

# Reference ranges and cutoff levels of pneumococcal antibody global serum assays (IgG and IgG<sub>2</sub>) and specific antibodies in healthy children and adults

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**Abstract** Pneumococcal antibodies represent the acquisition of natural immunity. Determination of pneumococcal antibodies is an important screening tool for immunodeficiencies. Our study generated reference ranges and cutoff levels for pneumococcal antibody global serum assays correlated to a specific pneumococcal antibody ELISA. Specific pneumococcal antibody levels were measured from 457 children undergoing elective surgery and 46 healthy adult volunteers (88 with previous pneumococcal immunization from both groups), 22 severe immunodeficient subjects with ataxia telangiectasia (A-T, negative controls), and age-matched 36 healthy allergic asthmatics. We determined a representative panel of serotype-specific pneumococcal antibodies (serotype 4, 5, 6B, 7F, 14, 18C, 19F, 23F) by ELISA and global pneumococcal IgG and IgG<sub>2</sub> antibodies by EIA. In vaccine-naïve healthy subjects, initial pneumococcal IgG geometric mean concentrations of 13.1 µg/ml were low in the first year of life and increased over the time, reaching adult levels (70.5 µg/ml) at age 8–12 years. In parallel, IgG<sub>2</sub> antibodies increased from 20.7 % (0.5–1 year old) to adult proportions (>30 %)

in preschoolers. Correlation between the pneumococcal IgG screening assay and specific pneumococcal antibody levels was acceptable (Pearson's coefficient  $r = 0.4455$ ;  $p = 0.001$ ). Cutoff levels showed high sensitivity, whereas specificity was high to moderate calculated from correlations with the specific ELISA. We provide reference ranges and cutoff levels for the interpretation of specific antibody determinations in the clinical setting. The global pneumococcal IgG/IgG<sub>2</sub> assay is a suitable screening tool and correlates with the ELISA serotype-specific pneumococcal antibodies. However, results below our cutoff values should be re-evaluated by serotype-specific ELISA testing.

**Keywords** Pneumococcal antibodies · Cutoff levels · Immunodeficiency · Reference values · Screening

## Introduction

The determination of specific antibody levels is an important tool to estimate immune competence, as expressed by the European Primary Immunodeficiencies Consensus Conference [1], demanding quality assurance for assays to measure specific antibodies to common pathogens and immunization antigens. Interpretation of the results may be difficult. In order to diagnose primary antibody failure, it is important to show that a patient is unable to produce antibodies to pathogens, using either common exposure antigens or those used for immunization. This includes patients with primary immune deficiency syndromes (PIDSs), who are particularly susceptible to infections caused by encapsulated bacteria. Although patients with congenital immunodeficiencies (e.g., common variable immunodeficiency, selective immunoglobulin G subclass deficiency) typically have low

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levels of serum antibodies, also subjects with normal immune function may have low antibody levels. The physiological delay of the immune response especially to polysaccharide capsular antigens in immunologically immature infants and a decrease in specific antibody titers with time has to be considered when interpreting results [2].

*Streptococcus pneumoniae* is a pathogen that is estimated by the WHO to be responsible for over one million acute deaths worldwide. Thus, routine immunization of infants with pneumococcal conjugate vaccine has been introduced and is recommended in many countries worldwide. Assays for antibody responses to *S. pneumoniae* are of two types: Those that measure total pneumococcal IgG antibodies to the 23 most prevalent pneumococcal serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F (as included in the 23-valent pneumococcal polysaccharide vaccine, PPV-23) by EIA (here called “global pneumococcal assay”), and those that determine serotype-specific antibodies by ELISA. The current European Society for Immunodeficiencies (ESID) guidelines recommend the use of the PPV-23 for diagnosing PIDSs by monitoring IgG responses to booster immunizations [1].

Moreover, routine global pneumococcal EIAs became available that even allow pneumococcal antibodies to be assigned to IgG subclasses. So far, no threshold antibody levels could be established for the pneumococcal global EIA. Existing ELISA reference values also do not consider antibodies directed against the C-polysaccharide, a variant form of the pneumococcal cell wall polysaccharide. Thus, there is a need of reference values controlling for such antibodies, which can be removed by adsorbing sera against pneumococcal serotype 22F polysaccharide. In order to develop quality assurance for pneumococcal assays and to lay the foundation for a comparison of available assays for test immunization in the diagnosis of PIDSs, the present study was designed to establish the pattern of specific antibody responses to pneumococcal polysaccharide antigens in a large cohort of immunologically healthy subjects from Germany. In addition, we correlated threshold levels from the ELISA with corresponding EIA antibody concentrations in order to provide calculated cutoff levels for the interpretation of the EIA results.

