Characterisation of Sabin based inactivated polio vaccines

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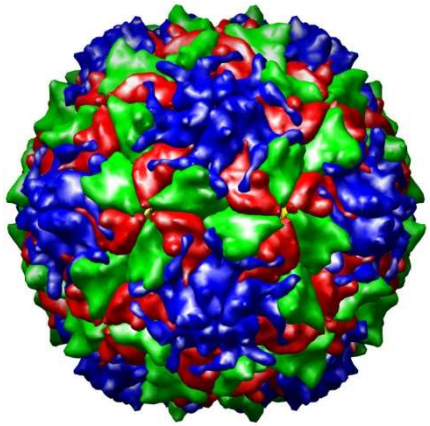

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IPV quantity

- Protein ≠ Antigen ≠ Immunogen

Active	Unit	Assay
Amount: protein or virus concentration	gram, mole	protein assays, UV adsorption
Antigenicity: recognition by antibodies	DU	Immunoassay (ELISA, biosensor analysis)
Immunogenicity: induction of antibodies	VN titer, relative potency	Immunization plus serology

- If all protein is D-ag and no adjuvant is used:
  Protein = a*(Antigen) = b*(Immunogen)

- The correlation is within the same strain and serotype

How much virus is one D-antigen unit?

Relationship D-units and amount of virus based on A260nm of purified vaccine.

	ng virus/DU		ng virus/DU
Sabin 1	30	Salk 1	18
Sabin 2	112	Salk 2	49
Sabin 3	70	Salk 3	26

The D-ag unit represents different quantity of virus both between serotypes and vaccine strain.

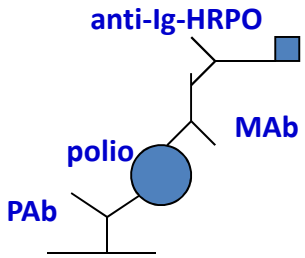
Intravacc approach to assess Sabin-IPV antigenic quality

Antigenic fingerprints were made to:

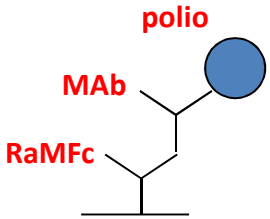
- select antibodies for quantification purposes
- demonstrate batch consistency

Reagents and references are based on classical IPV:

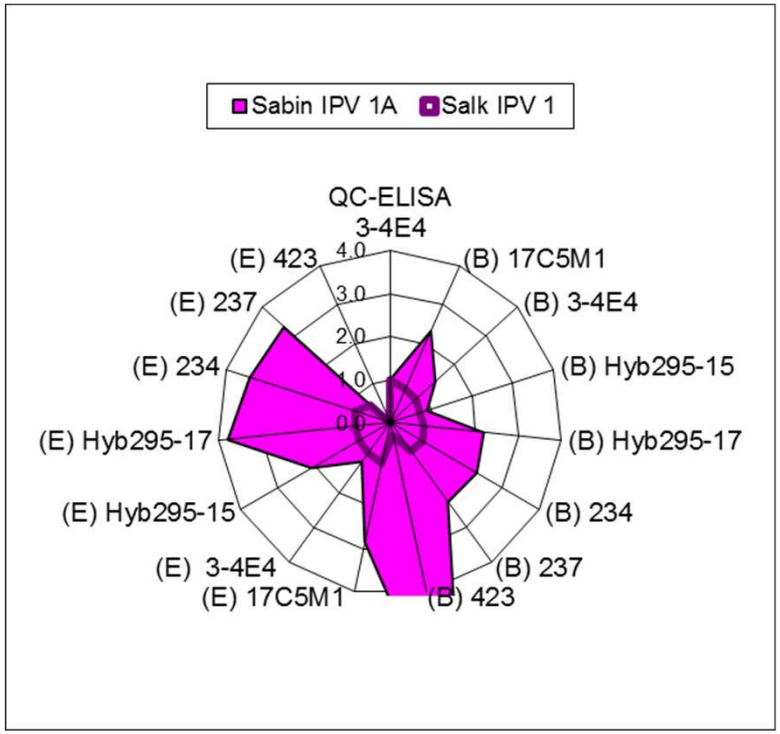
- Reference Pu 91-01
- Home made and commercially available antibodies
- Two assay formats: sandwich ELISA and BIAcore

