

Development of Validated Assays for Measuring the Human Antibody Response to Polysaccharide Conjugate Vaccines

Helena Käyhty, PhD

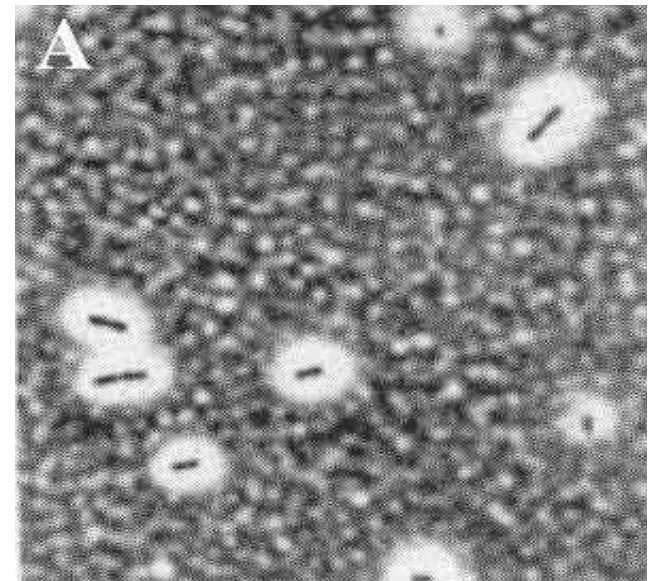
National Public Health Institute

Helsinki, Finland



- ***Haemophilus influenzae* type b (Hib)**
- ***Neisseria meningitidis* (Men)**
- ***Streptococcus pneumoniae* (Pnc)**

- For all, antibody to capsule has been proven protective via complement mediated mechanisms
 - Bactericidal activity (lysis)
 - Opsonizing for phagocytosis



- **Serum bactericidal activity (SBA)**
 - The ability of antibodies to mediate killing of bacteria in the presence of complement
= antibody and complement mediated lysis
- **Serum opsonophagocytic activity (OPA)**
 - The ability of antibodies to mediate opsonization of bacteria for phagocytosis by phagocytosing cells (PMNL, HL-60) in the presence of complement
= antibody and complement mediated phagocytosis
- **OBS! Animal experiments** do not (always) correlate with the properties of the conjugate and polysaccharide vaccines humans

- For all, **antibody concentration** can be measured by radioimmunoassay (RIA) or enzyme immunoassay (EIA)

- Important for long term protection together with sustaining antibody concentrations
- Challenge of memory B cells with a polysaccharide or conjugate vaccine → measure antibodies by EIA
- Determination of **antibody avidity maturation**

- Carefully validated
- Standardized and with good QC
- Give comparable results in different laboratories
 - Reagents (e.g. EIA antigens and reagents, complement source)
 - Reference and QC materials
 - Strains

Hib conjugate vaccines



Four different Hib conjugate vaccines



	PRP-D	HbOC	PRP-OMPC	PRP-T
Trade name	ProHIBit	HibTiter	PedvaxHIB	ActHib, Hiberix
Manufacturer		Wyeth	Merck	sanofi pasteur, GSK
Carrier	Diphtheria toxoid (D)	Mutant diphtheria Toxin (CRM)	Men OMP complex	Tetanus toxoid (T)
PS µg	25 (sized)	10 (OS)	7.5 (native)	10 (native)

© World Health Organization

WHO Technical Report Series, No. 897, 2000

<http://www.who.int/biologicals/publications/meetings/areas/vaccines/en/>

Annex 1

Recommendations for the production and control of *Haemophilus influenzae* type b conjugate vaccines¹