## Methods

### Subjects

The study population encompassed clinically healthy children who were admitted to the hospital for minor

surgery or healthy adult volunteers. Only subjects who were free of recurrent infections or chronic disease conditions, as assessed by a standardized questionnaire and a physical examination, and whose C-reactive protein concentrations were within the normal limits were included in the study. As negative controls, we included a group of ataxia telangiectasia (A-T) patients, who are characterized by severe polysaccharide-specific immunodeficiency, and an age-matched group of immunologically healthy allergic asthmatics. Peripheral venous blood was drawn after an overnight fast; sera were separated by centrifugation and then stored in aliquots at  $-20^{\circ}\text{C}$  until use. In addition, previous pneumococcal immunizations were recorded. The study was approved by the Frankfurt University Research Ethics Committee and registered with ClinicalTrials.gov, number NCT00567632. Written informed consent from participants, parents, or guardians was obtained before enrollment.

### ELISA

We determined a representative panel of specific serum anti-pneumococcal antibodies (serotype 4, 5, 6B, 7F, 14, 18C, 19F, 23F) via a modified ELISA technique using Nunc CovaLink<sup>®</sup> microtiter plates (Nunc, Germany) as described [3]. Our choice included six of the seven PCV-7 serotypes (4, 6B, 14, 18C, 19F, 23F), which represent both strong and weak immunogenic serotypes, and are among the most prevalent causing invasive disease. Additionally, we included serotypes 5 and 7F, which are responsible for invasive disease in elderly children and adults. They were meant to show naturally acquired immunity against pneumococci. Serum samples were preincubated with 10  $\mu\text{g}/\text{ml}$  pneumococcal polysaccharide C (CPS, Statens Serum Institut, Denmark) and serotype 22F capsular polysaccharide (a rare serotype, not included in any conjugate vaccine) to neutralize antibody binding to CPS and other common contaminants present in the pneumococcal polysaccharide coating antigens [3, 4]. Reference serum 89-SF (kindly provided by Dr. Frash, Rockville, USA) was used for assay standardization. The minimum pneumococcal antibody detection level was 0.05  $\mu\text{g}/\text{ml}$ ; titers below were arbitrarily assigned to 0.049  $\mu\text{g}/\text{ml}$ .

### EIA

For the measurement of pneumococcal IgG and IgG<sub>2</sub> antibodies, commercially available test kits (Human anti-pneumococcal IgG EIA No. MK012, Human anti-pneumococcal IgG<sub>2</sub> EIA No. MK013; The Binding Site GmbH, Schwetzingen, Germany) with precoated microtiter plates were used according to the manufacturer's instructions [5]. Throughout the procedures pyrogen-free

water was used to avoid non-specific background binding [6]. The antigen used in the assay for pneumococcus-specific antibodies was composed of a mixture of capsular polysaccharide serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F, which is the same composition as that of the licensed 23-valent polysaccharide vaccine. Standard sera were calibrated against human affinity-purified anti-pneumococcal capsular polysaccharide antibodies. Interfering non-specific C-polysaccharide antibodies were absorbed by prediluting the serum (1:100) in a buffer containing pneumococcal cellular wall antigen. After incubation for 30 min and three washing steps, affinity-purified sheep anti-human IgG or anti-human IgG<sub>2</sub> antibody coupled to horseradish peroxidase was added. After a washing step, 100 µl H<sub>2</sub>O<sub>2</sub> and 3,3', 5,5'-tetramethylbenzidine substrate buffer was added to all wells. Measuring range was 3.3–270 mg/l.

#### Statistical analysis—data management and presentation

According to WHO recommendations, a threshold-specific pneumococcal antibody concentration of >0.35 µg/ml was assumed as predictive for seroprotection [7]. Since this level is only valid for invasive pneumococcal infections in young children and after conjugate vaccine immunization, we additionally used serum antibody concentrations of >1.0 µg/ml as estimate for protection from non-invasive (e.g., mucosal) infections [8]. For both limits, the percentage of subjects reaching these concentrations is presented for each age group. Since antibody titers did not conform to a Gaussian distribution, geometric mean antibody concentrations (GMCs) were calculated with 95 % CIs by taking the antilog of the mean of the log concentration or titer transformations. The *U* test (Mann–Whitney) or a multi-dimensional *H* test (Kruskal–Wallis) was performed for between-group comparison. Statistical analysis was conducted with SPSS for Windows (SPSS, Inc., Chicago, IL). For exploratory immunogenicity comparisons, statistical significance was based on the non-overlap of the 95 % CIs. Probability values (*p*) <0.05 were considered as statistically significant. As a negative control, we determined pneumococcal antibodies with the screening EIA in a group of A-T patients, who are definitely immunodeficient [9]. Their geometric mean concentrations can be safely considered as lower limits of the pneumococcal EIA, where no protection can be expected at all. To visualize our results, we also provide a color plot, demonstrating assumed levels of protection via a color scale. Data from immunodeficient A-T patients were illustrated in comparison with a group of age-matched healthy allergic asthmatics.

