

# Functional antibody activity as measured by opsonophagocytosis

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Respiratory Diseases Immunology  
Laboratory



# Induction of antibodies

- Disease
- Vaccination
- Passive immunization
- Colonization
- Cross reactive



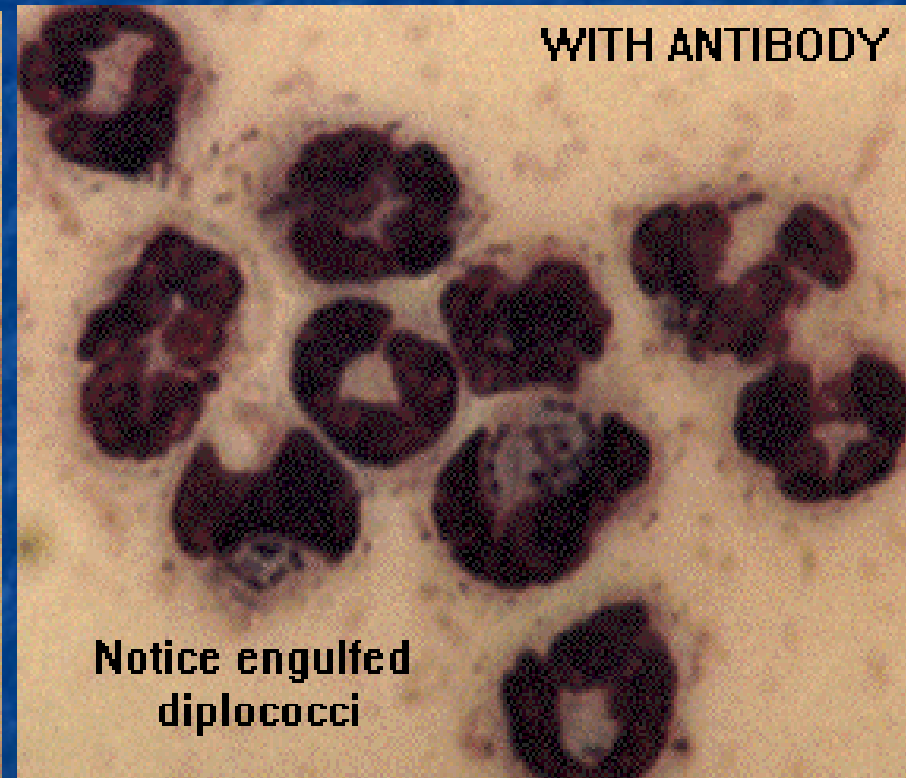
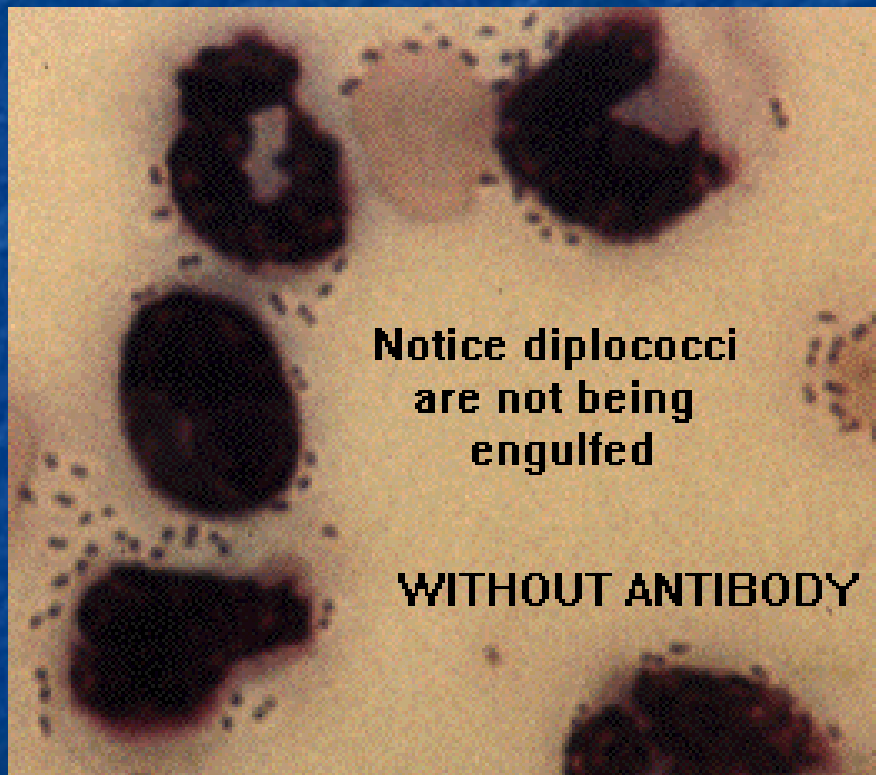
# Antibody Measurements

- **Quantitative measurement**
  - (ELISA) =  $\mu\text{g/ml}$
- **Functional determinations**
  - How well do those Abs protect?
  - Animal protection
  - Opsonophagocytosis
- **Indicator of Memory**
  - Antibody avidity
  - B-cell stimulation (Elispot)

