

Quality Assurance for Pneumococcal Assays in Europe

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- Pneumococcal pathogen
- Pilot pneumococcal serotype study

A European QA Scheme for Pneumococcal Assays

- EU funded Consensus conference (Langen 2006) on Primary Immunodeficiency Diseases (PIDs) outlined:
- A lack of quality assurance for the assays that are used for measuring specific antibody assays to common pathogens and immunization antigens – within PID community!

A European QA Scheme for pneumococcal assays

- These assays are an important aid for the diagnosis of primary antibody deficiencies.
- Provision of standardized assay protocols with a reliable quality assurance scheme will enable progress in determining diagnostic criteria for these diseases.
- For this reason: A study to develop a quality assurance scheme for pneumococcal assays in Europe was initiated.

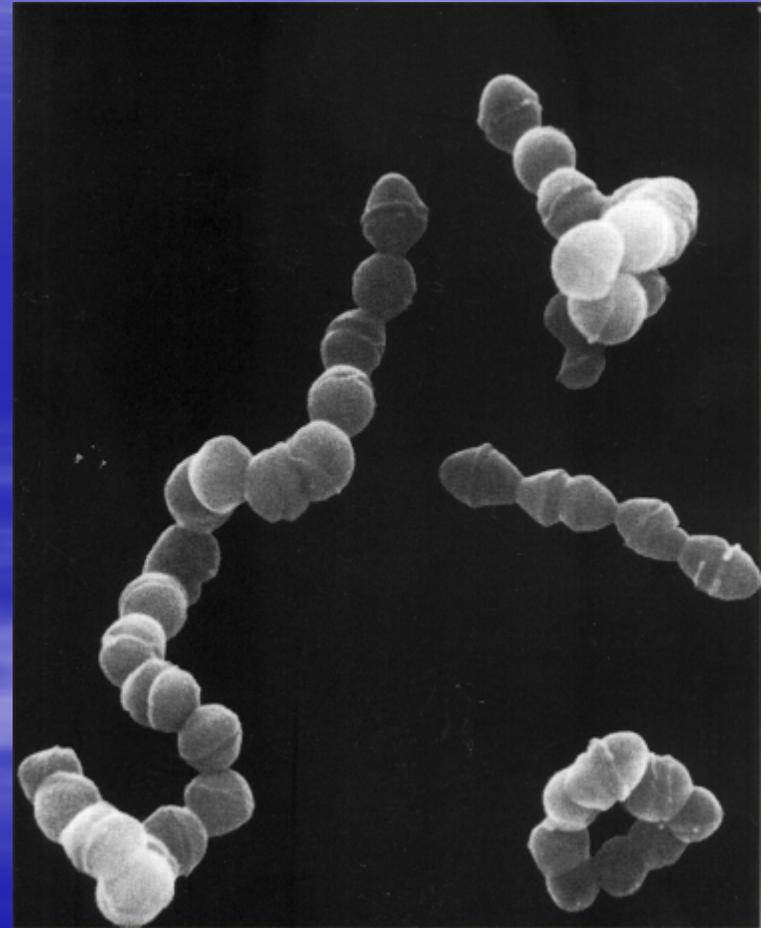
ESID guidelines

- Currently pneumovax unconjugated vaccine is included in the ESID guidelines for PIDs. E. De Vries 2005
- Is used to determine the severity of humoral immunodeficiency in a patient by way of looking at the result from booster vaccinations
- However the authors recognise that the protocols will need to be revised
- Pneumococcal serotype assays could be incorporated

The Pathogen - *Streptococcus pneumoniae*

What Is it?

- Common microflora bacteria that has >90 known serotypes
- Because of this variation it has strong pathogenicity



Streptococcus pneumoniae

What does it cause?

- Causes pneumonia, meningitis and systemic sepsis.
- Prevalent in individuals at the extremes of age
- 1 million deaths are accountable to pneumococcal infections worldwide
- This is mostly in developing countries

PIDs

- Are highly susceptible to infections caused by encapsulated bacteria
- Especially those with X-linked agammaglobulinaemia and common variable immune deficiency

Invasive Pneumococcal Disease

What is it and what are the symptoms?

