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Laboratory diagnosis of specific antibody deficiency to pneumococcal capsular polysaccharide antigens by multiplexed bead assay

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Abstract We evaluated a multiplexed bead-based assay (xMAP® Pneumococcal Immunity assay from Luminex) for the simultaneous determination of antibodies against 14 capsular polysaccharides. Post-vaccination (Pneumovax®) antibody concentrations were measured in 35 healthy children, 40 healthy adults, 99 consecutive patients with increased susceptibility to respiratory infection, and 24 patients with a deficient anti-polysaccharide antibody response. The serotype-specific lower 5th percentile (cutoff) value for the post-immunization antibody concentration was determined in healthy individuals. Eleven of 99 patients (11%) failed to mount a response that was >5th percentile of controls for at least 6 of the 14 serotypes tested, whereas only 3 of 75 controls (4%) failed to do so. All patients with known anti-polysaccharide antibody deficiency failed to mount a response that was >5th percentile of controls for at least 6 of the 14 serotypes tested. The XMAP® pneumococcal immunity panel appears useful for identifying individuals with a low response to the unconjugated pneumococcal vaccine.

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Introduction

Streptococcus pneumoniae (pneumococcus) causes common benign infections of the upper respiratory tract. It also causes lobar pneumonia and, in rare cases, invasive disease, such as septicemia and meningitis. Risk groups for invasive pneumococcal disease include young children, the elderly,

Abbreviation: Caps-PS, capsular polysaccharides

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and patients with immune defects [1,2]. Invasive pneumococcal disease is the highest risk factor, as recurrence occurs in 2–4% of the cases [3].

The immune response to *S. pneumoniae* relies on both innate and adaptive components. Antibody-initiated complement-dependent opsonisation is an important immune mechanism protecting the host against pneumococcal infections [4,5]. The capsular polysaccharides (caps-PS) of *S. pneumoniae* are a major determinant of the virulence of *S. pneumoniae*. High levels of antibodies directed against caps-PS confer clinical protection against invasive infections [6] and caps-PS-based vaccines are efficacious in reducing *S. pneumoniae* infection in the elderly and children after the age of 2. Although the 23-valent Pneumovax®/Pneumo23® vaccine is immunogenic in adults and children, young children under 2 years of age have an impaired antibody response to the non-conjugate vaccine. Children with a persisting defect in the production of antibodies specific for pneumococcal capsular antigens after 2 years of age have “specific antibody deficiency with normal immunoglobulins” [7,8]. These patients suffer from recurrent pneumococcal infections, although their immunoglobulin and immunoglobulin subclass levels and responses to protein antigens are normal [9]. It is estimated that 5% to 10% of the patients with recurrent pneumococcal infections have a defective antibody response to pneumococcal polysaccharides [10].

A number of defects in the Toll like receptor and NF- κ B pathway associated with increased susceptibility to recurrent pneumococcal infection have been described. Mutations in genes encoding the interleukin-1 receptor-associated kinase 4 (IRAK-4), MyD-88, and nuclear factor kappa B essential modulator protein (NEMO) have been associated with recurrent invasive pneumococcal infections and with decreased antibody response to caps-PS [11–15].

The response to pneumococcal polysaccharides is important in the evaluation of patients with documented immune abnormalities and of patients with normal total immunoglobulins [16]. A deficient antibody response to caps-PS is determined by measuring the anti-caps-PS antibody concentrations after vaccination with the 23-valent unconjugated vaccine [10]. Classically, a deficient response to pneumococcal caps-PS is identified by determining antibodies to a panel of pneumococcal serotypes. A standardized enzyme linked immunosorbent procedure (ELISA) to measure anti-pneumococcal antibodies has been developed. This procedure includes absorption of patient serum samples with polysaccharide C and serotype 22F [17]. However, the number of serotypes tested and the criteria used to define a deficient response differ from institution to institution [18–29].