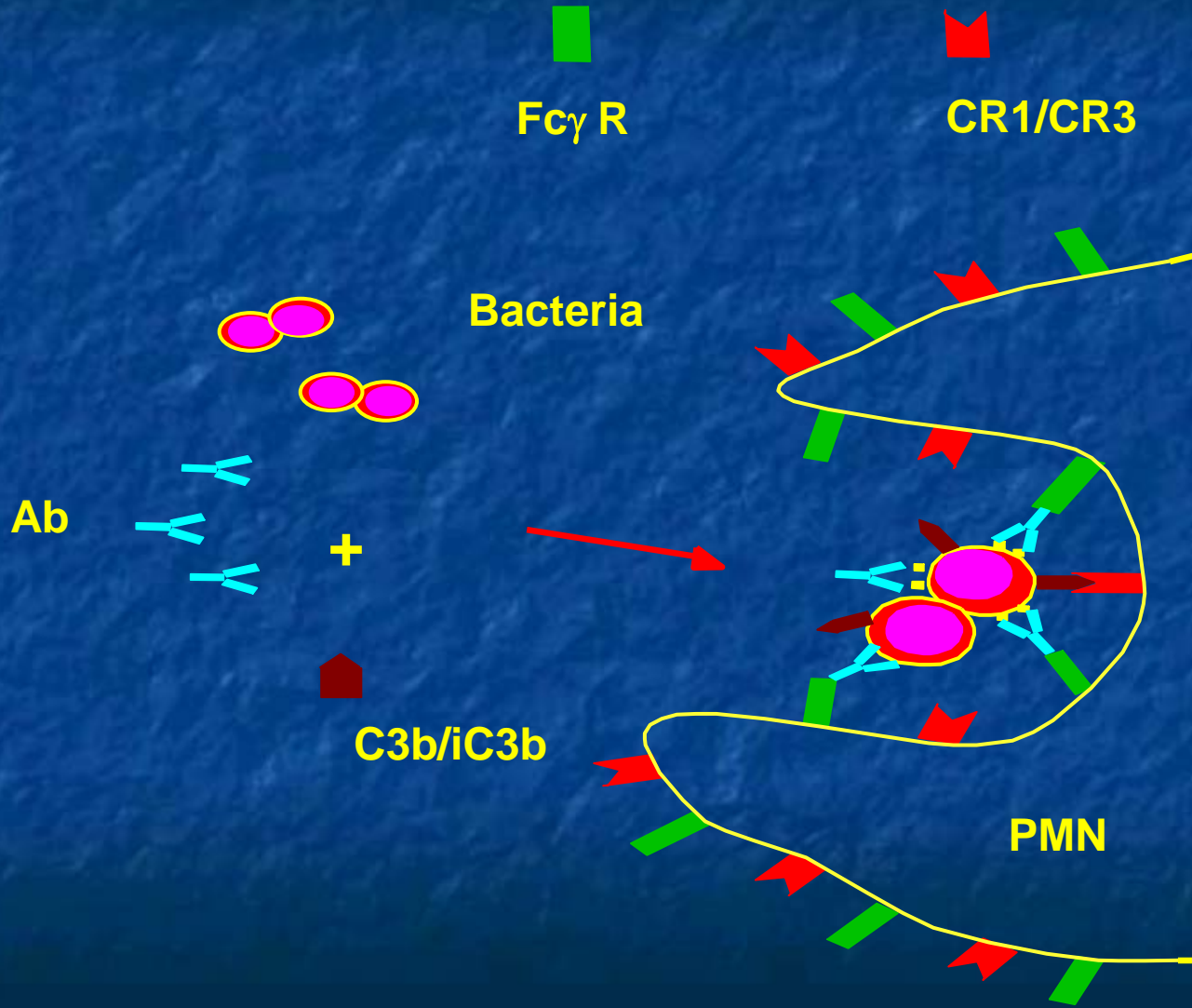




# Opsonophagocytosis



# OPA Players



# Opsonophagocytosis

- **Romero-Steiner *et al.***  
CDLI 1997;4:415-22
- **Four components**
  - Serum
  - Bacteria
  - Complement
  - Culturable phagocytes
- **Internalized Pnc are killed**
- **OPA titer**





2048

Unk1 Unk2 Unk3 Unk4 QC  $\gamma$ globulin

1 2 3 4 5 6 7 8 9 10 11 12

1:8

1:16

1:32

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1:1024

1:64

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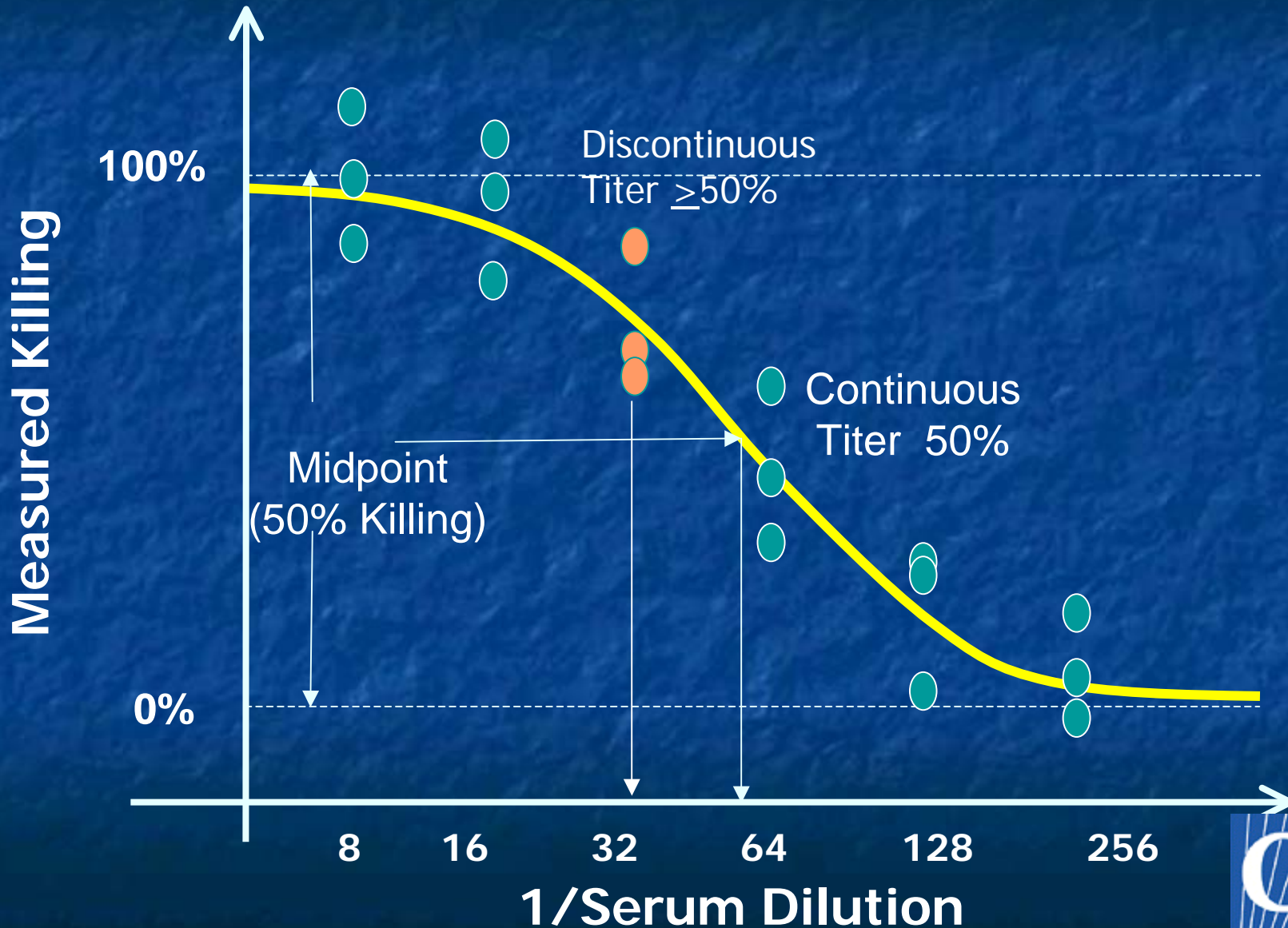
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C' controls