- The most severe form of pneumococcal infection
- Three major conditions: pneumonia, bacteremia, and meningitis.
- They are all caused by infection with the same bacteria, but have different symptoms.



Vaccines

- 2 available pneumococcal vaccines: pneumococcal polysaccharide vaccine (PPV) or Pneumovax® and the pneumococcal conjugate vaccine (PCV) or Prevenar®
- Pneumovax® contains chains of polysaccharides that make up the surface capsule of the bacteria.
- The 23 types of pneumococci that are included cause 88% of invasive pneumococcal disease.
- This vaccine is not effective in children younger than 2 years in whom 80% of invasive pneumococcal disease in childhood occurs – so it is often given to elderly patients

Vaccines and Serotype Prevalence in USA and Europe

- Prevenar ® includes purified capsular polysaccharide of seven types of the bacteria conjugated to a harmless variety of diphtheria toxin.
- The seven types: 4, 6B, 9V, 14, 18C, 19F and 23F account for 86% of bacteremia, 83% of meningitis, and 65% of acute otitis media among children less than six years of age in the United States.
- These seven serotypes are responsible for 83% of invasive pneumococcal disease in children <4 years old in the US and cause the majority of invasive disease in Europe
- All healthy infants and toddler should receive four doses of Prevenar ® vaccine

Pilot Study

Aims

- To develop a quality assurance scheme for pneumococcal assays in Europe that aid the diagnosis of PIDs.
- To compare pneumococcal serotype results with those from the existing 23 valent Pneumovax ELISA assay, in order to determine the relative usefulness of these assays for test immunisation in the diagnosis of PIDs
- To determine if it is possible to establish levels of protection in patients at high risk of invasive pneumococcal disease

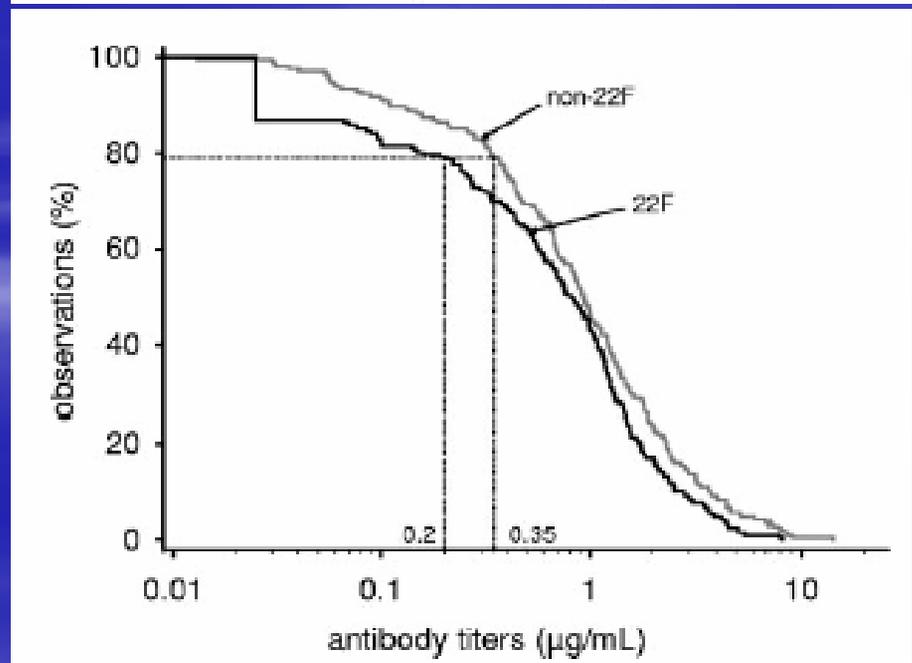
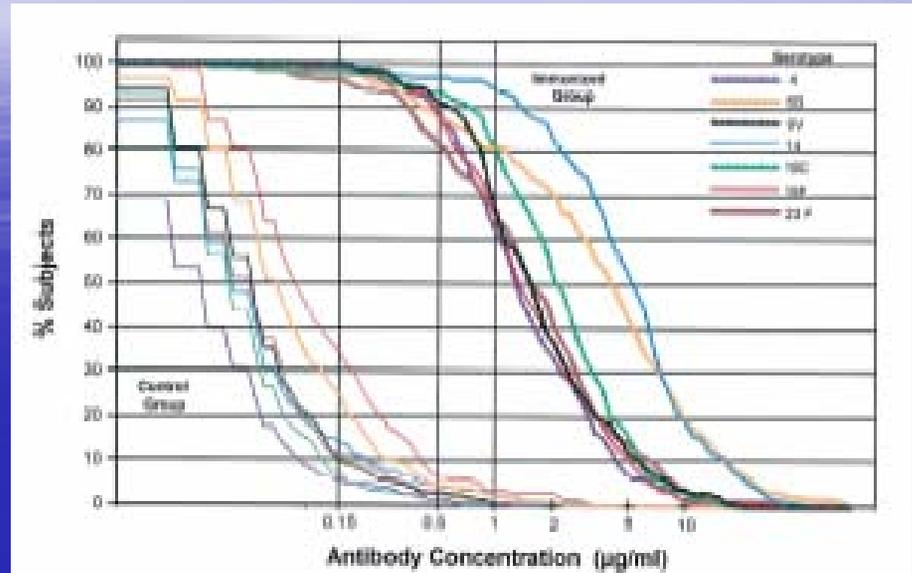
Pilot Study