## Results

### Study population

The study population encompassed healthy adult volunteers (27 females and 19 males; age 18–57 years) or clinically healthy children (178 females and 279 males, age 2 months to 18 years). Among all age groups, a total of 88 individuals had received pneumococcal immunization in advance (see Table 1). As negative controls, we investigated a group of 22 subjects (9 females, 13 male, median age 13.0 years (Q25 11.6, Q75 17.5) with A-T, who are characterized by a polysaccharide-specific immunodeficiency, that is, thus being at a severely increased risk to acquire infections from encapsulated bacteria. The group of age-matched healthy allergic asthmatics encompassed 36 subjects (21 female, 15 male).

### Serotype-specific and global pneumococcal antibody assay

For healthy children and adults, the distribution of IgG anti-PnC and IgG<sub>2</sub> antibody concentrations in relation to age and the putative normal ranges for each of the assays are shown in Tables 2 and 3. In unimmunized subjects, geometric mean concentrations of pneumococcal IgG were low at age 0.5–2 years (age 0.5–1 years: 13.1 µg/ml; age 1–2 years: 13.6 µg/ml) and increased steadily to reach adult levels (70.5 µg/ml) at age 8–12 years (Table 2). These data correspond with a summarized concentration of the eight serotype-specific pneumococcal antibodies of 3.57 µg/ml (age: 0.5–1 year), 5.34 µg/ml (age 1–2 years), and 7.56 µg/ml (age 8–12 years) with a mean percentage of subjects reaching a protection level (>0.35 µg/ml) of 48.8, 60.0, and 80.5 %, respectively. Subjects with previous pneumococcal immunization had comparable antibodies against PCV-7-included serotypes as immunological mature individuals.

**Table 1** Subjects with previous pneumococcal immunization

| Age [years] | <i>N</i> | Gender         | Median age | Vaccine | Time since vaccination [months; median (range)] |
|-------------|----------|----------------|------------|---------|-------------------------------------------------|
| 0 to <1     | 11       | f = 2, m = 9   | 0.7        | PCV-7   | 6 (1–6)                                         |
| 1 to <2     | 30       | f = 10, m = 20 | 1.4        | PCV-7   | 9.5 (1–17)                                      |
| 2 to <3     | 12       | f = 4, m = 8   | 2.5        | PCV-7   | 9.0 (2–15)                                      |
| 3 to <4     | 10       | f = 3, m = 7   | 3.4        | PCV-7   | 11 (2–25)                                       |
| 4 to <8     | 15       | f = 2, m = 13  | 5.6        | PPV-23  | 24 (3–63)                                       |
| ≥8          | 10       | f = 6, m = 4   | 11.8       | PPV-23  | 43 (6–107)                                      |

PCV-7 = 7-valent pneumococcal conjugate vaccine; PPV-23 = 23-valent pneumococcal polysaccharide vaccine

**Table 2** Serum levels [ $\mu\text{g/ml}$ ] of pneumococcal global antibody assays (PnC-IgG, IgG<sub>2</sub>) and serotype-specific antibody concentrations in geometric mean concentrations (5–95 % confidence intervals) in subjects without antecedent pneumococcal immunization (in bold) and percentage of subjects reaching threshold levels