# OPA titer determination (50% killing)

Tom Taylor, PAOAW, June 2005

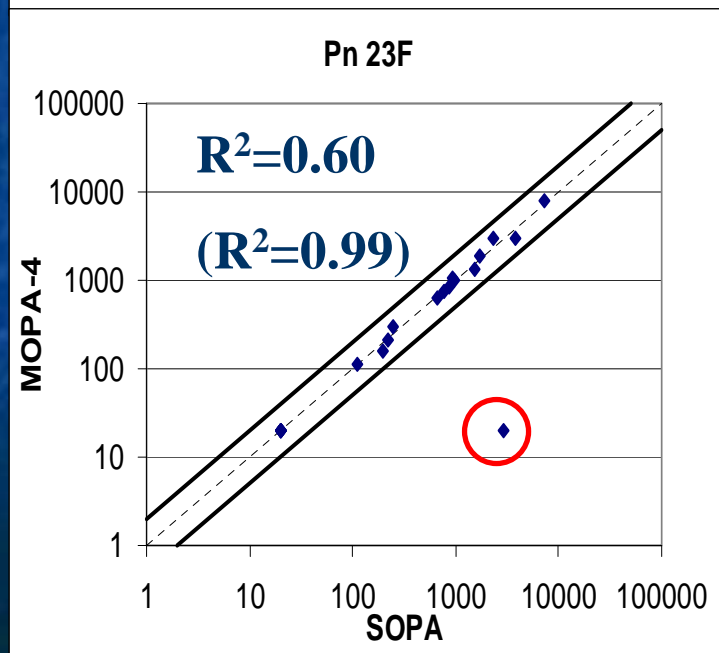
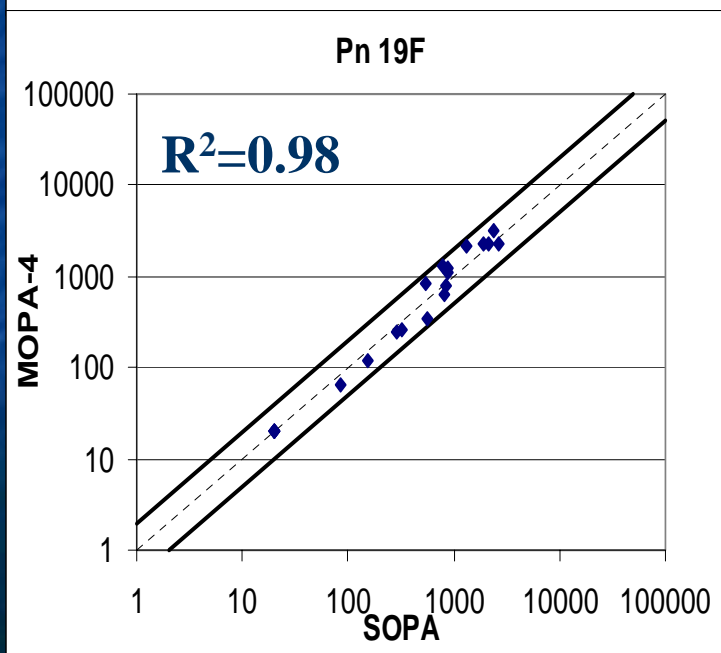
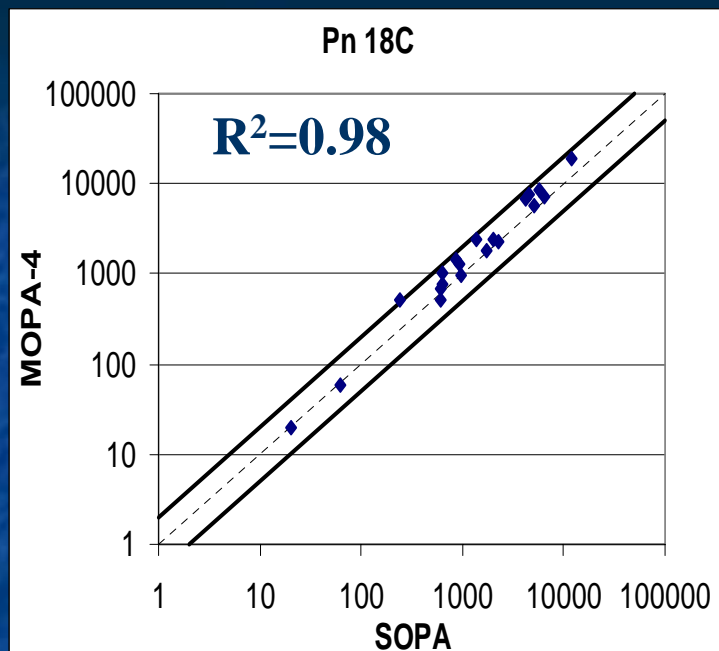
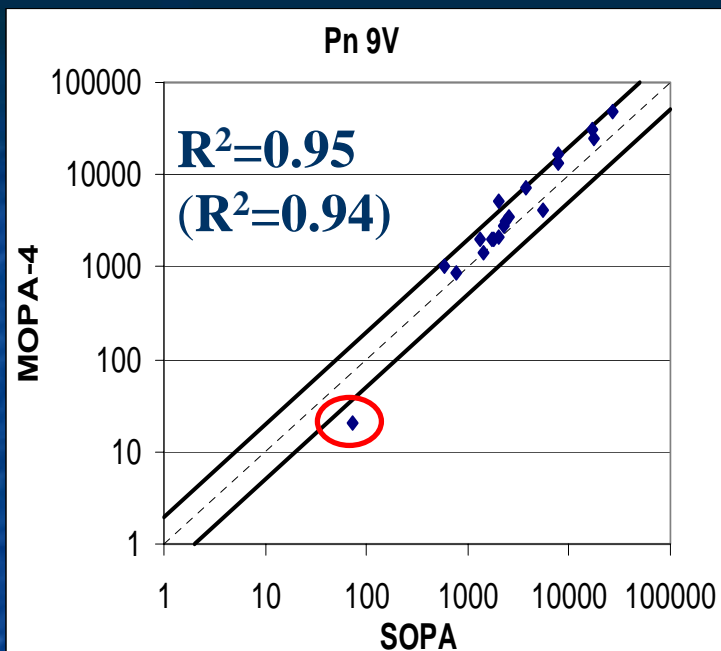




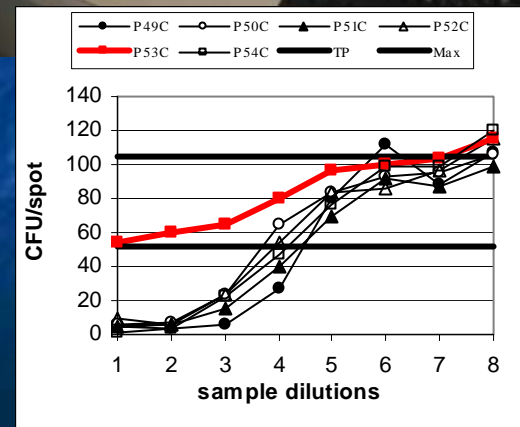
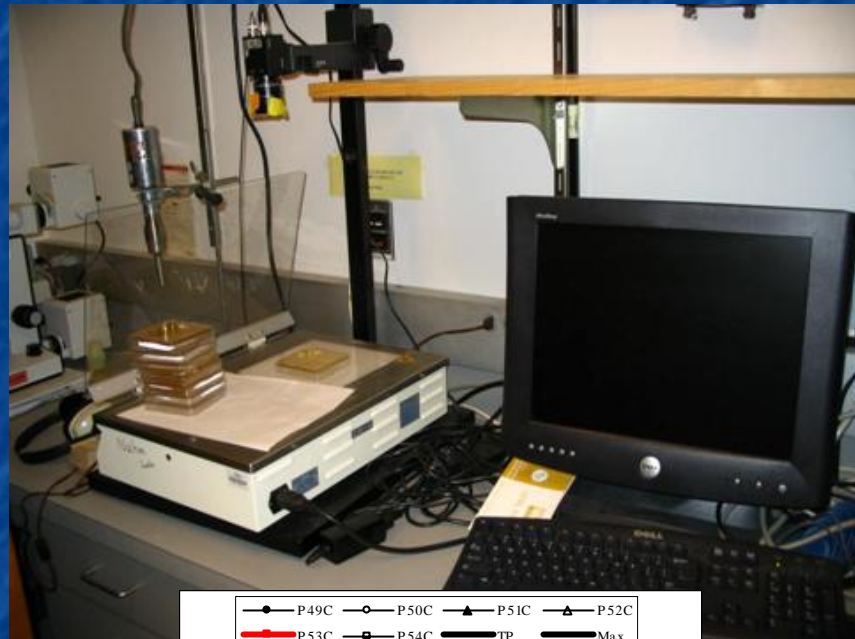
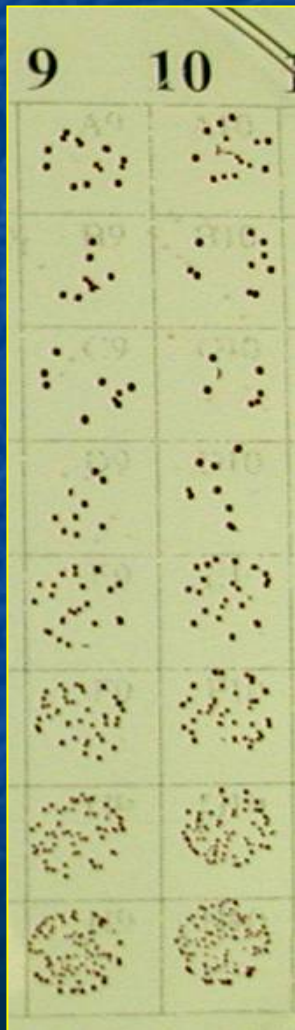
# Killing OPAs

