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

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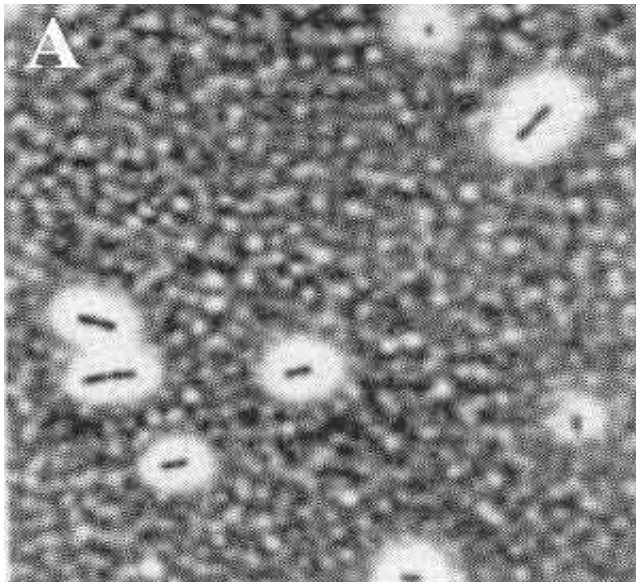
Conjugate vaccines

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

The capsules

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



Functional activity of antibodies

- **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

Antibody concentration

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

Immunological memory

- 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**

Assays need to be

- 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)

WHO Recommendation

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 vaccines[1]