Recently, the xMAP® Pneumococcal Immunity assay became available on the Luminex® system. This assay allows the simultaneous determination of antibodies in serum to 14 different caps-PS serotypes: 1, 3, 4, 6B, 7F, 8, 9N, 9V, 12F, 14, 18C, 19A, 19F, 23F. Serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F are included in the conjugated vaccine Prevnar as well as in the unconjugated vaccine Pneumovax®/Pneumo23®. Serotypes 1, 3, 7F, 8, 9N, 12F, and 19A are included in Pneumovax / Pneumo23 but not in Prevnar. The Luminex® xMAP® Pneumococcal Immunity assay adheres to the international recommendations regarding

preabsorption with polysaccharide C and serotype 22F. The technology is based on the use of color-coded tiny polystyrene microspheres. Each color-coded set of microspheres is coated with a different polysaccharide, allowing the simultaneous capture and detection of different specific antibodies. Lasers excite the internal dyes that identify each microsphere particle. The amount of sample antibodies captured during the assay is assessed. This is the first commercially available assay for measurement of serotype-specific anti-polysaccharide antibodies. In the present paper, we evaluated the Luminex® xMAP® Pneumococcal Immunity panel for the quantification of anti-caps-PS antibodies and the evaluation of the response to pneumococcal polysaccharides.

Methods

Control and patient populations

We measured the anti-caps-PS antibody response in 35 healthy children aged between 3 and 15 years (mean 8 years) and 40 healthy students aged between 19 and 30 years (mean 21 years) (recruited between 2000 and 2005). This control population has been described previously [18].

We measured the anti-caps-PS antibody response in 99 consecutive patients aged between 3 and 80 years (mean age 11 years) who were referred to our institution because of recurrent infections of the upper and/or lower airways (between 2007 and 2008). Recurrent infections of the upper respiratory tract were defined as at least 5 episodes (in a 1-year period) of upper respiratory tract infections complicated by otitis media or chronic (longer than 3 weeks duration) draining ears. Recurrent infection of the lower respiratory tract was defined as at least 3 lower respiratory tract infections in a 1-year period with radiographic evidence of pneumonia in at least 2 of these periods.

A final group of patients consisted of 24 historically selected patients aged between 2 and 26 years (mean 9 years) who had previously been diagnosed with deficient anti-polysaccharide antibody response according to the criteria described by Jeurissen et al. [18]. This included 6 patients with well-defined immune deficits: 3 patients (age: 26, 26, and 13 years old) with a mutation in IRAK-4 and 3 patients (ages 5, 7, 7 years old) with a mutation in NEMO.

In all individuals, antibody response was measured 3 weeks after vaccination with Pneumovax/Pneumo23.

Determination of anti-Caps-PS antibodies

Measurement of serotype specific anti-caps-PS antibodies by ELISA

Anti-caps-PS antibodies to serotypes 3, 4, 9N, 18C and 19F were determined by ELISA according to WHO-guidelines as described by Jeurissen et al. [18].

Measurement of total anti-caps-PS antibodies by ELISA

Anti-caps-PS antibodies to the mix of the 23 serotypes contained in Pneumovax®/Pneumo23® were determined as described by Jeurissen et al. [18] for the determination of serotype-specific antibodies, except that the plates were

coated with Pneumo23®. The incubation of the plates after coating was 1 h at room temperature and overnight at 4 °C.

Measurement of serotype-specific anti-caps-PS antibodies by bead assay

Anti-caps-PS antibodies to serotypes 1, 3, 4, 6B, 7F, 8, 9N, 9V, 12F, 14, 18C, 19A, 19F, 23F were measured by xMAP® Pneumococcal Immunity assay (Luminex®) (Oosterhout, The Netherlands) according to the instructions of the manufacturer. All reagents were stored as prescribed by Luminex® and used at room temperature. Fluid was removed from the 96-well filter plates (millipore) with a vacuum pump system. Microspheres (20 µL/well) were added to pre-wetted (150 µL wash solution per well) 96-well filter plates. Samples and controls were 1:100 diluted in diluent and incubated for 30 min at room temperature before addition to the filter plates (100 µL per well). Standards were not diluted. After 20- to 25-min incubation, wells were washed 3 times (100 µL wash solution/well) and 50 µL of conjugate was added to each well, followed by an incubation step of 20–25 min. Afterwards, plates were washed 3 times (100 µL wash per well) and read on the Luminex200 IS device. Absorbance data were converted to antibody concentrations with Luminex100 IS software (version 1996–2004).