- Single serotype
  - Time consuming
  - Reagent demanding
- Multivalent assays
  - 2 serotypes
  - 4 serotypes
  - 7 serotypes

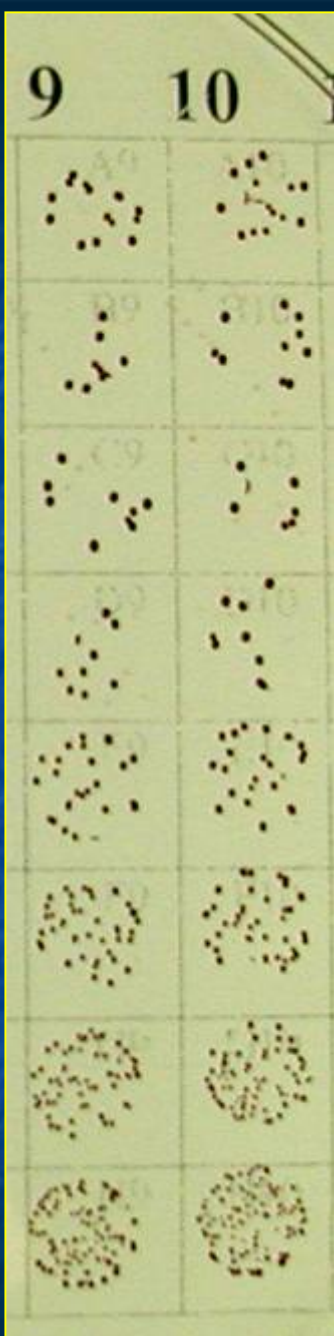
# SOPA vs MOPA-4 (Burton, R. PAOAW, June 2005)



# Automation of killing OPAs







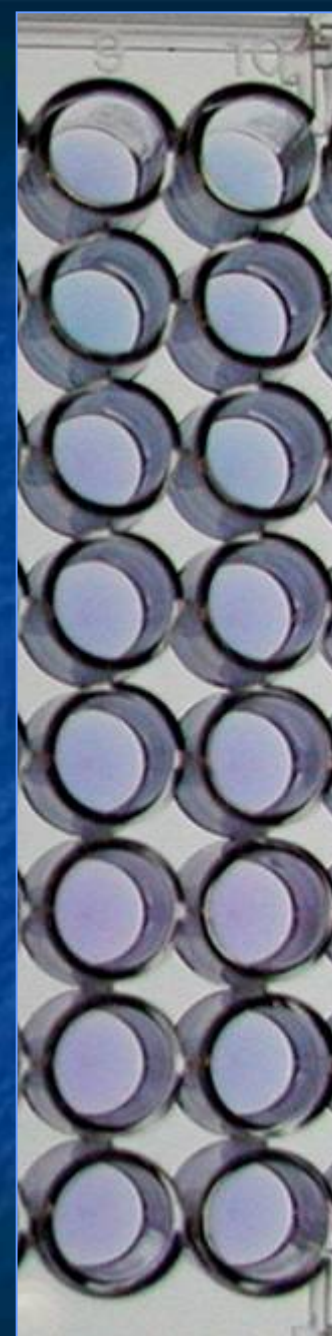
**vOPA**



**Bieging, K. et al.  
CDLI 2005; 10:1238-42**

**$r = 0.76$  to  $0.97$   
7-valent fOPA**

**Bogaert, D. et al,  
Vaccine 2004; 22:4014-20**



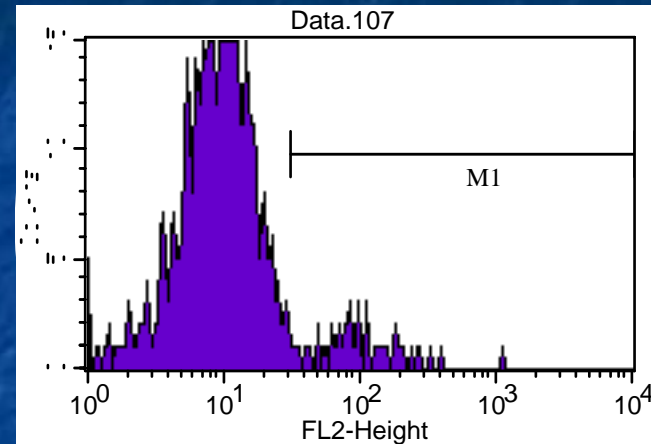
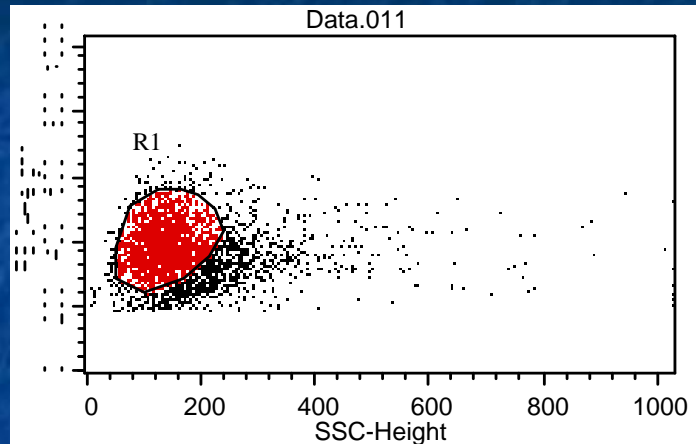
**fOPA**

# Uptake OPAs

- Flow cytometric methods
- Uptake vs killing
  - Killed bacteria
  - Ps-coated particles
- Single serotype
- Multivalent assays
  - 4 serotypes