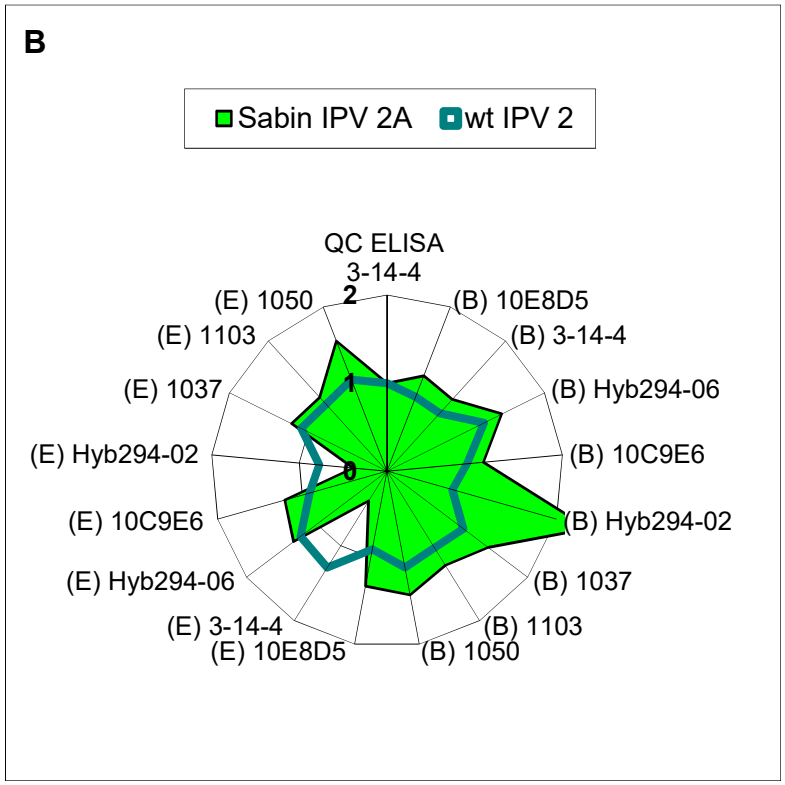


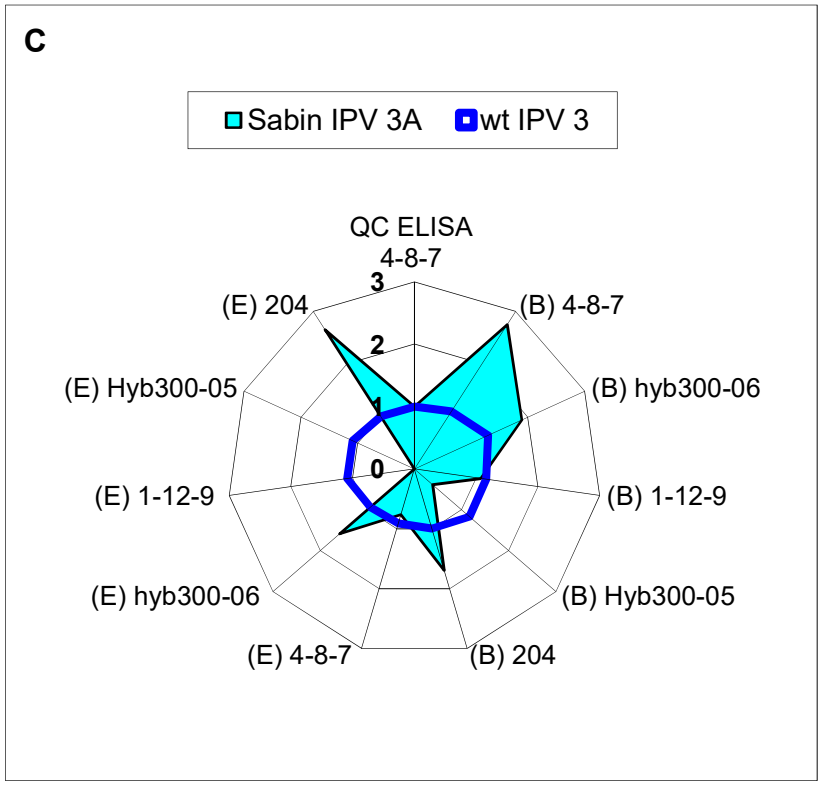
ELISA



Biacore




B
Sabin IPV 2A
wt IPV 2
QC ELISA
3-14-4
(B) 10E8D5
(B) 3-14-4
(B) Hyb294-06
(B) 10C9E6
(B) Hyb294-02
(B) 1037
(B) 1103
(B) 1050
(E) 10E8D5
(E) 3-14-4
(E) Hyb294-06
(E) 10C9E6
(E) Hyb294-02
(E) 1037
(E) 1103
(E) 1050
2
1
0


C
Sabin IPV 3A
wt IPV 3
QC ELISA
4-8-7
(B) 4-8-7
(B) hyb300-06
(B) 1-12-9
(B) Hyb300-05
(B) 204
(E) 4-8-7
(E) hyb300-06
(E) 1-12-9
(E) Hyb300-05
(E) 204
3
2
1
0

Conclusion: the D-ag unit is not one unit

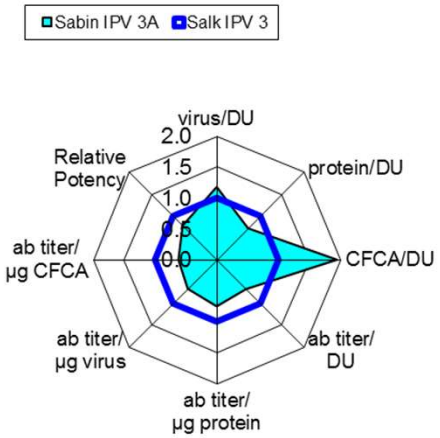