The Danish nomenclature is used for the description of the serotypes.

Statistical methods

Imprecision was calculated as described by Tholen DW et al. [30]. Differences in antibody response between groups were evaluated by Mann–Whitney test or, when indicated, by χ^2 using Analyze It for Excel (version windows 2003).

Results

Linearity of the Luminex assay for determination of anti-caps-PS antibodies

Linearity for the xMAP® Pneumococcal Immunity assay was determined by diluting a serum sample containing a high anti-caps-PS antibody level with increasing amounts (from 0% to 100%) of a serum sample containing low concentrations of anti-caps-PS antibodies. The ratio of the volume of sample containing a high level of anti-caps-PS antibodies to the total volume was plotted against the measured anti-polysaccharide antibody concentration. The results are shown in Fig. 1 for a selection of 4 serotypes. In general, a good linearity was observed with correlation coefficients (linear regression analysis) ranging between 0.92 and 1. The correlation coefficients were 0.99, 0.92, 0.99, 0.99, 0.97, 0.98, 0.95, 1, 0.98, 0.97, 0.99, 0.99, 0.95, and 0.98 for caps-PS 1, 3, 4, 8, 9N, 12F, 14, 19F, 23F, 6B, 7F, 18C, 19A, 9V, respectively. The best linearity was found for serotype 19F and the worst linearity for serotype 3.

Imprecision of the Luminex assay for determination of anti-caps-PS antibodies

Estimates of total imprecision (as CV, $n=7$ runs per serotype, with duplicate determinations per run) of the xMAP® Pneumococcal Immunity panel are summarized in Table 1. The calculations were performed for the control samples supplied with the assay (low, medium, and high antibody concentration). Total imprecision was lowest for the low control sample.