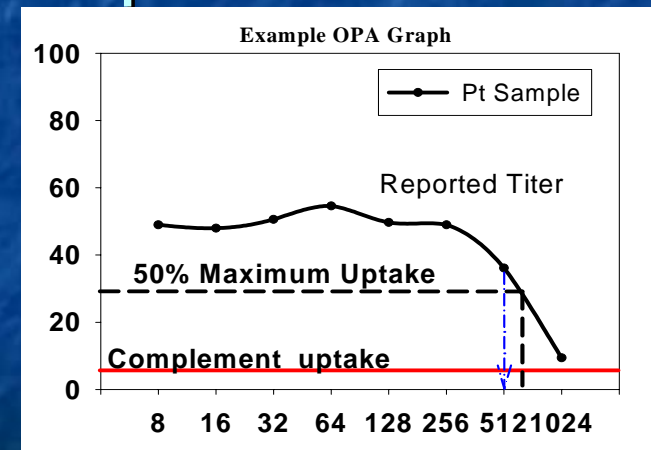
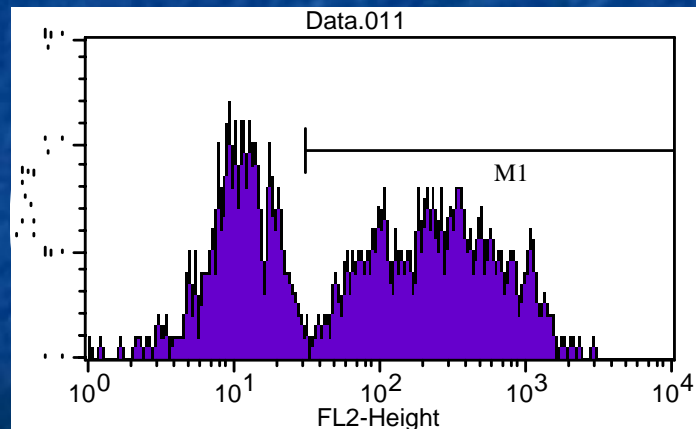
# Flow cytometric OPA

(Uptake of killed bacteria or Ps-coated particles)



Phagocytic cells

Complement control



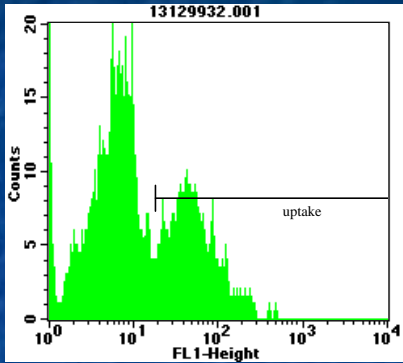
Positive Rx

OPA titer

Martinez, J. *et al.* CDLI 1999; 6:581-6.

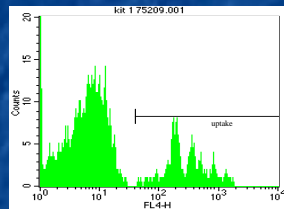
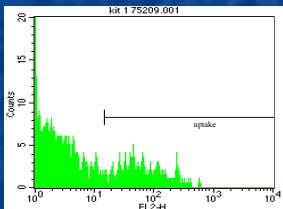
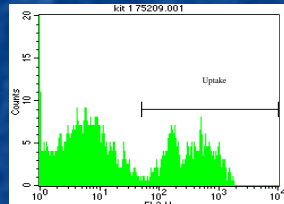
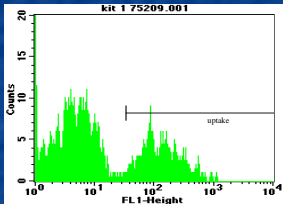
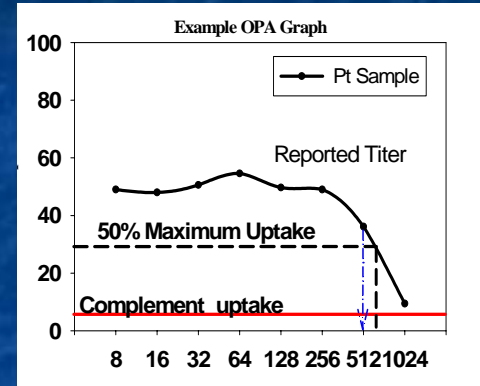


# Multiplexed Flow OPA



$r = 0.53$  to  $0.95$

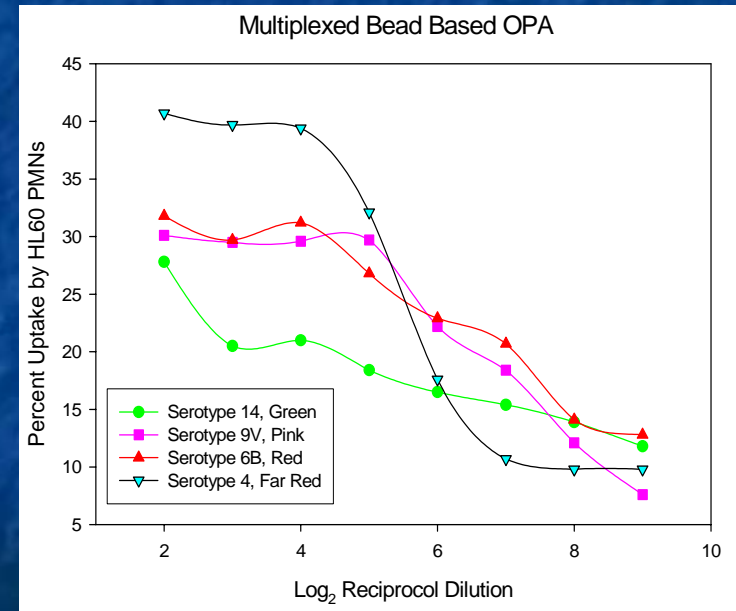
Monovalent



$r = 0.61$  to  $0.91$

$r = 0.68$  to  $0.88$

Multivalent



# Current validation status

- **Single serotype killing assay**
  - Developed, standardized, evaluated and validated at the GLP level
- **Multivalent killing assays**
  - Developed and standardized
- **Fluorescent killing assays**
  - Developed and standardized
- **Flow cytometric (uptake) assays**
  - Developed and standardized

# Multi-laboratory Evaluation

Romero-Steiner *et al.* CDLI 2003; 10:1019-24

<i>S. pneumoniae</i> Serotype	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5
4	88 <sup>a</sup> , 100 <sup>b</sup>	79, 88	100, 100	96, 96	96, 100
6B	75, 88	83, 100	56, 72	83, 88	88, 92
9V	63, 71	83, 88	75, 94	83, 96	83, 92
14	63, 83	75, 83	81, 94	92, 92	88, 92
18C	88, 96	88, 96	56, 69	88, 92	88, 100
19F	79, 96	88, 96	94, 100	96, 100	88, 100
23F	100, 100	100, 100	100, 100	96, 100	92, 96