Methods

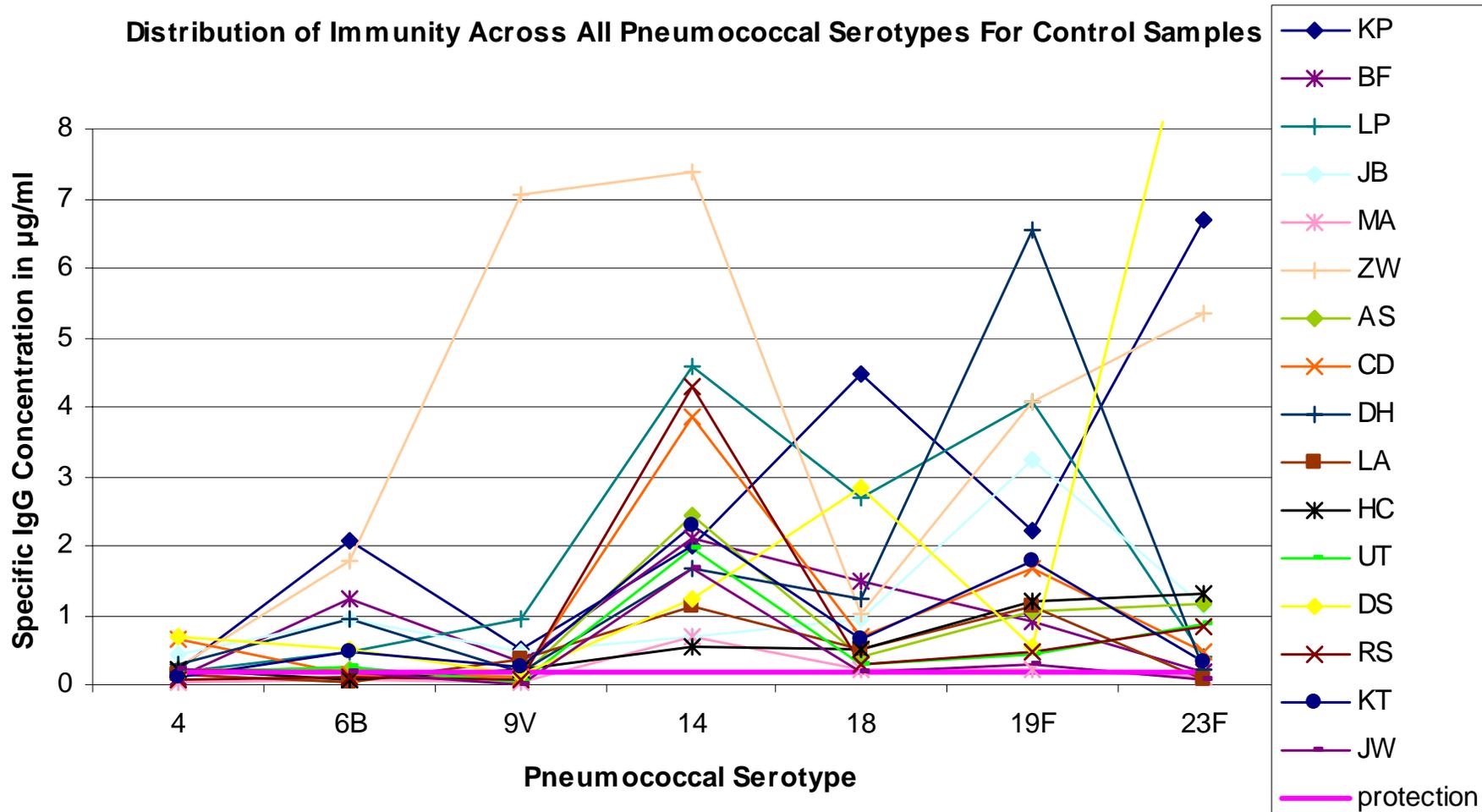
- Development of ELISA assays, in accordance with WHO protocol, for the detection of IgG reactive to 7 common pneumococcal serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) in human serum
- Acquired and tested 16 control serum samples along with 16 known positive pneumovax serum samples
- Concentrations of specific IgG to certain serotypes, within sera, were calculated using the reference serum from WHO (89-SF)