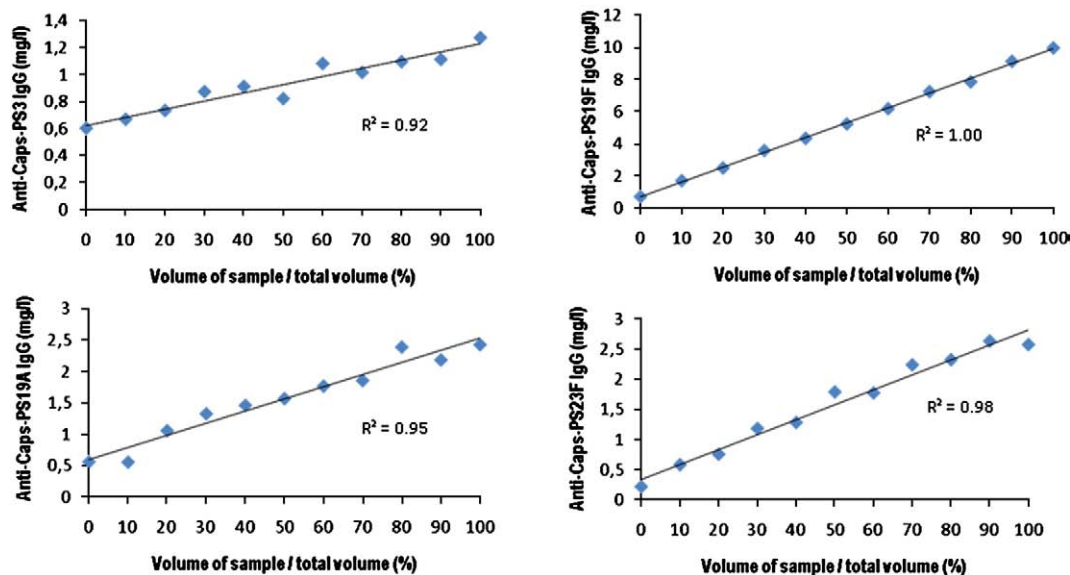


Figure 1 Linearity of xMAP®. Linearity for the xMAP® assay was determined by diluting a serum sample (A, B or C) containing a high anti-polysaccharide antibody level with increasing amounts (from 0% to 100%, in increments of 10%) of a serum sample that contained low concentrations of anti-polysaccharide antibodies. The ratio of the volume of sample containing anti-polysaccharide antibodies to the total volume was plotted against the measured anti-polysaccharide antibody value. The figure shows the results for 4 serotypes.

Table 1 Total imprecision (coefficient of variation [CV]) of xMAP® was assessed by duplicate measurements of 3 control materials (supplied by the manufacturer) in 7 separate runs.

	Low control		Medium control		High control	
	Mean (mg/L)	CVtot (%)	Mean (mg/L)	CVtot (%)	Mean (mg/L)	CVtot (%)
PS1	0.71	4.71	1.15	14.35	2.59	15.76
PS3	0.67	7.74	1.29	14.45	3.16	14.97
PS4	0.42	9.00	0.79	13.90	1.80	10.08
PS6B	1.17	8.68	2.13	11.56	4.58	13.33
PS7F	3.10	10.16	6.67	24.72	19.99	18.32
PS8	1.60	9.45	3.22	23.02	10.45	15.46
PS9N	0.71	4.71	1.36	12.70	3.17	10.83
PS9V	0.63	7.36	1.16	0.20	2.73	17.10
PS12F	2.30	8.75	3.95	20.97	11.63	27.58
PS14	1.38	9.13	2.52	14.11	5.64	10.05
PS18C	0.80	7.33	1.51	17.11	3.91	18.30
PS19A	1.68	9.25	3.02	18.81	8.14	13.90
PS19F	0.86	16.71	1.49	13.17	3.21	12.94
PS23F	0.43	6.79	0.76	14.29	1.59	18.53

Comparison of the Luminex assay with conventional serotype-specific ELISA

The values obtained for the healthy children and adults with the Luminex xMAP® Pneumococcal Immunity assay were compared with the values obtained with conventional ELISA described by Jeurissen et al. [18]. Such comparison was made for 5 serotypes (3, 4, 9N, 18C, and 19F). The results are shown in Fig. 2 and show a correlation coefficient of -0.046,

0.7139, 0.7643, 0.6502 and 0.6127 for serotype 3, 4, 9N, 18C and 19F, respectively. For serotype 3, no correlation was observed (correlation coefficient -0.046).

Post-vaccination antibody concentrations in controls. Cut-off values

Table 2 shows anti-caps-PS antibody concentrations to serotypes PS1, PS3, PS4, PS8, PS9N, PS12F, PS14, PS19F, PS23F, PS6B, PS7F, PS18C, PS19A, PS9V in 35 healthy children and 40 healthy students immunized with Pneumovax®/Pneumo23®. We evaluated age-dependent differences in the ability to mount antibodies against different polysaccharides. In children <16 years of age, no statistically significant difference was observed among age groups (data not shown). The 5th percentile value (cutoff value) obtained with the xMAP® Pneumococcal Immunity assay was comparable to the 5th percentile value obtained with conventional ELISA as described by Jeurissen et al. [18]: 0.64 mg/L vs. 0.36 mg/L, 0.49 mg/L vs. 0.45 mg/L, 0.63 mg/L vs. 0.46 mg/L, 0.34 mg/L vs. 0.31 mg/L and 0.74 mg/L vs. 1.04 mg/L, respectively for serotype 3, 4, 9N, 18C and 19F.

Sixty (80%) of the 75 controls had a value higher than the 5th percentile (cutoff) value for all 14 serotypes. Seventy (93%) had a value higher than the 5th percentile (cutoff) value for at least 11 serotypes. Seventy two controls (96%) had a value higher than the 5th percentile (cutoff) value for at least 9 serotypes. These results are shown in Table 3. A "normal" antibody response was determined to be an antibody level of >5th percentile of controls for 9 or more polysaccharide serotypes. The least immunogenic serotypes were serotype 6B and serotype 23F (data not shown).