- Dependent on reference
- Dependent on antibodies
- Dependent on product

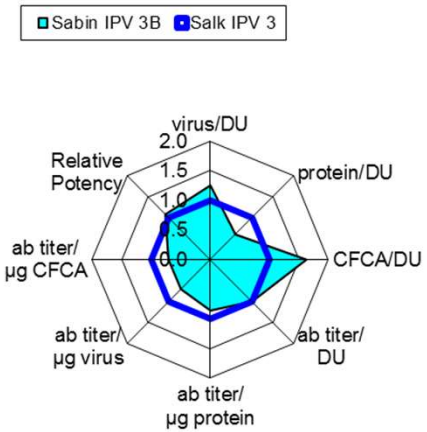
- For a manufacturer the amount of active biomass produced is relevant
- How much viral protein is a D-ag unit?

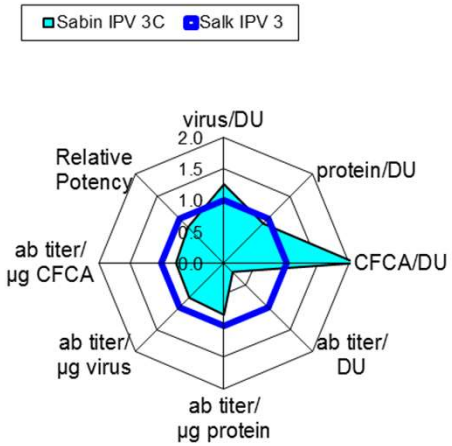
Antigenic fingerprints obtained with a panel of antibodies can be helpful

- to demonstrate batch consistency
- in stability studies
- to demonstrate products consistency i.e.
  - from different production sites
  - after process optimization or scale up
  - between different manufacturers