Protection Level

- Needed to have a protection level in order to establish positive and negative results
- Black et al. 2000 found that >95% of patients receiving the pneumococcal conjugate vaccine developed >0.15 $\mu\text{g}/\text{ml}$ after 3rd dose
- Henckaerts et al. 2005 found that the putative protection level was higher (3.5) in the non 22F assay
- Therefore 0.2 $\mu\text{g}/\text{ml}$ was used as a putative protection level



Results



- Wide range between controls, but also between serotypes

Serotype Specific Immunoglobulin G

Serotype								
Control	Pneumovax	4	6B	9V	14	18	19F	23F
Range	4.16-104.29	0.04-0.68	0.06-2.07	0.02-7.07	0.53-7.38	0.18-4.47	0.23-6.53	0.07-10.47
JB	104.29	0.42	0.93	0.46	0.69	0.95	3.23	1.11
HC	30.22	0.22	0.07	0.23	0.53	0.52	1.20	1.31
JW	11.22	0.21	0.17	0.02	1.66	0.18	0.31	0.07
ZW	13.24	0.27	1.77	7.07	7.38	1.02	4.08	5.33

Statistical Correlation with 7 serotypes and Pneumovax Assay: (Kappa Coefficients)

	PNPS	4	6B	9V	14	18C	19F	23F
PNPS	-----							
4	0.24	-----						
6B	0.35	0.10	-----					
9V	0.26	0.02	0.39	-----				
14	N/A	N/A	N/A	N/A	-----			
18C	0.16	-0.12	0.20	0.10	N/A	-----		
19F	N/A	N/A	N/A	N/A	N/A	N/A	-----	
23F	0.60	0.33	0.43	0.18	N/A	0.33	N/A	-----

Pilot study

Next Steps

- Samples with adequate volumes and concentrations will be selected for distribution to 15 centres in Europe
- This is so that our assay can be compared and validated with those of our potential European collaborators

Pilot study

Next Steps

- Will attempt to make a selection of sera including 2 with high concentrations of specific anti-pneumococcal antibodies, 2 with low and 2 with intermediate concentrations
- There are anomalies, but we will inform collaborators of these

Conclusions

- Observed high immunity for serotypes 14 and 18C
- Found that immunity against the serotypes varies between serotypes and individuals
- Immunity against most serotypes generally indicates a high pneumovax result but not always
- Compared serotype findings to results obtained from the existing total Pneumovax ELISA assay and found a correlation with 23F serotype

Acknowledgements

- Dr Helen Chapel
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