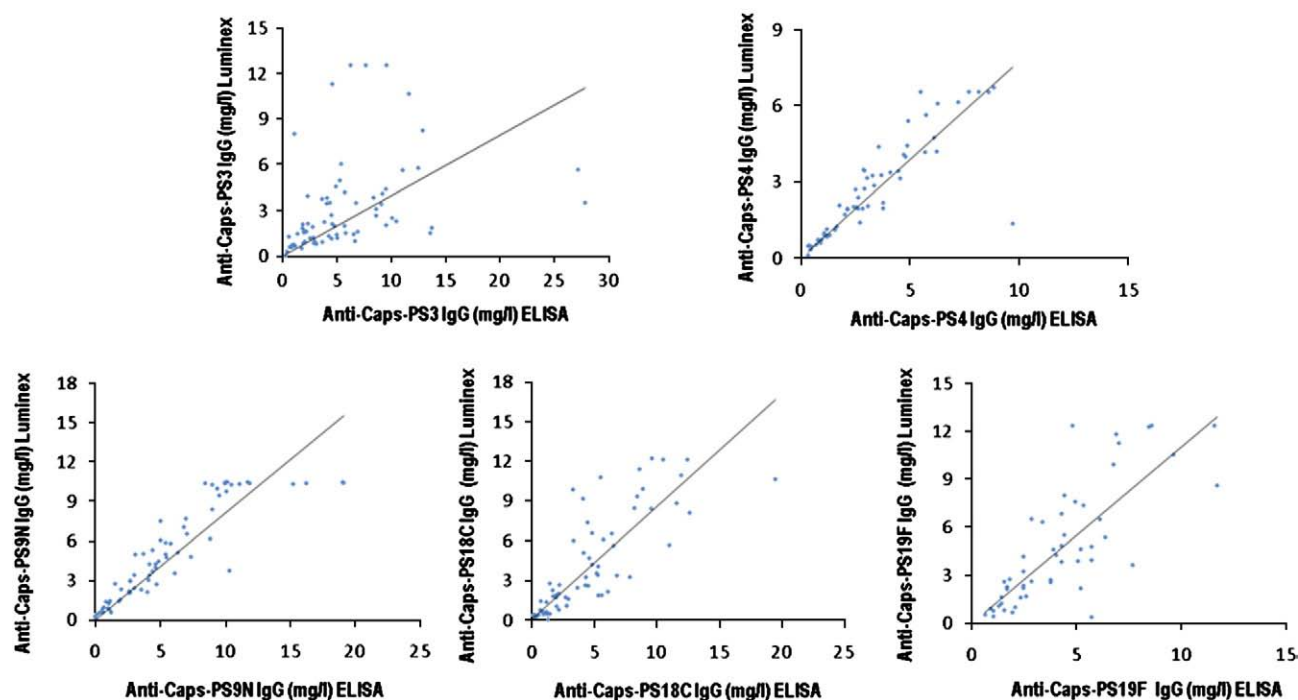


Figure 2 Correlation between xMAP® and serotype-specific ELISA. The samples of the healthy individuals were analyzed by xMAP® (x-axis) and by WHO-based method [18] (y-axis).

Table 2 Median values, 5th and 25th, and 75th percentile for the antibody responses to caps-PS in control individuals.

Controls (n=75)	median (mg/L)	5th percentile (mg/L)	25th–75th percentile (mg/L)
PS1	2.51	0.53	1.34–5.00
PS3	2.07	0.64	1.21–4.06
PS4	3.25	0.49	1.39–6.56
PS6B	3.90	0.80	1.83–10.41
PS7F	6.50	1.45	3.69–14.32
PS8	7.07	1.02	3.14–10.46
PS9N	4.82	0.63	2.27–10.27
PS9V	2.75	0.69	1.43–7.71
PS12F	1.21	0.46	0.70–2.18
PS14	11.34	0.57	2.46–19.76
PS18C	3.29	0.34	1.10–8.68
PS19A	3.76	0.96	2.14–9.15
PS19F	6.52	0.74	2.59–12.40
PS23F	2.48	0.24	0.99–6.31

Thirty-five healthy children and 40 healthy students were immunized with Pneumovax®/Pneumo23®, and antibody titers were measured against 14 serotypes by xMAP®.