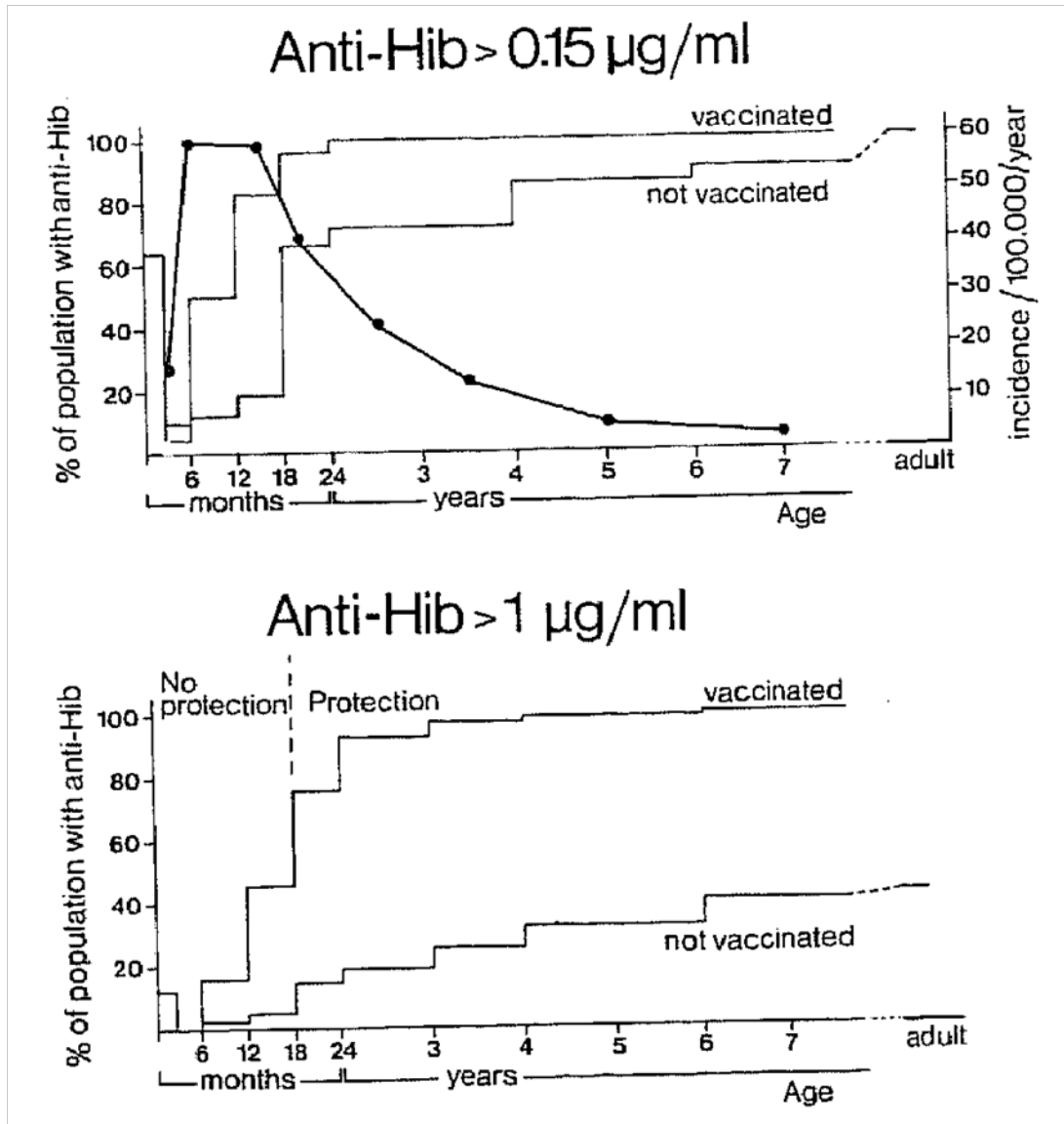
Appendix 1

Evaluation of immunogenicity of *Haemophilus influenzae* type b conjugate vaccines in humans

- Primary endpoint: antibody concentration post primary vaccination series
 - Thresholds 0.15 and 1.0 $\mu\text{g/ml}$
- Functional activity of antibodies measured by SBA (or OPA)
- Immunologic memory
 - Booster response
 - Avidity