Product profile of three batches sabin IPV





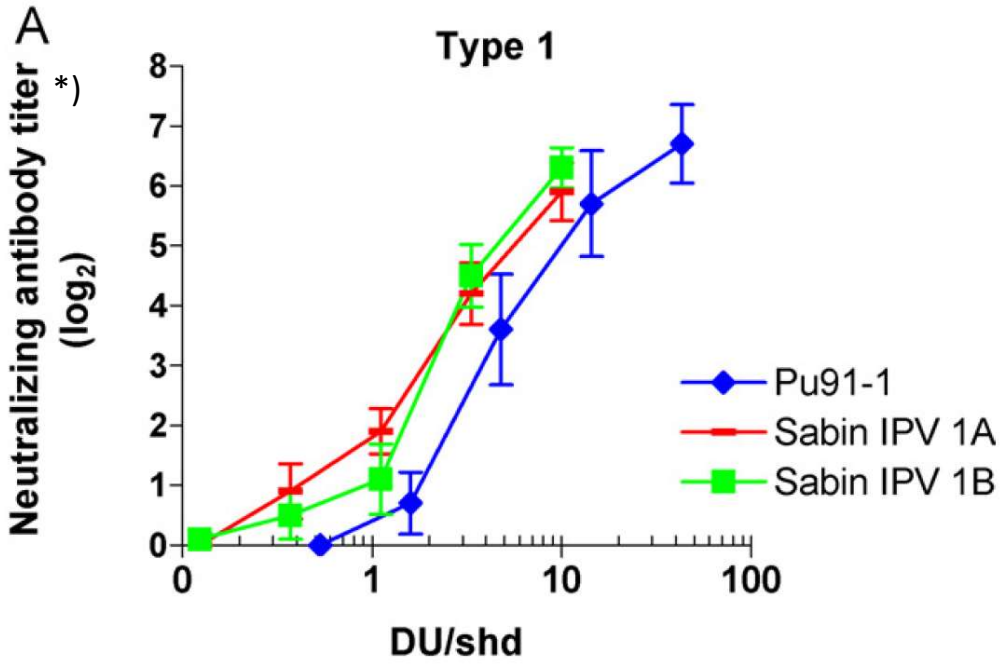


Sabin-IPV immunogenicity (European Pharmacopoeia procedure)

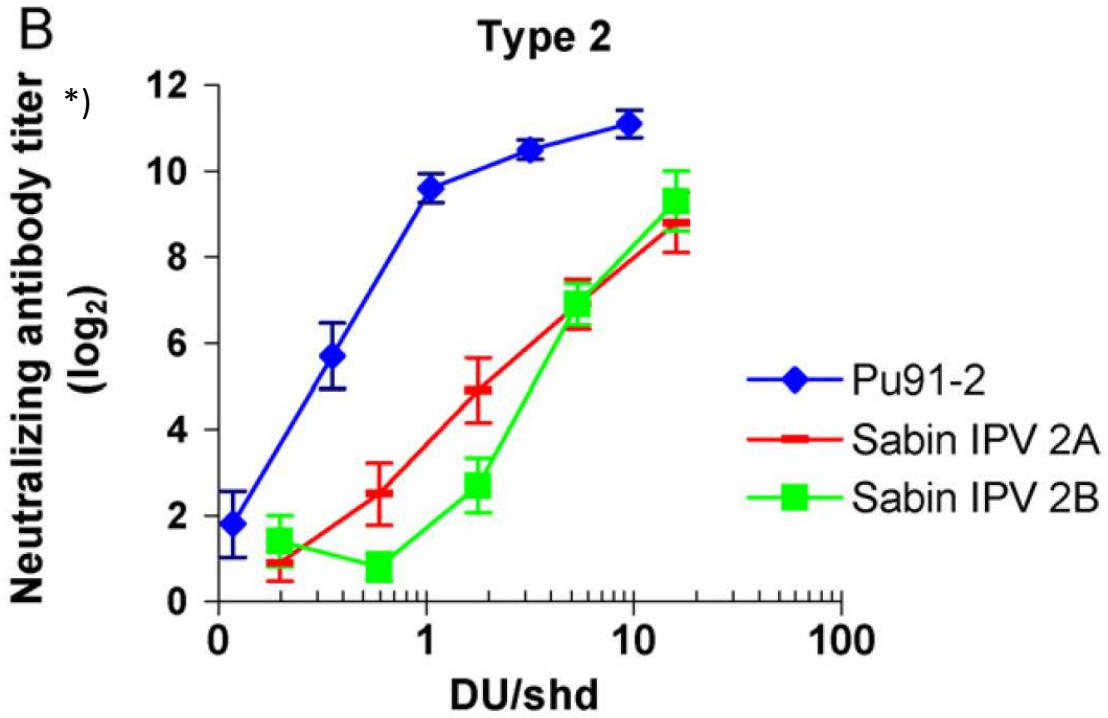
- The potency of IPV can be determined in rats, (guinea pigs or chickens)
- Dose titration.
  - 5 Dilutions per vaccine
  - 10 Rats per group
  - Reference vaccine
  - One immunisation
- In vitro Sabin virus neutralisation on Vero cells