Evaluation of the anti-caps PS antibody response in consecutive patients and in selected patients

The anti-caps-PS antibody response after vaccination with Pneumovax in 99 patients in whom measurement of anti-caps-PS antibodies was ordered in the context of evaluation of the humoral immune function is summarized in Table 3. The response to all 14 serotypes tested was >5th percentile

Table 3 Controls, consecutive patients with recurrent respiratory infections, and a group of selected patients with a defective anti-polysaccharide antibody response were vaccinated with Pneumovax.

Number of serotypes. to which a response <5th percentile was generated	Controls (n=75)	Consecutive patients (n=99)	Selected patients (n=24)
14			7
13		1	4
12			4
11	2		1
10		2	3
9		1	1
8		1	3
7	1	2	
6		4	1
5	1	3	
4	1	9	
3	2	11	
2	1	9	
1	7	22	
0	60	34	

Antipolysaccharide antibodies to 14 serotypes were measured by xMAP® 3 weeks after vaccination. The table shows the number of patients and controls with a response <5th percentile for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 serotypes.

of controls in 34 (34%) of the 99 patients, whereas 56 (56%) had a response <5th percentile of controls for at least 1 serotype. Responses <5th percentile of controls for 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 serotypes were seen in 9, 11, 9, 3, 4, 2, 1, 1, 2, 0, 0, 1 and 0 patients, respectively. One patient had a response <5th percentile of controls for 13 of the 14 serotypes tested, a condition not observed in controls. Eleven patients (11%) had a response that was <5th percentile of controls for at least 6 of the 14 serotypes tested, a condition observed in only 3 (4%) of the controls. The ages of these controls were 12, 23 and 22 years of age.

Finally, we evaluated the anti-caps-PS antibody concentration in 24 patients who had previously been diagnosed with a defective anti-polysaccharide antibody response according to the criteria described by Jeurissen et al. [18]. All of these patients had a response that was <5th percentile of controls for at least 6 of the 14 serotypes tested. Fifteen (62%) patients had a response that was <5th percentile of controls for at least 13 of the 14 serotypes tested, a condition not observed in healthy individuals. Seven of these patients had a complete lack of detectable antibodies.

Anti-caps-PS antibodies in patients with mutations in NEMO or IRAK4

Patients with mutations in IRAK-4 and NEMO suffer from invasive pneumococcal disease, and it has been suggested that they have a defect in the anti-polysaccharide antibody response, especially NEMO patients [12]. We evaluated the post-vaccination antibody concentration in 3 patients with a NEMO defect and in 3 patients with a mutation in IRAK-4. Analysis of the anti-caps-PS antibody concentration with the xMAP® Pneumococcal Immunity panel revealed that all three NEMO-patients had a response that was <5th percentile of controls for at least 13 of the 14 serotypes tested. The IRAK-4 patients had response that was <5th percentile of controls for either 6, 8 or 10 serotypes.

Comparison of the Luminex assay with conventional ELISA for anti-caps-PS antibodies to the mix of 23 serotypes

Anti-caps-PS antibodies to the mix of the 23 serotypes contained in Pneumovax®/Pneumo23® were determined in the 75 control individuals. Median values, 5th, 10th, 25th, and 75th percentile for the antibody responses was 115 mg/L, 8.3 mg/L, 18.5 mg/L, 45 mg/L, and 576.5 mg/L, respectively. The antibody response to 14 serotypes quantified by the xMAP® Pneumococcal Immunity assay was compared with the antibody response to the mix of the 23 serotypes measured by ELISA. The comparison was done for 75 controls, 99 consecutive patients and 22 selected patients with a defective anti-polysaccharide antibody response. The results are shown in Fig. 3. The higher the number of serotypes to which the response that was <5th percentile of controls, as measured with the xMAP® Pneumococcal Immunity assay, the lower the antibody level to the mix of the 23 serotypes, as measured by ELISA. The antibody response to the mix of 23 serotypes was lower in the patients with a response that was <5th percentile of controls for at least 12 serotypes than the antibody response in patients with a response that was <5th