Appendix 1

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

Evaluation criteria

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

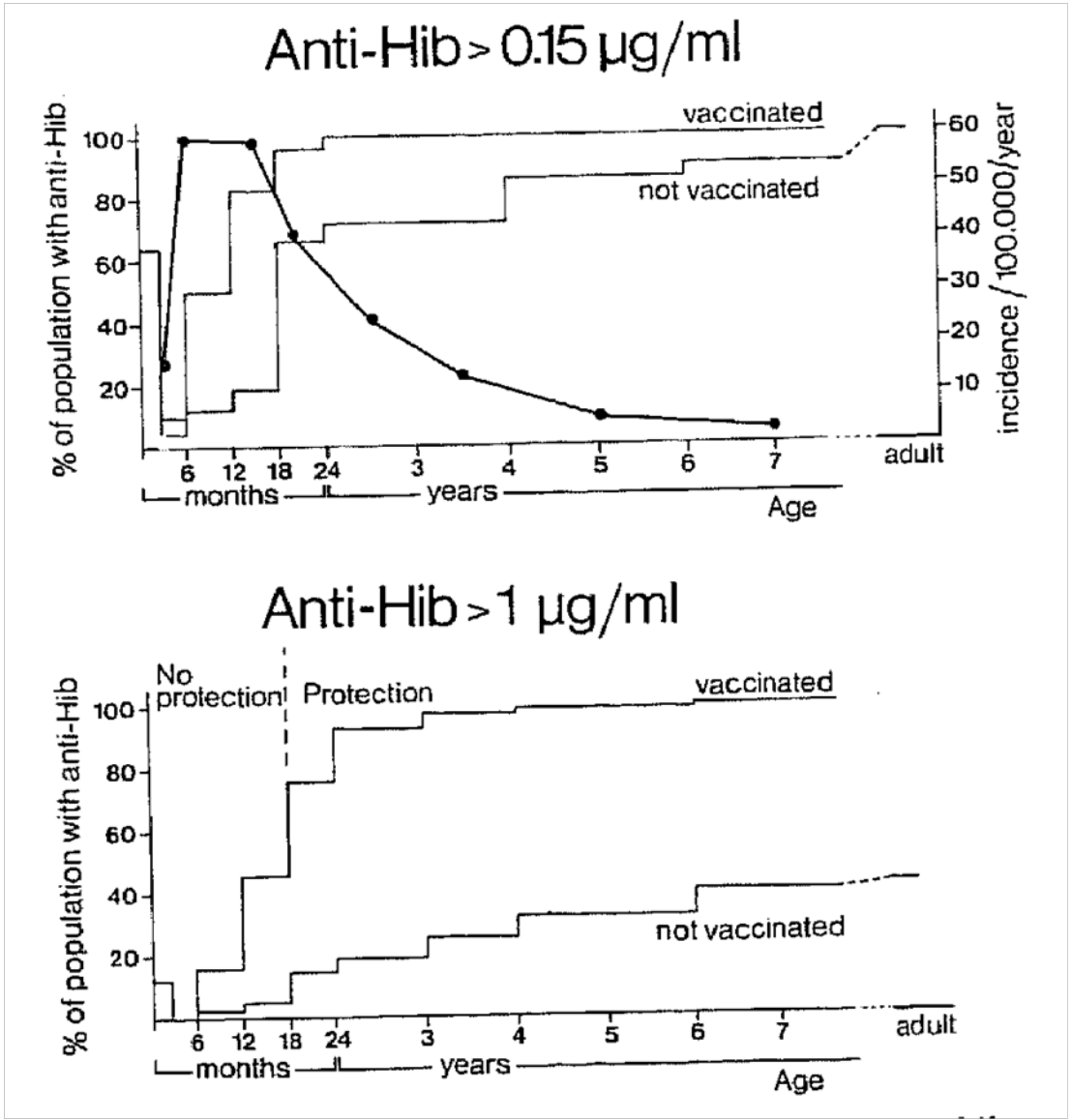
Challenge 1

- 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

Search of correlates for Hib PS vaccines



Käyhty ea, JID 1983

Challenge 1

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Challenge 2.

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**

Useful address

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