- All Intravacc immunogenicity data shown are anti-wild type virus

What about immunogenicity?



*)Against wild-type virus



*)Against wild-type virus

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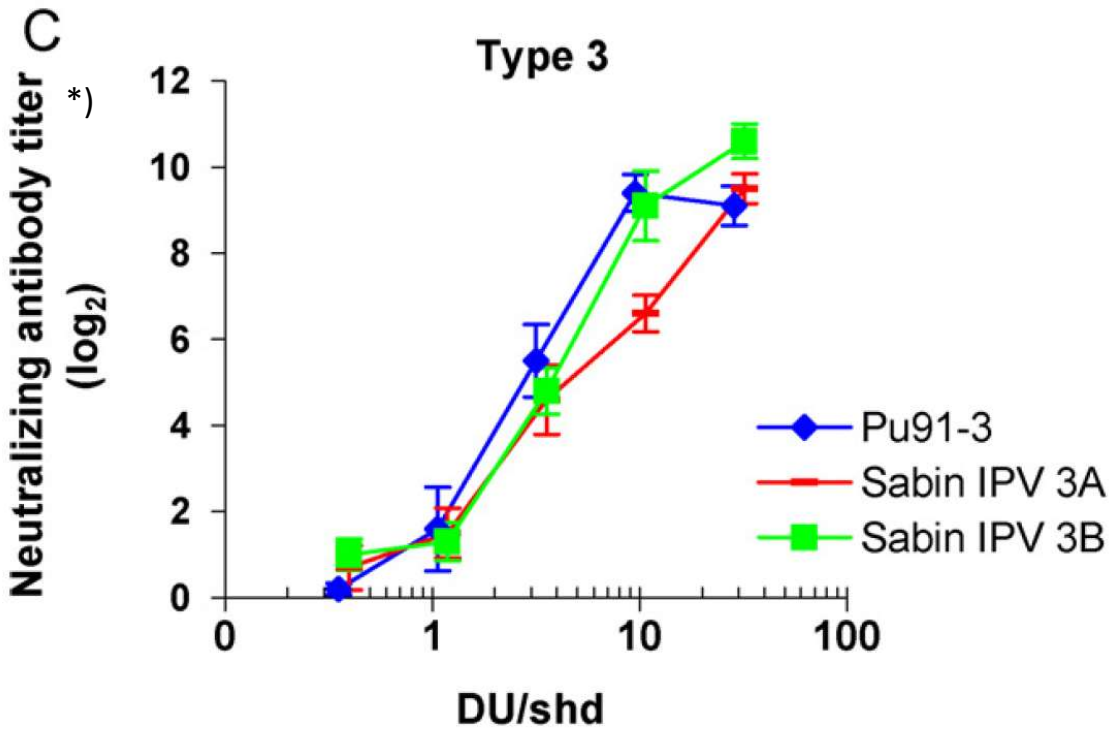

*)Against wild-type virus

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Potency of Sabin-IPV and Salk-IPV in rats

	Dose Sabin/Salk		Neutralizing antibodies (^{2}log)	
Type	(DU)	A260 (mug)	Sabin-IPV	Salk-IPV
1	10/40	300/720	6.3 ± 1.1	6.5 ± 1.4
2	16/8	1790/390	9.3 ± 2.2	11.4 ± 1.0
3	32/32	2240/830	10.6 ± 1.3	9.6 ± 1.1