- Radioimmunoassay (RIA) used in 1970-80's
→ concentrations associated with protection
- **Enzyme immunoassays (EIA)** replaced RIA
 - Interlaboratory comparisons
 - Different protocols
 - Should give equivalent results to RIA
- Reference serum lot 1983 and QC sera from US FDA
- EIA antigen (HbOHA) from NIBSC, UK

Search of correlates for Hib PS vaccines



Challenge 1



- Radioimmunoassay (RIA) used in 1970-80's -
> concentrations associated with protection
- **Enzyme immunoassays (EIA)** replaced RIA
 - Interlaboratory comparisons
 - Different protocols
 - Should give equivalent results to RIA
- Reference serum lot 1983 and QC sera from US FDA
- EIA antigen (HbOHA) from NIBSC, UK

Continuing need of immunogenicity evaluation

- Several Hib conjugate vaccines licensed and in use in developed and developing countries
- Licensure of **new vaccines** based on immunogenicity data and safety
- Component of **combination vaccines**
- **Schedules**
- Use in **resource poor countries with high risk** of Hib disease
- **Risk groups among adults**

<http://www.vaccine.uab.edu/>

**Information on hemophilus and
pneumococcal assays**

Meningococcal conjugate vaccines



- **MenC**
 - carriers: mutant diphtheria toxin (CRM) , tetanus toxoid (T)
 - used widely in Europe
- **MenACW135Y**
 - Carrier: diphtheria toxoid (D)
 - CRM conjugate in clinical trials
 - Used in USA for adolescents; clinical trials in infants ongoing
- **MenA**
 - Planned to be used in African meningitis belt
 - The Meningitis Vaccine Project

WHO Technical Report Series, No. 926, 2004

**Recommendations for the production and control
of group C meningococcal conjugate vaccines**

APPENDIX 1

**Evaluation of the immunogenicity of group C
meningococcal conjugate vaccines**

WHO/BS/06.2041- 2006 Final

**RECOMMENDATIONS TO ASSURE THE QUALITY,
SAFETY AND EFFICACY OF GROUP A
MENINGOCOCCAL CONJUGATE VACCINES**

Thresholds associated with protection for MenC CVs:

- SBA is a gold standard
 - Baby rabbit complement: titer of ≥ 8 (rSBA)
 - Additional indicators: ≥ 4 - fold rise or a titer of ≥ 4 with human complement (hSBA)