Information on hemophilus and pneumococcal assays

Meningococcal conjugate vaccines

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

World Health Organization

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

WHO Expert Committee:

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)

Meningococcal SBA in cases and controls

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

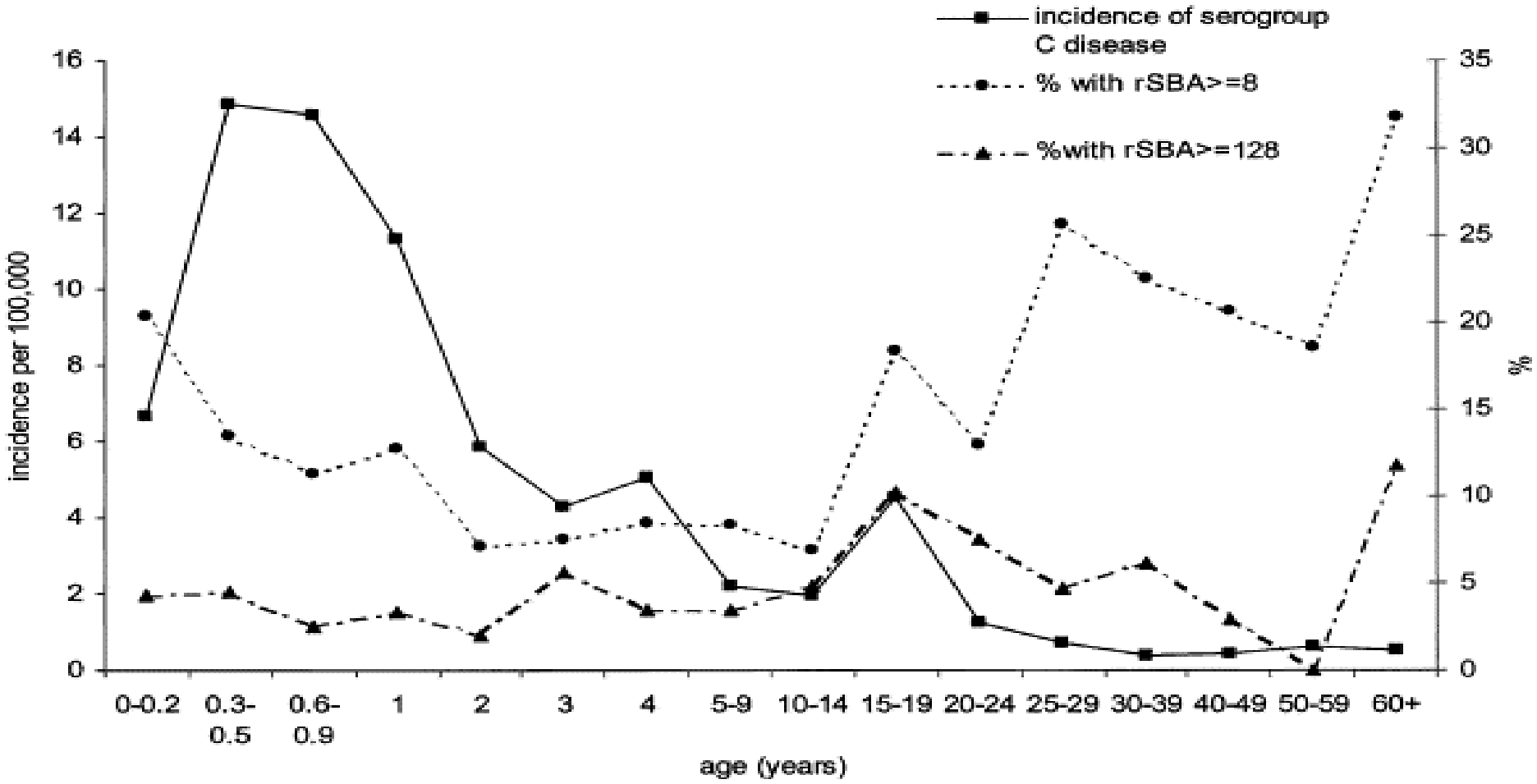
Goldschneider I *et al. J Exp Med* 1969; 129: 1307–26