Standardisation of antigenicity (1)

- D-antigen definition is dependent on
  - Antibody
  - Reference
  - Method to measure amount of (virus) protein
- Alternative: any high affinity, D-antigen specific antibody allows absolute quantitation.
- This is called calibration free concentration analysis (CFCA)

Standardisation of antigenicity (2)

Principle of CFCA:

- Measure diffusion dependent accumulation of antigen to an antigen binding surface.
- Signal is a function of concentration, molecular weight and diffusion coefficient of antigen.
- Antigen is depleted through a D-antigen specific surface -> ACTIVE concentration (D-antigen per ml)

Development CFCA for IPV

Dimensions IPV:

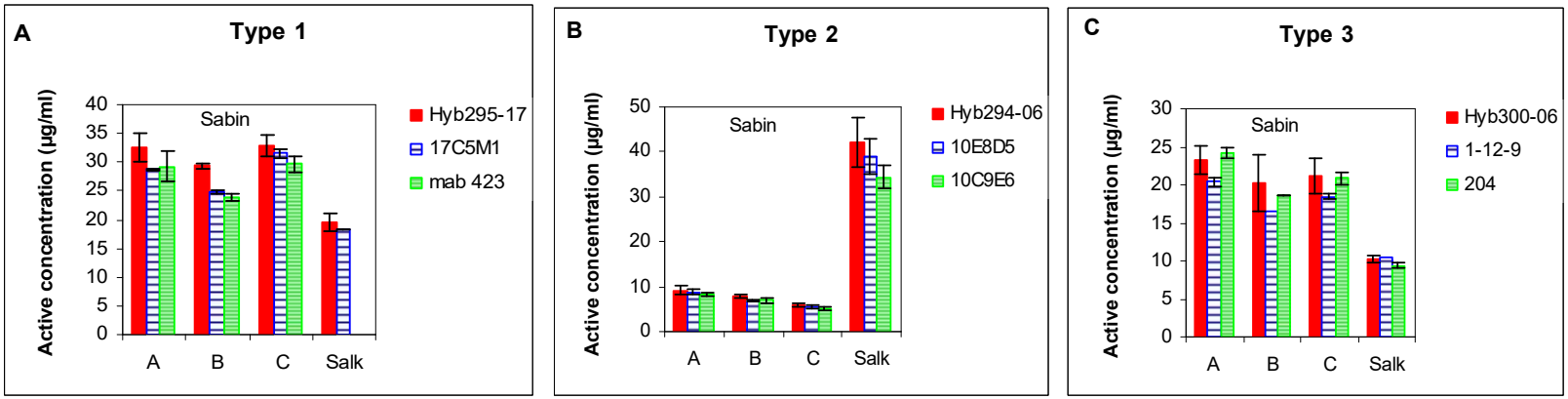
- Molecular weight: 8250 kD
- Diffusion coefficient: 1.44^{-11} M^2/s

(Source: Koch F, Koch G. The molecular biology of poliovirus, 1985)

- Type of sensor chip
- Selection of monoclonals
- Type of immobilisation (direct or indirect immobilisation)
- Specificity
- Reproducibility