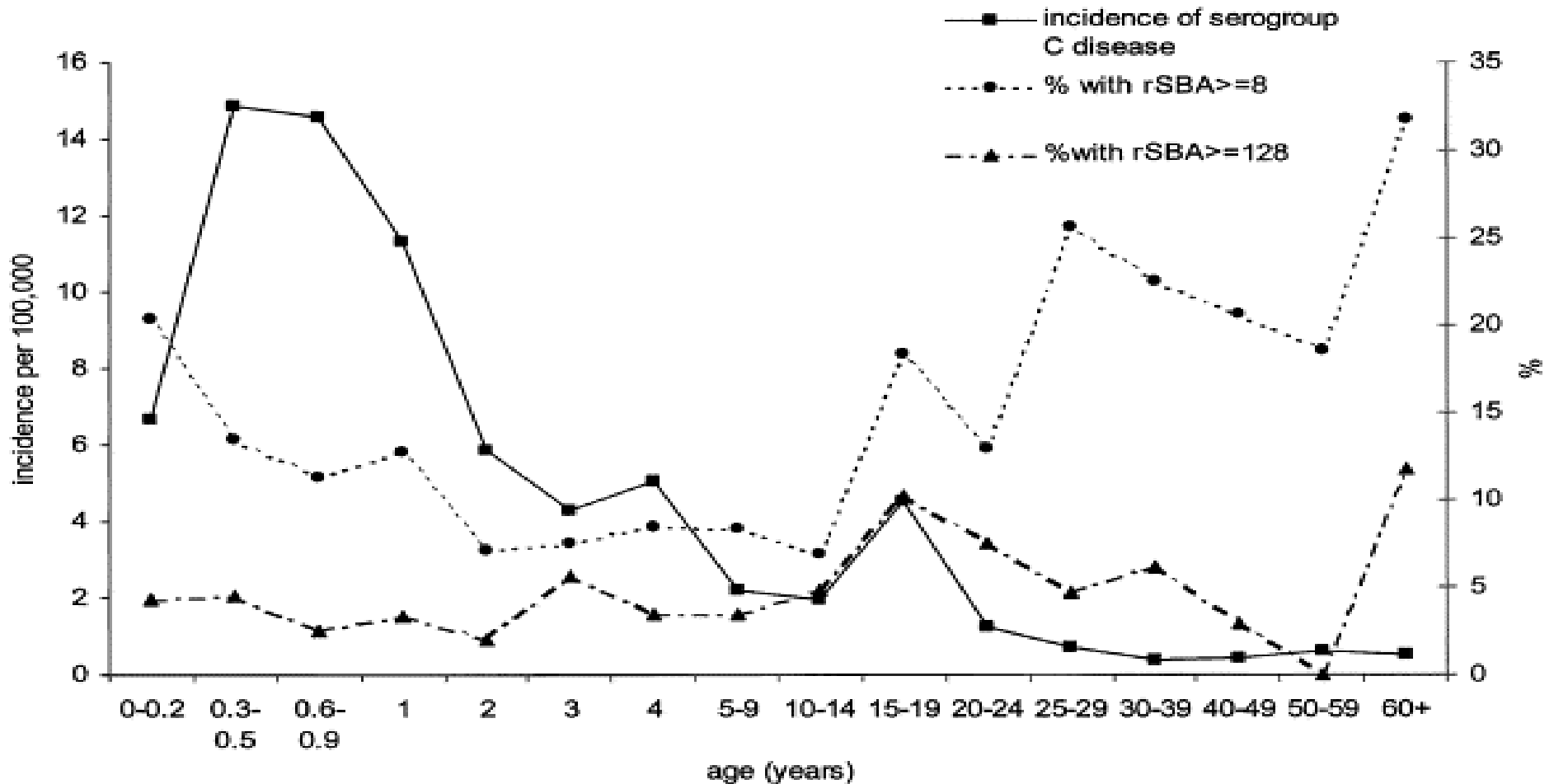
	Sera of cases	Sera of healthy controls
Homologous strain	3/54 (5.6%)	444/540 (82.2%)

Goldschneider I *et al.* *J Exp Med* 1969; 129: 1307–26

Human or rabbit complement?

- Original Goldschneider study used human complement (hSBA)
- Difficult to get and standardize
- Baby rabbit complement commercially available... but gives higher SBA titers
- UK experience with rSBA and Menc conjugates:
 - rSBA titer of $\geq 1:8$ in immunized infants and children correlates with effectiveness in post licensure surveillance (Andrews et al. Clin Diag Lab Immunol 2003)

MenC disease and prevalence of rSBA



Challenge 2.



Serogroups A, W135, Y

- Are criteria set up for MenC valid for other serogroups?
- Which strains are the relevant to be used in SBA?

EIA or comparable assays for isotype specific antibody concentrations

- Concentration associated with protection not determined
- **OBS!** Should correlate with SBA
- To increase correlation use
 - Pure polysaccharide antigens
 - Derivatized polysaccharide antigens
 - Chaotropic agents (thiocyanate) to detach antibodies of low affinity (assumed to be not protective)

NIBSC, Potters Bar, UK

- Polysaccharides
- Reference serum
- Methylated serum albumin for enhancing the binding of PS to solid phase

Pneumococcal conjugate vaccines



Pneumococcal conjugate vaccines



- **PncCRM; Wyeth.**
 - Carrier: mutant diphtheria toxin, CRM197
 - At present 7-valent vaccine licensed (Prevenar, Prevnar)
 - 13-valent vaccine in clinical trials
- **PncPD; GSK**
 - Carrier: *H. influenzae* Protein D
 - 11-valent vaccine tested in clinical efficacy trials
 - 10-valent mixed carrier vaccine filed for licensure
- **PncOMPC; Merck**
 - Carrier: meningococcal outer membrane protein complex
 - 7-valent vaccine tested in clinical efficacy trial
 - Company has stopped the development
- **PncDT, sanofi pasteur**
 - Carriers: diphtheria and tetanus toxoids
 - 11-valent vaccine tested in clinical efficacy trial
 - Company has stopped the development