<sup>a</sup> Within one dilution about the median OPA titer – 75% overall

<sup>b</sup> Within two dilutions about the median OPA titer – 88% overall

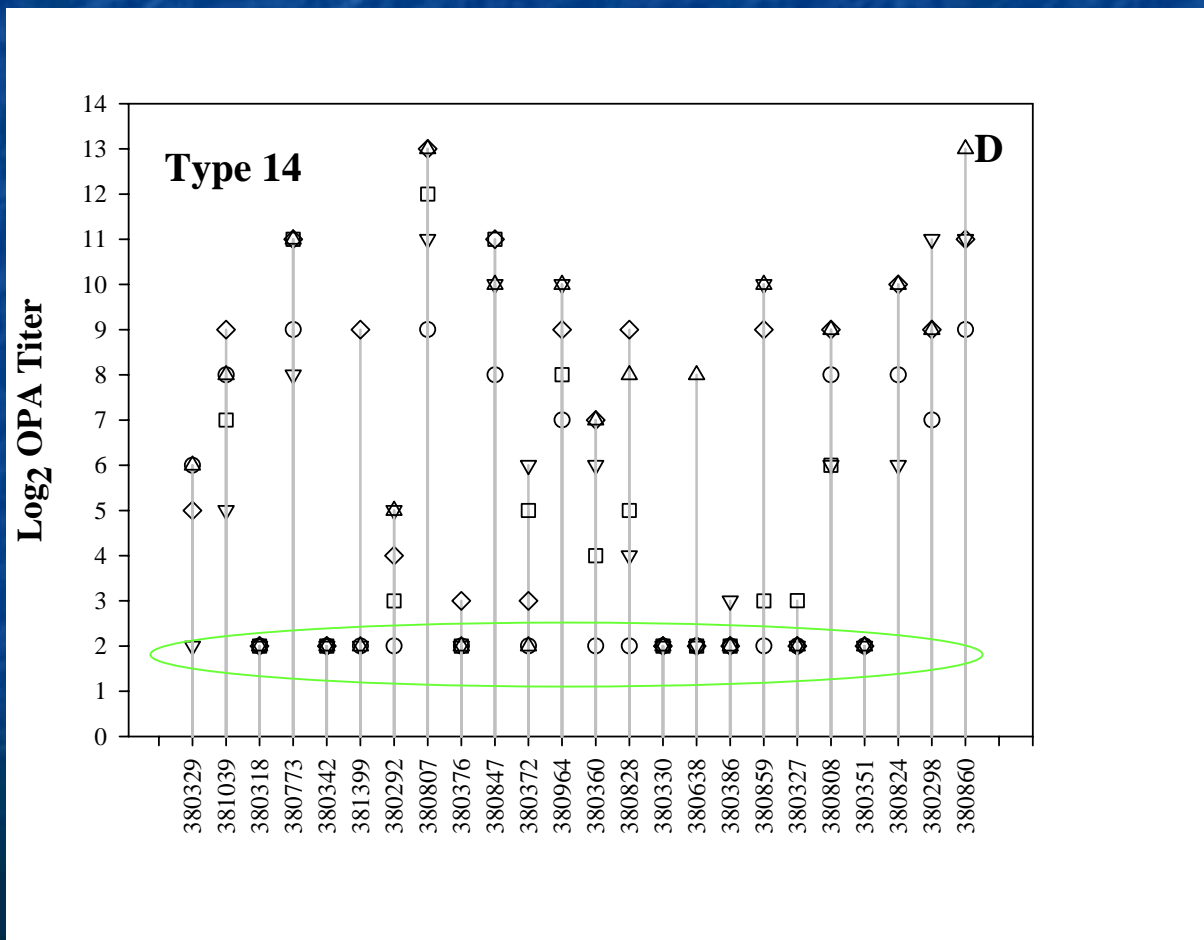




# Multi-laboratory Evaluation

Romero-Steiner *et al.* CDLI 2003;10:1019-24

- Higher agreement in sera with **low titers**
- Lower agreement in sera with high titers



# Validation of killing OPA

B. T. Hu et al. CDLI 2005, 12: 287-295.

- Specificity  $\geq 80\%$  ( $\geq 87\%$  CDLI, 1997)
  - Heterologous Ps  $\leq 20\%$
- Intermediate Precision
  - Overall 81% of titers within 2 dil. of median
- Linearity (9 serotypes)
  - $r = 0.982$  to  $1.000$ , slopes =  $-0.850$  to  $-1.350$
- Accuracy (9 serotypes)
  - All types 100% except 14 (81%) & 23F (75%)
- Robustness



# OPA as a correlate of protection

- ELISA in healthy populations
- Passive protection in animals
- Minimum level needed for vaccine efficacy in infants