CFCA: reproducibility

- 3 measurements on three different days

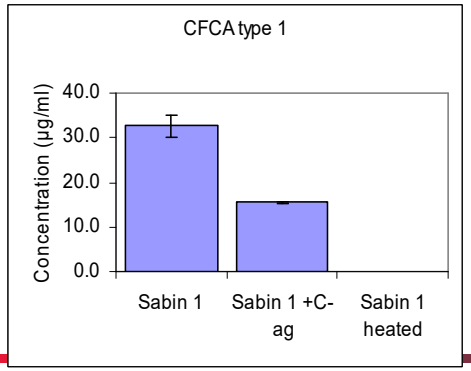


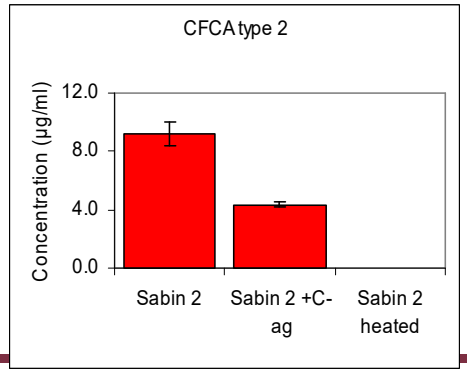
VC < 10%

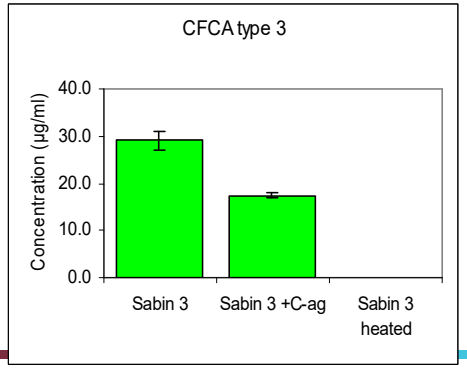
CFCA: specificity

	Samples	Mabs
Type 1	Sabin I Sabin I + C-ag (1:1) Sabin I heated (15 min 60 °)	Hyb 295-17
Type 2	Sabin 2 Sabin 2 + C-ag (1:1) Sabin 2 heated (15 min 60 °)	Hyb 294-06
Type 3	Sabin 3 Sabin 3 + C-ag (1:1) Sabin 3 heated (15 min 60 °)	Hyb 300-06

n= 3 (on different days)

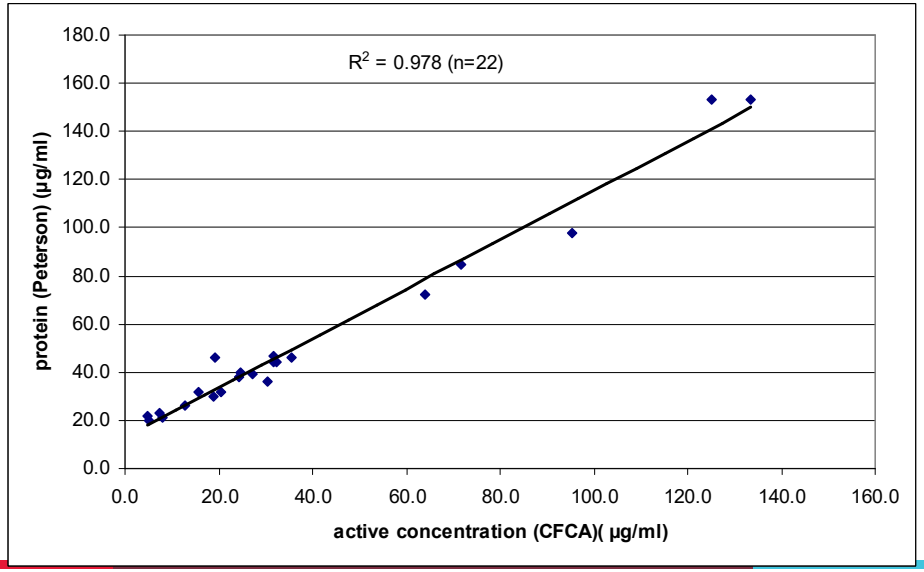






Correlation CFCA and protein (Peterson)

Active concentration vs. protein concentration (Peterson) of monovalent Sabin virus or IPV and Salk-IPV batches



Summary

- Salk and Sabin: different antigenic and immunogenic profiles.
- Antigenic fingerprinting helps to demonstrate batch consistency.
- D-antigen unit as antigenic quantity: less suitable from a standardization point of view.
- Antigen quantity expressed as ‘active concentration’ (amount or concentration of protein with D-antigenicity; microgram/ml D-antigen) is an attractive alternative.

- ITV offers assistance in characterizing polio vaccines:
  - Antgenic characterization (including the active concentration)
  - Determination of thermo stability by biophysical characterization