WHO Technical Report Series, No. 927, 2005

Annex 2

**Recommendations for the production and control
of pneumococcal conjugate vaccines**

Appendix

**Serological criteria for evaluation and licensure of
new pneumococcal conjugate vaccine formulations
for use in infants**

- Acceptance of new vaccines, schedules, combinations, based mainly on immunological studies
- Primary threshold for all serotypes = % subjects with 0.35 µg/ml of **antibody measured by EIA**¹
- Secondary thresholds
 - Demonstration of **functional activity of antibodies by testing OPA**
 - Demonstration of **immunological memory**

¹<http://www.who.int/biologicals/publications/meetings/areas/vaccines/en/>

Challenge 1:



- Noninferiority to a licensed vaccine
- Primary threshold for all serotypes = % subjects with 0.35 $\mu\text{g/ml}$ of **antibody measured by EIA**
- 0.35 $\mu\text{g/ml}$ threshold determined with non-22F EIA

Training manual for Enzyme linked immunosorbent assay for the quantitation of *Streptococcus pneumoniae* serotype specific IgG (Pn PS ELISA).

A guide to procedures for qualification of materials and analysis of assay performance.

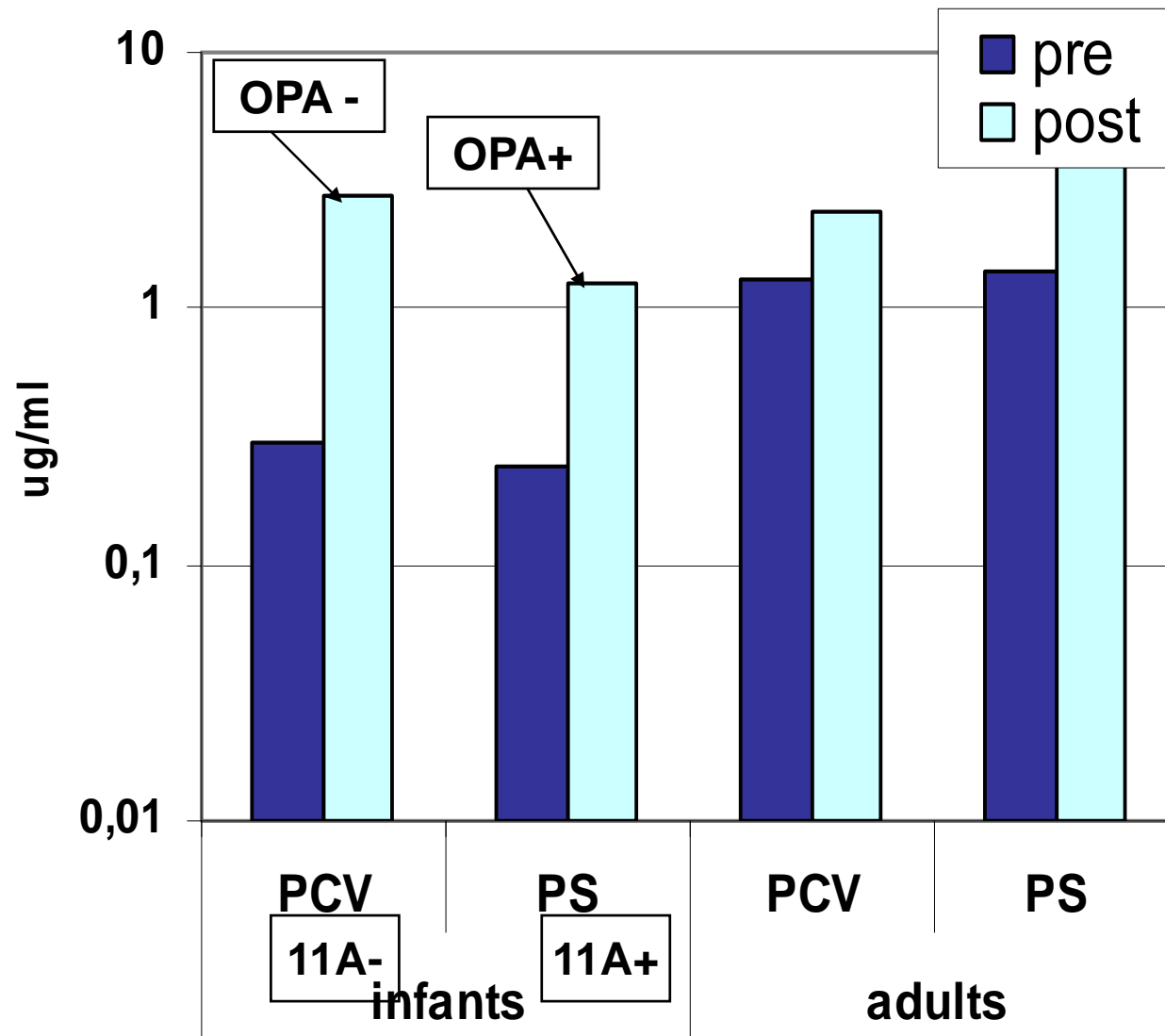


<http://www.vaccine.uab.edu/>

Prepared by the World Health Organization Pneumococcal Serology Reference Laboratories at the Institute of Child Health, University College London, London, England and the Department of Pathology at the University of Alabama at Birmingham, Birmingham Alabama, USA.

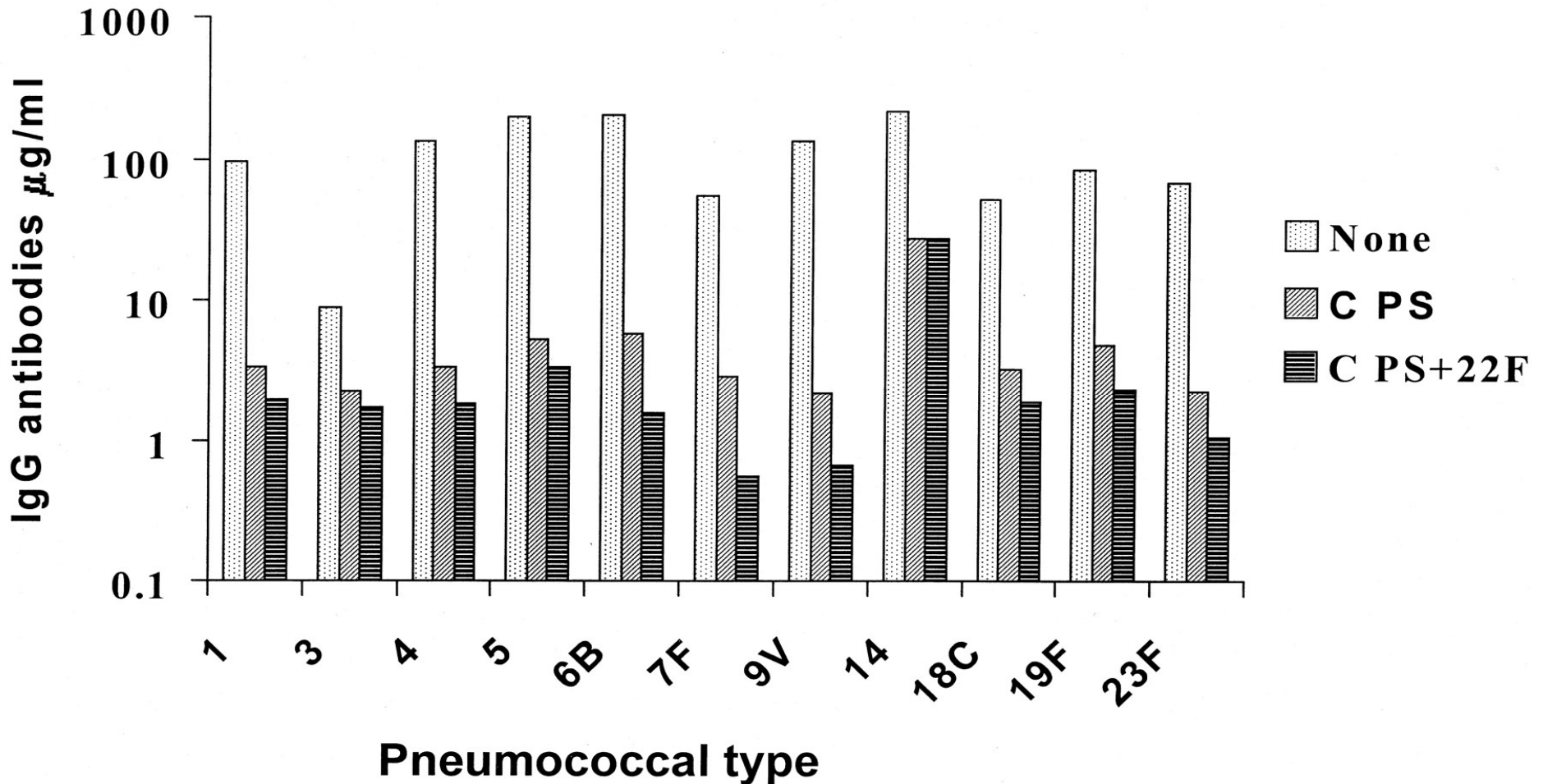
- **The commercial antigens are not pure**
- **To gain type specificity absorptions with cell wall polysaccharide (CPS) and 22F PS needed**
- **The effects of extra steps seem to vary in different labs**
- What to do when 22F is included in the Pnc conjugate vaccine
- Danish approach: CPS2 from 22F strain