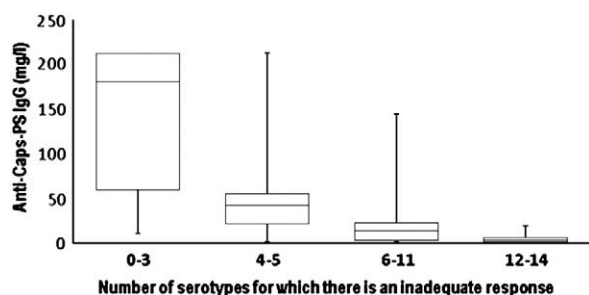


Figure 3 Correlation between xMAP® and ELISA for anti-polysaccharide antibodies. Controls and patients were vaccinated with Pneumovax. Antipolysaccharide antibodies to 14 serotypes by xMAP® were measured 3 weeks after vaccination. The x-axis shows the number of patients and controls with a response <5th percentile for 0–3, 4–5, 6–11, and 12–14 of the 14 serotypes tested. The y-axis shows the values obtained by ELISA that measures antibodies to the mix of 23 serotypes.

percentile of controls for 6–11 serotypes ($p=0.0256$; Mann–Whitney U test). Similarly, the antibody response in patients with a response that was <5th percentile of controls for 6–11 serotypes was significantly lower than the antibody response in patients with a response that was <5th percentile of controls for 4–5 serotypes ($p=0.0064$, Mann–Whitney U test). The antibody response in patients with a response that was <5th percentile of controls for 4–5 serotypes was significantly lower than the antibody response in patients with a response that was <5th percentile of controls for 0–3 serotypes ($p=0.0075$, Mann–Whitney U test).

Thirty five patients had a response that was <5th percentile of controls for at least 6 of the 14 serotypes tested (as determined by xMAP® Pneumococcal Immunity panel). Twenty of these patients had a total antibody concentration to the 23 serotypes that was less than 8.31 mg/L and 4 of these patients had a total antibody concentration to the 23 serotypes that was less than 18.5 mg/L. One patient had a total antibody concentration <8.31 mg/L with a response that was <5th percentile of controls for only 4 serotypes.

Discussion

Multiplex assays are emerging in laboratory medicine. The technology has several applications, such as cytokine quantification, autoantibody quantification, single nucleotide polymorphism genotyping, growth factor quantification, and characterization of the molecular interaction of nuclear receptors [31]. In this study we evaluated the Pneumococcal Immunity panel. The assay simultaneously measures the concentration of 14 serotype-specific anti-caps-PS antibodies. Compared with conventional serotype-specific ELISAs, multiplex assays require significantly less sample volume and are less labor intensive.

The xMAP® pneumococcal immunity assay showed good linearity. There was a good reproducibility, especially for the lower antibody concentrations. Total imprecision for low antibody concentration (values between 5% and 17%) was comparable to the imprecision values obtained with the serotype-specific WHO-based ELISA [18].

The antibody concentrations obtained with XMAP® Pneumococcal Immunity panel were compared with the antibody concentrations obtained with conventional WHO-based ELISA as described by Jeurissen et al. [18]. In general, there was a correlation between the methods, except for serotype 3. Serotype 3 is an immunogenic serotype and it has previously been reported that antibody levels against pneumococcal serotype 3 allows a good differentiation of patients who are able to develop anti-polysaccharide antibodies from those who are not [32]. Jeurissen et al. [18] also reported that an inadequate response to serotype 3 predicts unresponsiveness to other serotypes. The linearity of the Luminex® antibody assay to serotype 3 and the correlation with ELISA are points that could be improved. Such improvement might also improve the ability of anti-PS3 antibodies to predict unresponsiveness to other serotypes.