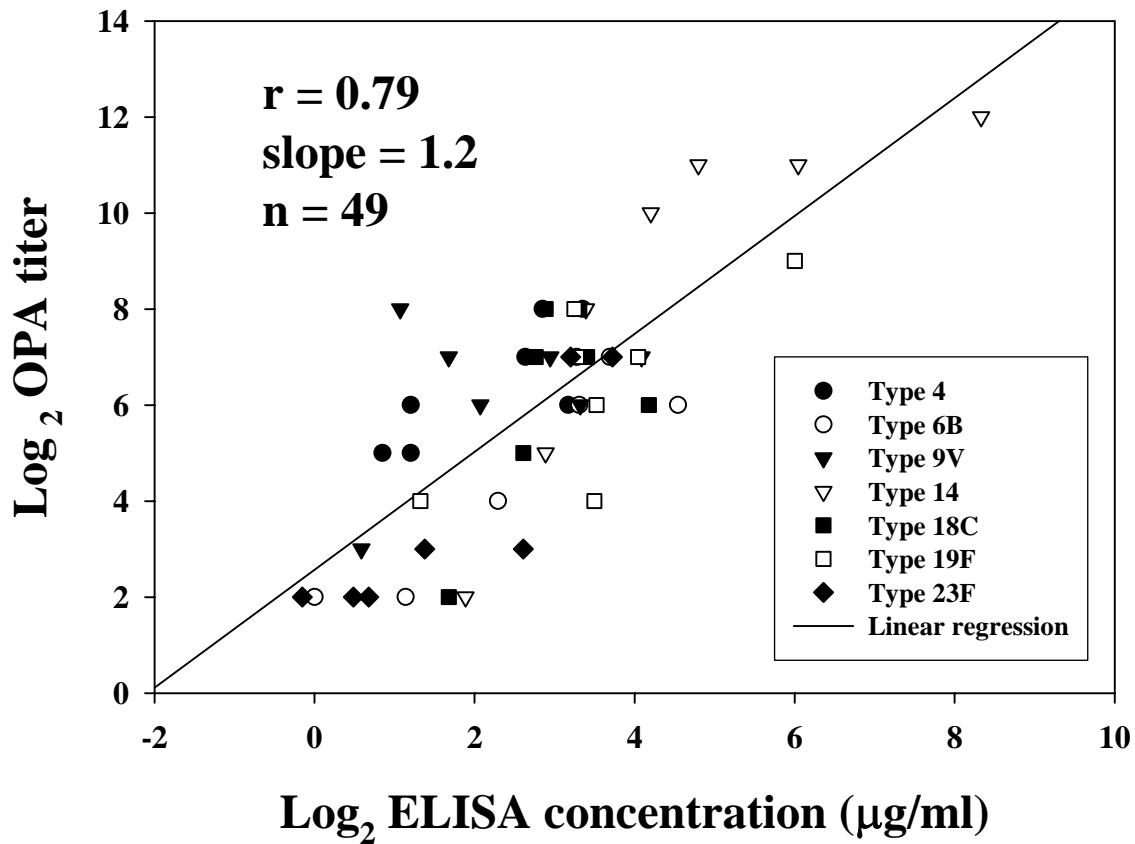
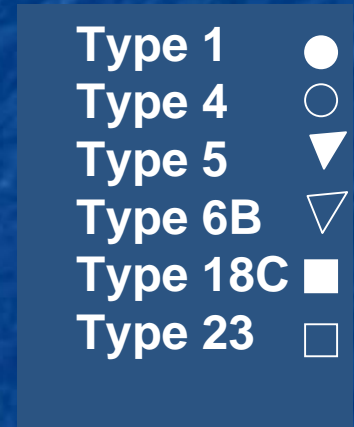
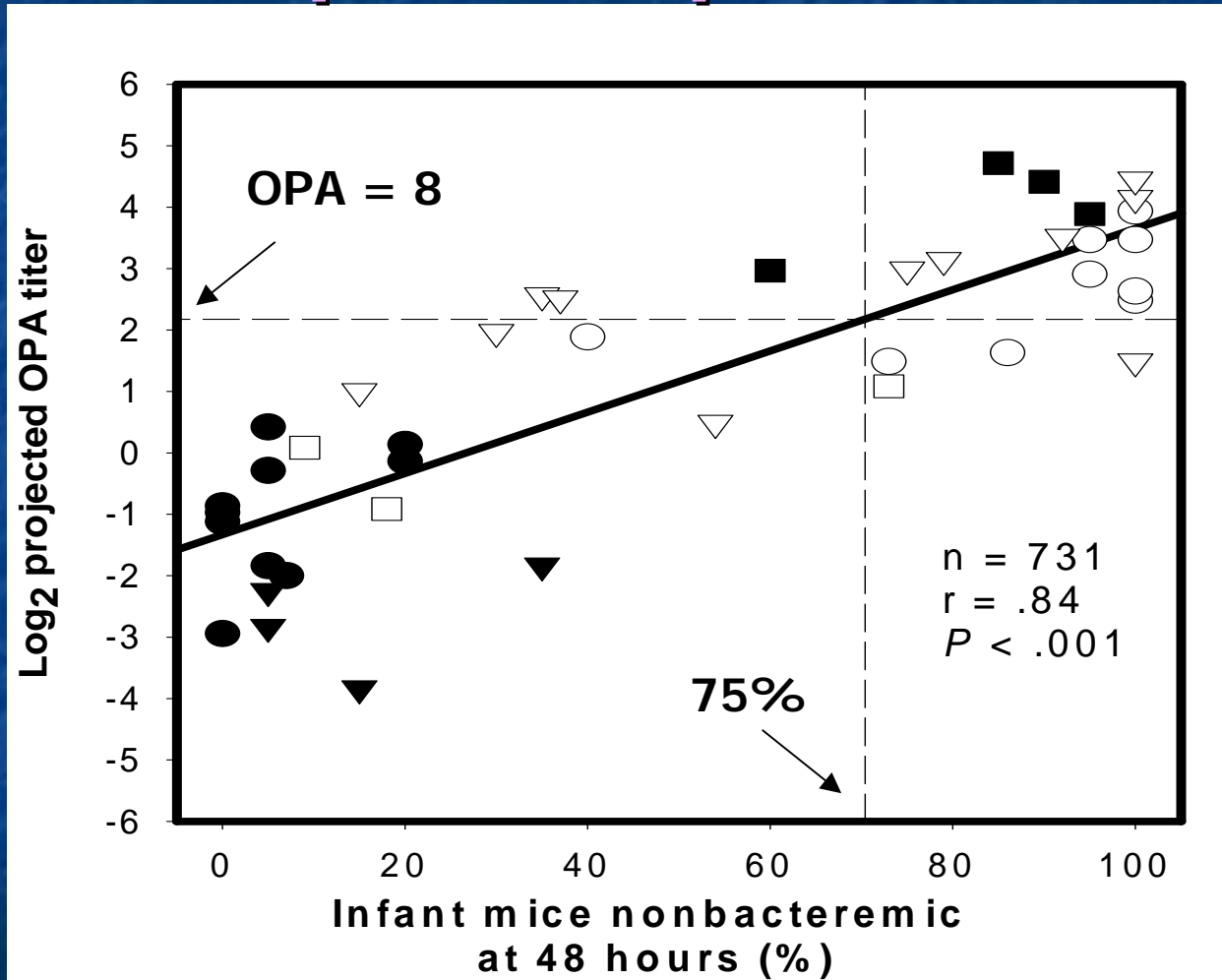


Figure 3.

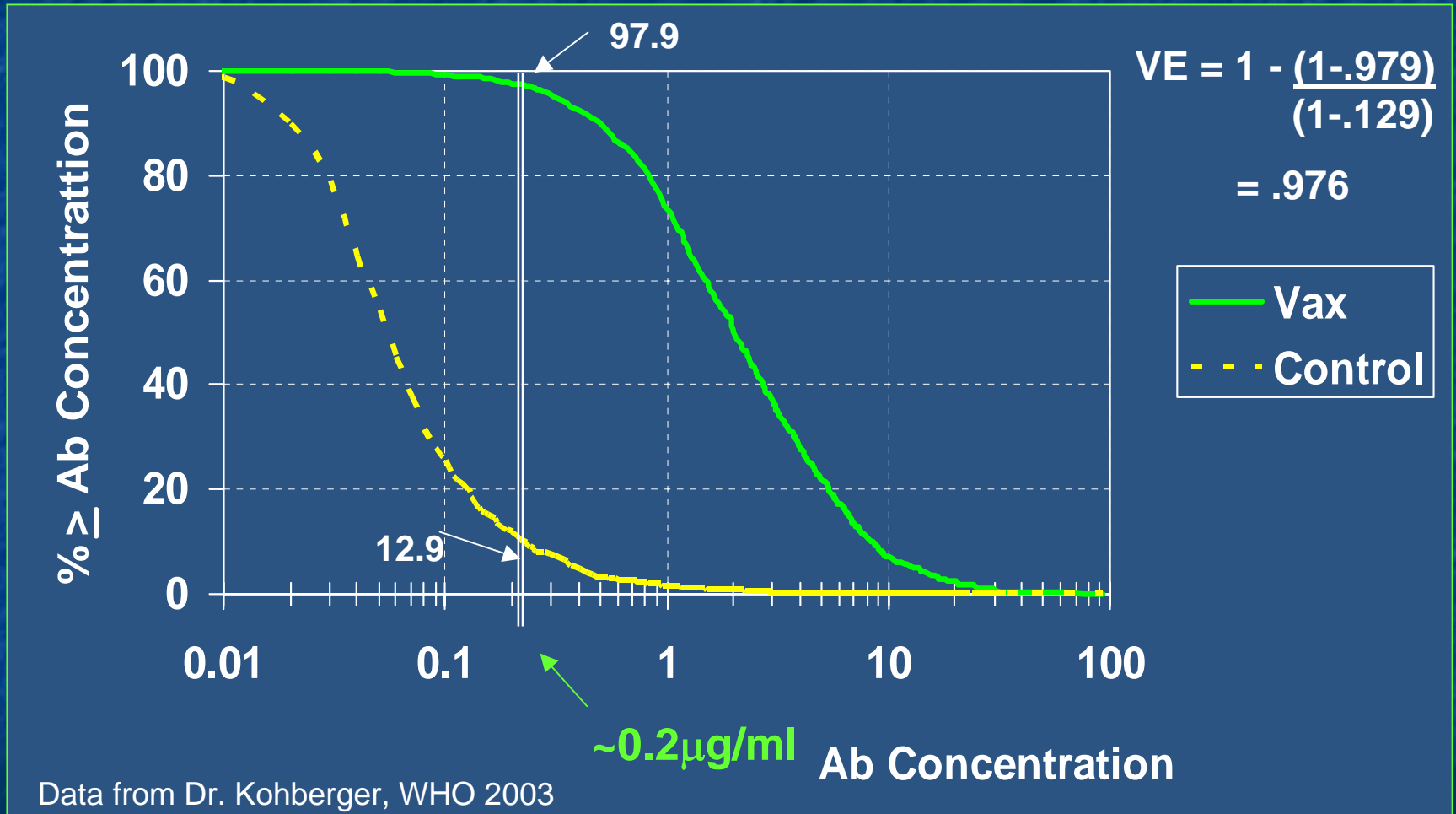
# Protective OPA titer in a mouse passive protection model