Antibody to type 11A



Absorption with CPS or with CPS+22F

Serum from an unimmunized adult

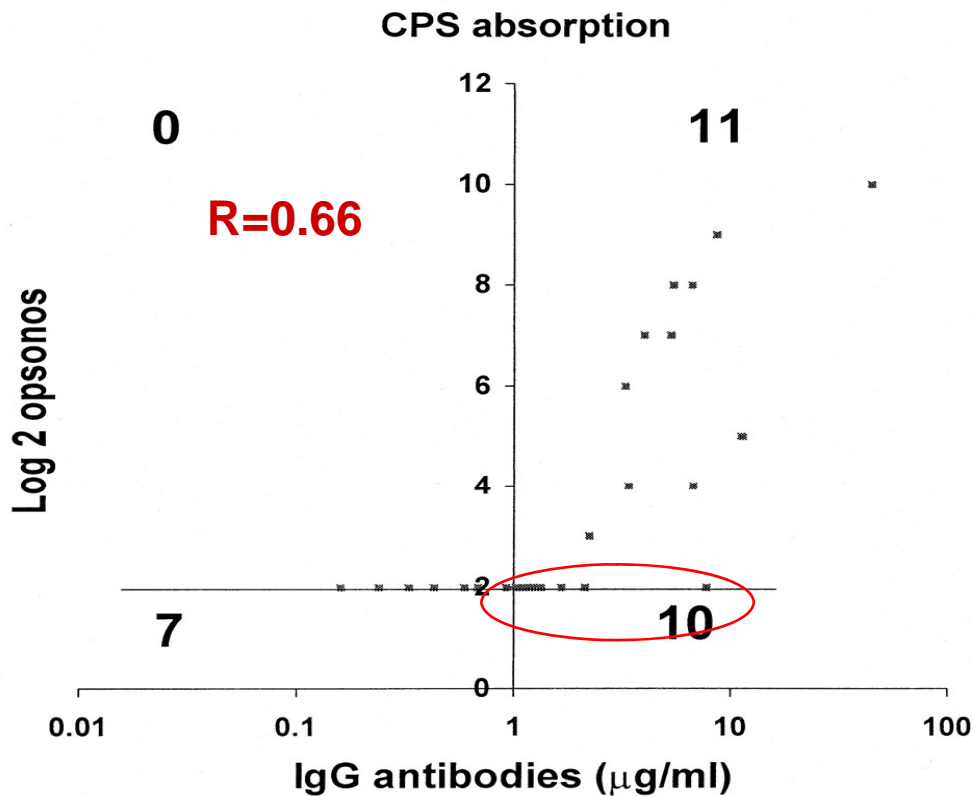


Correlation of EIA and OPA

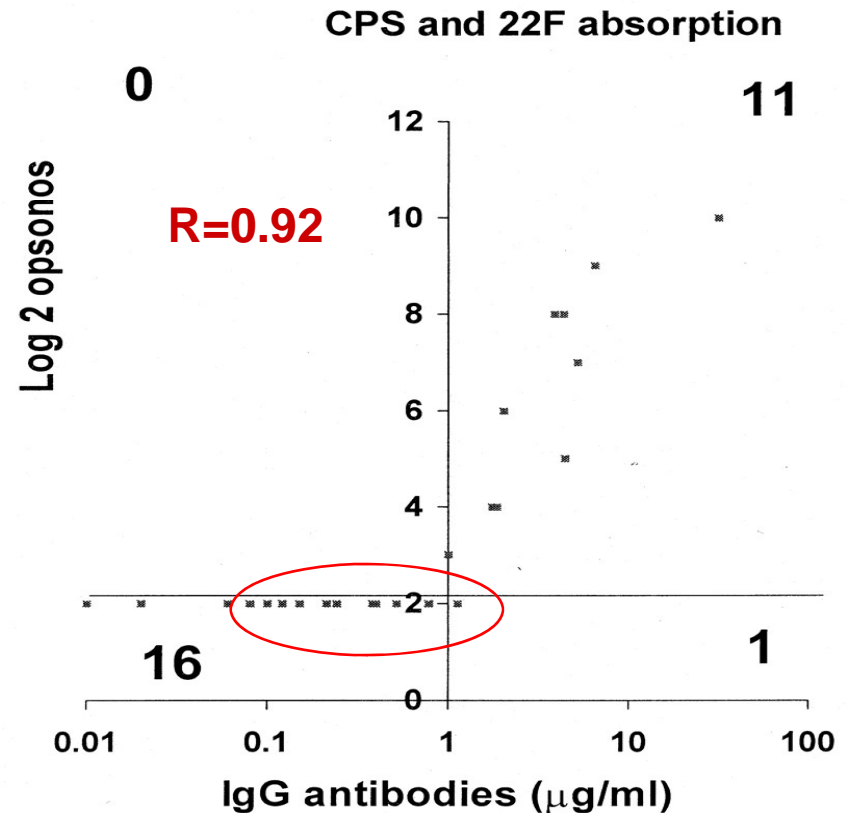


Serum samples from adults, type 4

A



B



- The commercial antigens are not pure
- To gain type specificity absorptions with Cell wall polysaccharide (CPS) and 22F PS needed
- The effects of extra steps seem to vary in different labs
- **What to do when 22F is included in the Pnc conjugate vaccine**
- **Danish approach: CPS2 from 22F strain**

Reagents for EIA available



- Polysaccharides from ATCC
- CPS from SSI, Denmark
- Reference serum 89S from US FDA
- QC sera from NIBSC

Opsonophagocytosis assay for demonstration of functionality of antibodies

- Validated by several groups
- Interlaboratory standardization and harmonization in process
- Strains, effector cells (HL60), detection, calculation of results
- High throughput assays needed

Challenge 3:



- Development of immunoassays that would predict protection against other than invasive disease

- The significance of demonstration of increase in avidity
- Standardization of the assays for measuring avidity
- Reliable and robust multiplexed assays for measuring concentration and functional activity of antibodies.
 - Should be able to be transferred easily to other labs