The serotype-specific cutoff concentrations (5th percentile) determined in our study varied between 0.24 and 1.45 mg/L. These cutoff values corresponded to the cutoff values obtained by ELISA [18]. Protective concentrations of anti-caps-PS antibodies against bacteremia are serotype dependent. An antibody concentration of 0.35–0.4 mg/L is generally considered to be protective [33]. Esposito et al. [25] and Wasserman et al. [10] estimated a protective concentration to be 1.3 mg/L, whereas Epstein [9] and Zora et al. [26] estimated a protective concentration to be 0.3 mg/L. Esposito et al. [25] suggested that IgG anti-capsular antibody concentrations >1 mg/L are required for protection for the majority of the serotypes (particularly serotype 1 and 19), whereas for serotype 4, an IgG antibody concentration of 0.15 mg/L seemed to be protective.

All individuals of our control population had a response that was >5th percentile of controls for at least 7 of the 14 tested serotypes except 1 adult and 1 child who had a response that was >5th percentile of controls for only 3 serotypes. A response that was <5th percentile of controls for 6 or more of the 14 tested serotypes suggests a defective response to pneumococcal polysaccharides. All patients previously identified with a defective response to pneumococcal polysaccharides according to the criteria defined by Jeurissen et al. [18] had a response that was <5th percentile of controls for at least 6 of the 14 serotypes tested. SPAD could be defined as a clinical infectious disease caused by a specific pneumococcal serotype and a concomitant lack or insufficient serum antibody to this specific pneumococcal serotype.

Of the 24 selected patients with a defective anti-polysaccharide response, 3 had a mutation in IRAK-4 and 3 a mutation in NEMO. There was a difference in antibody response to caps-PS between IRAK-4 and NEMO patients. In NEMO patients, the defect was more profound than in IRAK-4 patients, as previously suggested by Ku et al. [12]. All three NEMO-patients tested had a response that was <5th percentile of controls for at least 13 of the 14 serotypes tested, whereas the IRAK-4 patients had a response that was <5th percentile of controls for either 6, 8 or 10 serotypes. In NEMO deficient patients the defect is more downstream than in IRAK-4 deficient patients. IRAK-4 deficient patients have only impairment of the TLRs pathway, whereas NEMO deficient patients may have a secondary impairment of e.g. the CD40-CD40L interaction or the B cell receptor pathway.

Pneumovax[®] is a pneumococcal conjugate vaccine indicated for infants and young children up to 2 years old to help prevent invasive pneumococcal disease. Seven serotypes are contained within Pneumovax[®] (serotypes 4, 6B, 9V, 14, 18C, 19F and 23F). There are seven serotypes to which antibodies are measured by XMAP[®] Pneumococcal Immunity panel that are contained within Pneumovax[®] but not in Pneumovax[®] (serotypes 1, 3, 7F, 8, 9N, 12F, 19A). Measurement of antibodies to these serotypes can be used to evaluate the antibody response to Pneumovax[®]/Pneumovax[®] in children who had previously received Pneumovax[®]. Two healthy controls (3%) had a response that was <5th percentile of controls for 6 of these serotypes, whereas 97% had a response that was <5th percentile of controls for 3 or less serotypes. Nine (9%) consecutive patients had a response that was <5th percentile of controls for at least 4 serotypes. All the selected patients with a defective anti-polysaccharide antibody response had a response that was <5th percentile of controls for at least 3 serotypes, and 17 (71%) had a response that was <5th percentile of controls for 6 or 7 serotypes. Thus XMAP[®] Pneumococcal Immunity panel also allows to evaluate the immune response to the unconjugated vaccine in children who previously had been vaccinated with the 7-valent conjugated vaccine.

In conclusion, the XMAP[®] pneumococcal immunity panel appears useful for identifying individuals with a low response to the unconjugated polysaccharide vaccine.

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