Johnson, et al. JID 1999; 180:133

# Reverse cumulative distributions of post dose 3 ELISA Ab for 7 serotypes in infants

(Black, et al., North Calif. Trial)



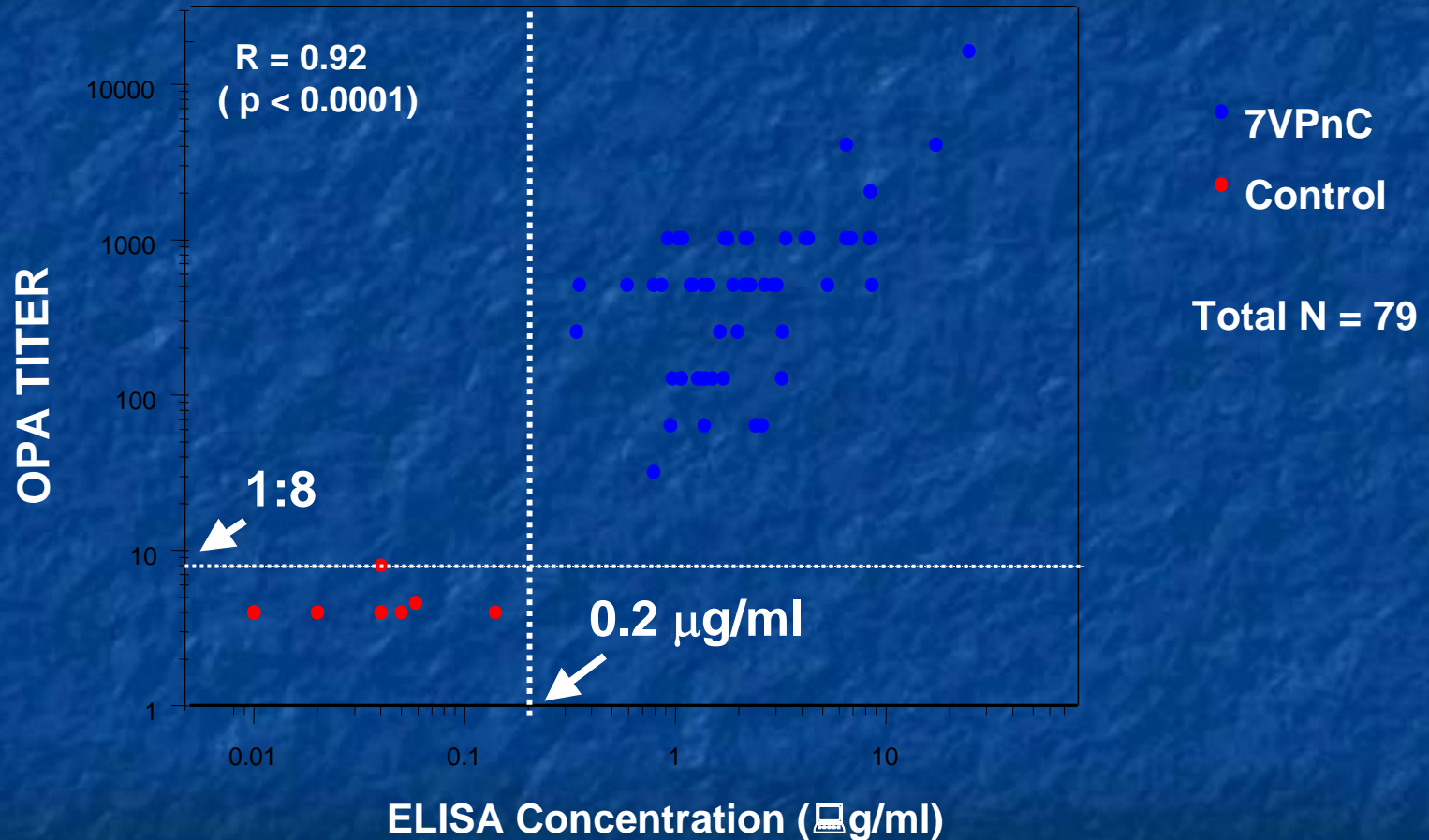
[Ab] for 97.9% protection in the target population = 0.20 μg/ml



# Correlation of ELISA and OPA

(North CA KP infant study)

Jodar, et al. Vaccine 2003; 21:3265-3272



ELISA concentration of 0.20  $\mu\text{g/ml}$   $\approx$  OPA titer of 1:8

# Functional Abs in the elderly

- Protective levels are unknown
- Reduced function in elderly receiving PPV-23 (CID 1999;29:281-8)
  - OPA (80-89 years &  $\geq 90$  years)
  - Lower Ab avidity
  - Poor correlation with ELISA IgG ( $r=0.3$  to  $0.8$ )
  - Do not protect mice
- Usinger and Lucas I&I, 1999;67:2366-70
- Absorption of cross-reactive antibodies (I&A 2005; 2:1-10)

# OPA in clinical vaccine trials

Ekstrom, N. PAOAW, June 2005

## ■ Killing OPA (Romero-Steiner et al. 1997)

- Various phase 2 studies with different Pnc-conjugate vaccines in Finnish infants (Anttila et al. 1999)
- 7-valent PncCRM and PncOMPC in Finnish infants in The Finnish Otitis Media Vaccine Trial (FinOM)
- 11-valent PncDT in Filipino infants (Puumalainen et al. 2003)
- 23-valent PS in HIV+ Ugandan adults (French et al. 2004)
- 11-valent PncDT in Finnish and Israeli infants (Wuorimaa et al. 2005)
- 11-valent Pn-PD in Finnish infants (Nurkka et al. 2005)

## ■ Flow cytometric OPA (Martinez et al. 1999)

- 11-valent PncDT in Filipino infants (Lucero et al. 2004)
- 9-valent PncCRM in South-African infants (Mahdi et al. 2005)



# Advantages of using OPA

- Laboratory correlate of protection
- Reduce numbers of efficacy studies
- Reference method (killing sOPA)
- [www.vaccine.uab.edu](http://www.vaccine.uab.edu)
- Standardized
- Validated
- Culturable phagocytes
- High through put
- GLP conditions
- Data analysis

# Disadvantages of OPA

- In vitro correlate
- Laboratory facilities
- Training of technical staff
- Multiplex assays need validation