Challenge 1.

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 ≥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



Trotter et al. Vaccine 2003

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?

Challenge 3:

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 polysaccahride antigens
  - Chaotropic agents (thiocyanate) to detach antibodies of low affinity (assumed to be not protective)

Reagents for EIA available

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 lisenced (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

World Health Organization

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

Pneumococcal conjugate vaccines

- Acceptance of nwe 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 µg/ml of **antibody measured by EIA**
- 0.35 µg/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.

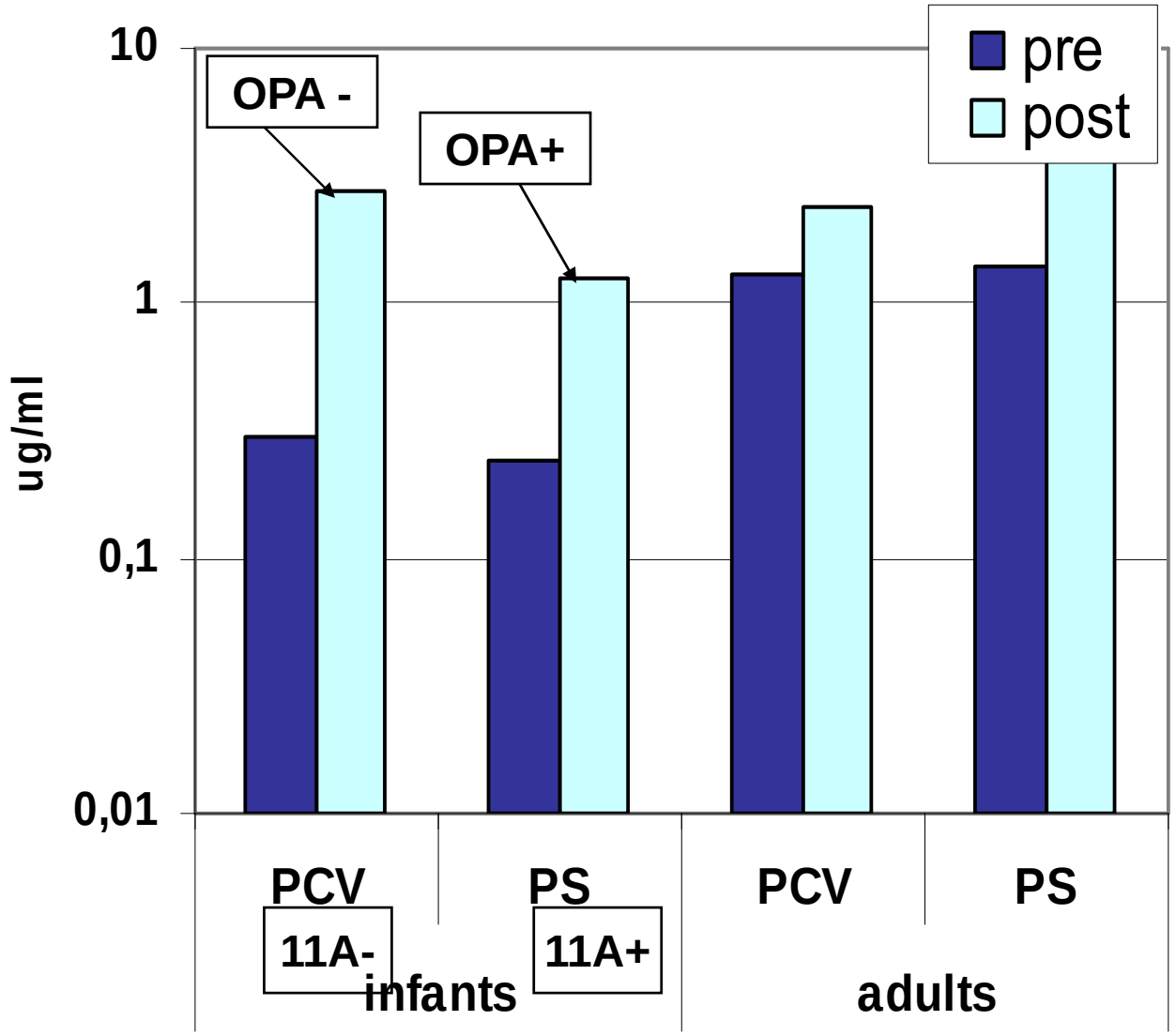
Prepared by the World Health Organization Pneumococcal Serology Reference Laboratories at the Institute of Child Health, University College London, London, England and the Department of Pathology at the University of Alabama at Birmingham, Birmingham Alabama, USA.

