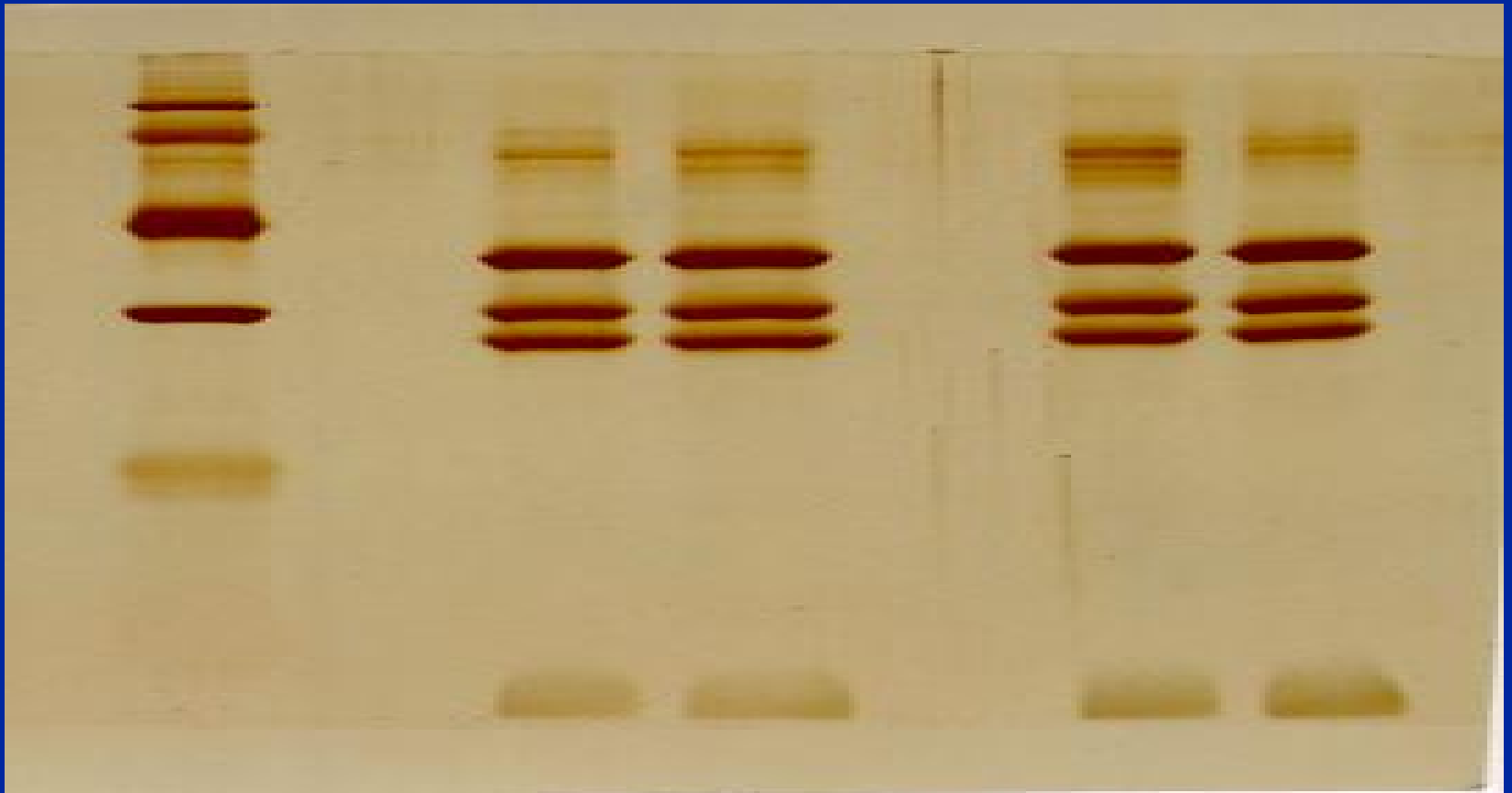
Preparation of monovalent pool



Comparing the 'Poliograms'



SDS-PAGE of type 3 polio virus: monkey kidney cells and Vero-cells



Identity of primary structure of capsid proteins

		VP1	VP2
	VP3		
Type 1	60%	47%	36%
Type 2	79%	91%	49%
Type 3	57%	58%	68%

(percentage of proteins tested) from the three types of poliovirus cultivated in tertiary monkey kidney cells and in Vero cells



Major characteristics experimental polio vaccine derived from Vero-cells

- ◆ Stability: no reduction in antigenicity (< 10%) after storage during 48 months after incubation at 37°C.
- ◆ Potency: no reduction in immunogenicity during the same period of time



Outcome fase I trial in adults

- ◆ No serious side effects
- ◆ No reactogenicity after a second dose



Serum neutralising antibody response in infants after three doses of IPV derived from Vero cells and from *MKC*

	Type 1	Type 2	Type 3
Before first dose	1.78; 1.71	1.63; 1.79	1.09; 0.50
After second dose	7.68; 7.46	5.94; 6.08	6.55; 6.08
Before third dose	5.65; 5.53	4.41; 4.73	4.34; 4.60
After third dose	9.15; 8.81	7.97; 8.06	8.21; 8.31



Conclusions

- ◆ It is unlikely that the virus synthesis is depending upon the cell substrate
- ◆ Process data of the two critical steps are comparable
- ◆ Virus suspensions from the two substrates are comparable
- ◆ No differences between the two vaccines are observed in humans



Conclusions in relation with type 1 variations

- ◆ *Changes with no impact on quality criteria*
- ◆ Changes with impact on in-process controls without impact on drug substance and/or drug product specifications
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- ◆ Changes with impact on quality criteria and anticipated consequences on safety/efficacy



References

- ◆ EMEA Document: CPMP/BWP/3207/00
- ◆ Ferguson et al., J Gen Vir 74, 685 (1993)
- ◆ Rueckert in Fields Virology (third edition) 609 (1996)
- ◆ Rümke et al., Scand J Inf Dis 30, 535 (1998)