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

Problems with EIAs

- **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

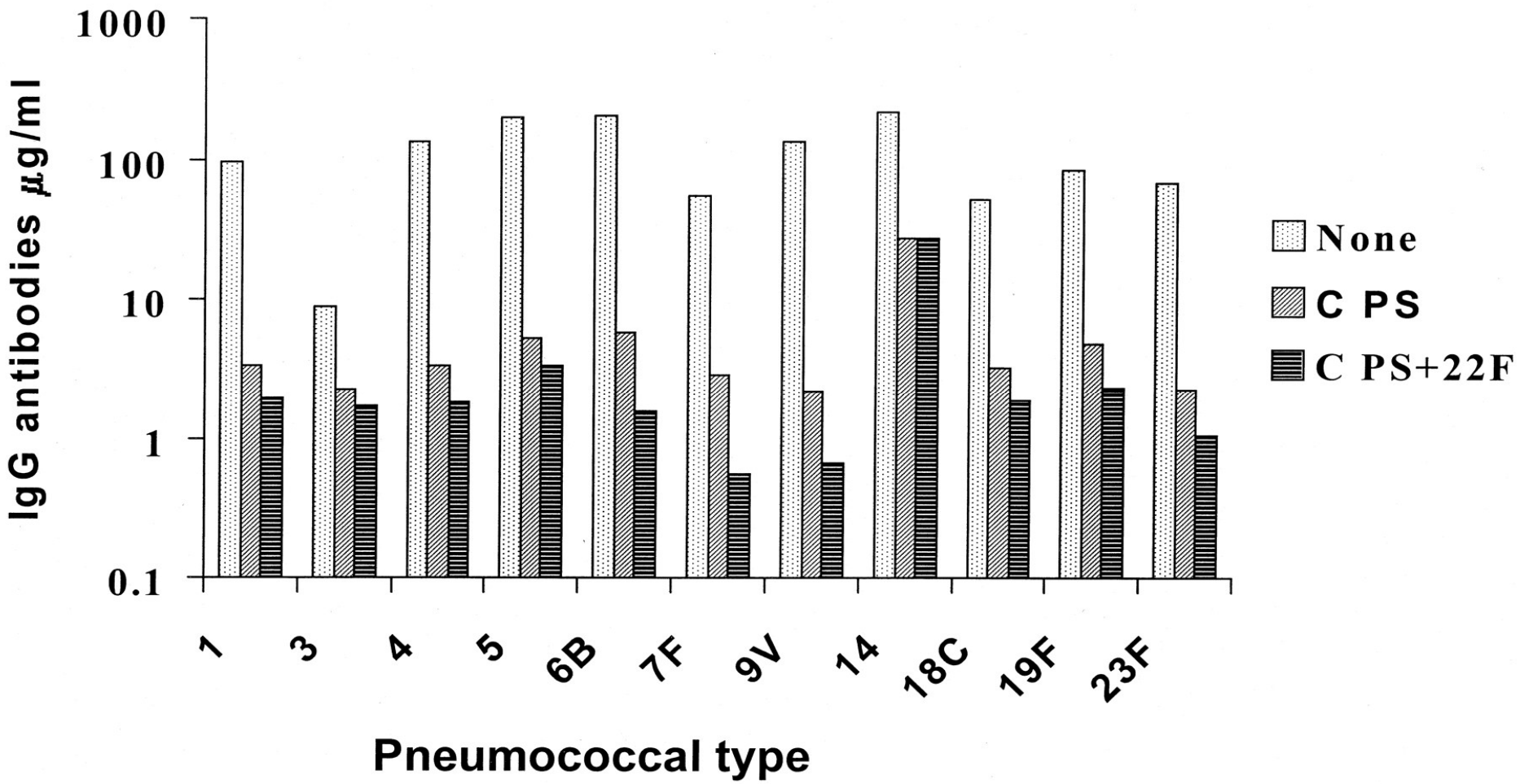
Antibody to type 11A



Soininen et al. Clin Diag Lab Imm 2001

Absorption with CPS or with CPS+22F

Serum from an unimmunized adult

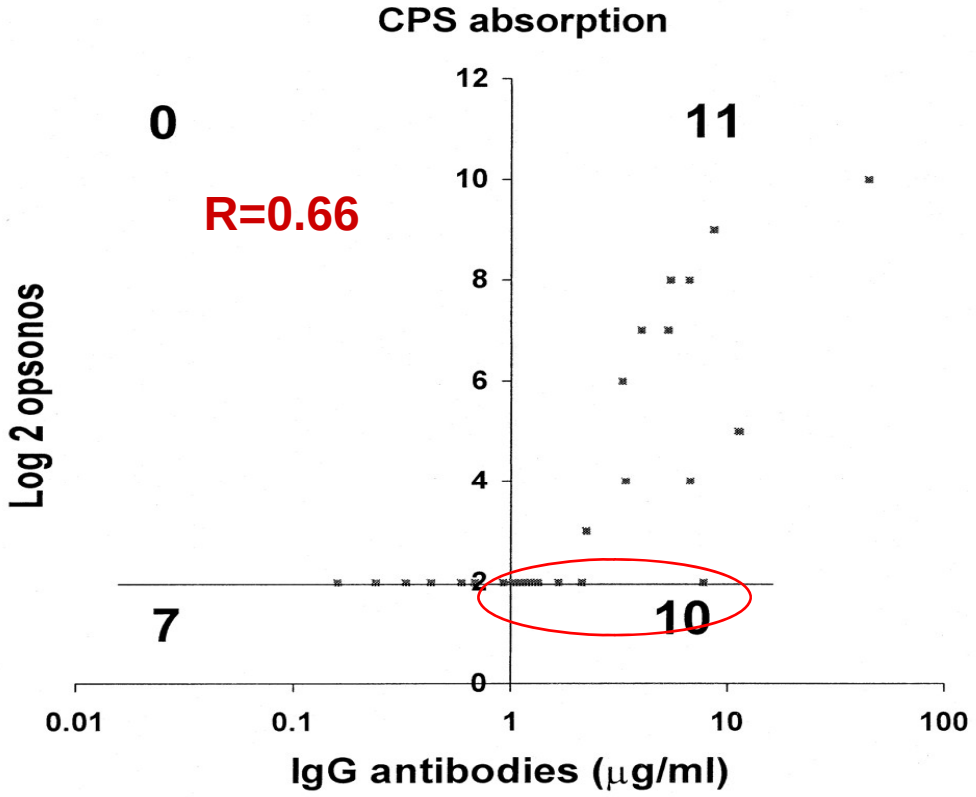


Concepcion & Frasch, Clin Diag Lab Imm 2001

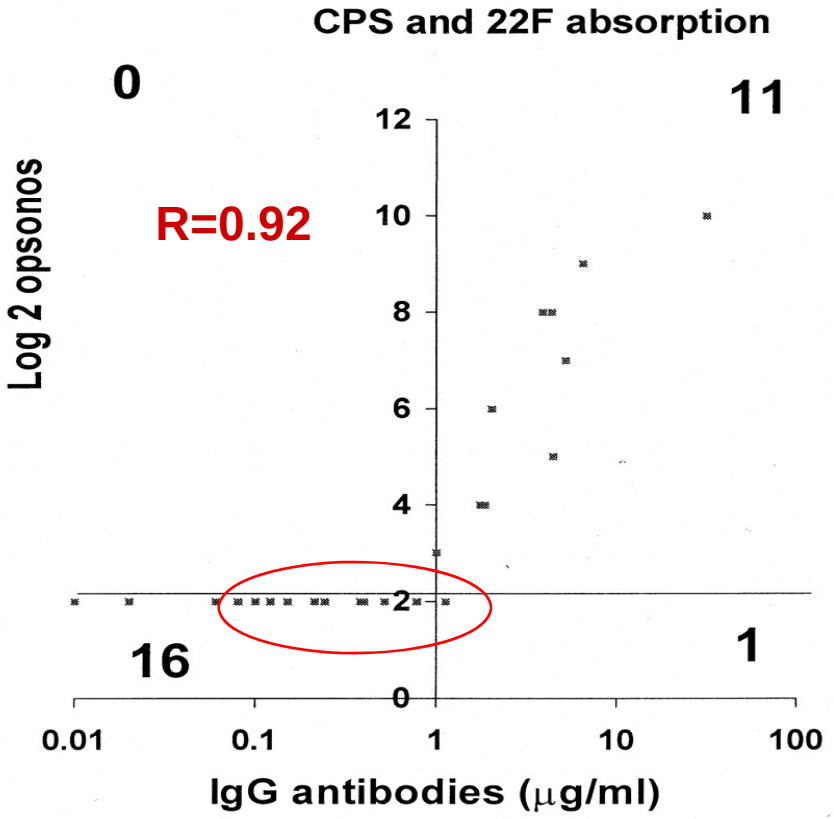
Correlation of EIA and OPA

Serum samples from adults, type 4

A



B



Concepcion & Frasch, Clin Diag Lab Imm 2001

Problems with EIAs

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

Reagents for EIA available

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

Challenge 2:

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

Challenges for all three

- 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 transfered easily to other labs