| Age [years] | N   | PnC-IgG                     | PnC-IgG <sub>2</sub>       | PnC-IgG <sub>2</sub> % | $\Sigma$ PnC 4–23 | PnC4                                 | PnC5                                 | PnC6B                                | PnC7F                                | PnC14                                | PnC18C                               | PnC19F                                | PnC23F                               |
|-------------|-----|-----------------------------|----------------------------|------------------------|-------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|--------------------------------------|
| <0.5        | 20  | <b>14.4</b><br>(4.46–24.3)  | <b>4.11</b><br>(0.69–7.53) | <b>28.6</b>            | <b>2.75</b>       | <b>0.20</b><br>(0.10–1.29)<br>25; 15 | <b>0.26</b><br>(0.13–0.38)<br>45; 5  | <b>0.14</b><br>(0.02–0.26)<br>30; 0  | <b>0.30</b><br>(0.16–0.44)<br>50; 5  | <b>0.50</b><br>(0.20–1.66)<br>65; 30 | <b>0.30</b><br>(0.20–0.90)<br>35; 25 | <b>0.82</b><br>(0.20–3.62)<br>65; 35  | <b>0.23</b><br>(0.10–0.53)<br>30; 10 |
| 0.5 to <1.0 | 10  | <b>13.1</b><br>(7.05–19.1)  | <b>2.70</b><br>(0.40–5.00) | <b>20.7</b>            | <b>3.57</b>       | <b>0.27</b><br>(0.20–0.64)<br>40; 20 | <b>0.34</b><br>(0.24–0.43)<br>40; 0  | <b>0.23</b><br>(0.40–1.71)<br>40; 20 | <b>0.31</b><br>(0.17–0.45)<br>40; 0  | <b>0.96</b><br>(0.40–2.00)<br>70; 40 | <b>0.43</b><br>(0.50–1.64)<br>50; 30 | <b>0.76</b><br>(0.40–1.78)<br>80; 30  | <b>0.27</b><br>(0.20–1.56)<br>30; 30 |
| 1.0 to <2.0 | 25  | <b>13.6</b><br>(1.80–31.1)  | <b>2.55</b><br>(0.40–6.10) | <b>18.8</b>            | <b>5.11</b>       | <b>0.61</b><br>(0.20–2.06)<br>48; 28 | <b>0.42</b><br>(0.31–0.52)<br>56; 4  | <b>0.47</b><br>(0.30–1.61)<br>56; 44 | <b>0.45</b><br>(0.05–0.84)<br>68; 4  | <b>1.44</b><br>(0.30–4.01)<br>84; 40 | <b>0.40</b><br>(0.20–1.17)<br>48; 36 | <b>0.89</b><br>(0.30–2.46)<br>68; 44  | <b>0.43</b><br>(0.20–1.47)<br>52; 44 |
| 2.0 to <3.0 | 28  | <b>23.6</b><br>(3.70–53.8)  | <b>6.38</b><br>(1.21–11.5) | <b>27.0</b>            | <b>3.97</b>       | <b>0.30</b><br>(0.01–0.59)<br>33; 22 | <b>0.50</b><br>(0.39–0.61)<br>78; 7  | <b>0.35</b><br>(0.03–0.74)<br>52; 22 | <b>0.51</b><br>(0.31–0.71)<br>70; 4  | <b>1.18</b><br>(0.20–2.16)<br>81; 44 | <b>0.25</b><br>(0.01–0.48)<br>33; 15 | <b>0.64</b><br>(0.07–1.20)<br>67; 30  | <b>0.24</b><br>(0.05–0.42)<br>33; 11 |
| 3.0 to <4.0 | 32  | <b>25.2</b><br>(9.06–41.2)  | <b>7.37</b><br>(2.96–11.8) | <b>29.3</b>            | <b>3.77</b>       | <b>0.23</b><br>(0.10–0.58)<br>22; 9  | <b>0.53</b><br>(0.40–0.67)<br>81; 19 | <b>0.30</b><br>(0.20–0.41)<br>56; 3  | <b>0.49</b><br>(0.35–0.63)<br>72; 9  | <b>0.80</b><br>(0.07–1.52)<br>78; 25 | <b>0.32</b><br>(0.20–0.95)<br>34; 22 | <b>0.77</b><br>(0.29–1.26)<br>90; 35  | <b>0.33</b><br>(0.20–4.11)<br>44; 16 |
| 4.0 to <6.0 | 36  | <b>28.1</b><br>(15.4–40.8)  | <b>8.50</b><br>(5.39–11.6) | <b>30.3</b>            | <b>4.33</b>       | <b>0.30</b><br>(0.11–0.49)<br>38; 9  | <b>0.58</b><br>(0.39–0.77)<br>82; 18 | <b>0.32</b><br>(0.08–0.56)<br>47; 15 | <b>0.55</b><br>(0.39–0.72)<br>82; 18 | <b>0.74</b><br>(0.37–1.10)<br>85; 32 | <b>0.26</b><br>(0.09–0.43)<br>35; 18 | <b>1.21</b><br>(0.21–2.21)<br>85; 50  | <b>0.37</b><br>(0.22–0.53)<br>50; 9  |
| 6.0 to <8.0 | 45  | <b>48.8</b><br>(19.3–78.3)  | <b>17.7</b><br>(9.80–25.5) | <b>33.1</b>            | <b>6.48</b>       | <b>0.33</b><br>(0.11–0.55)<br>47; 11 | <b>0.77</b><br>(0.40–1.15)<br>89; 22 | <b>0.55</b><br>(0.07–1.02)<br>67; 22 | <b>0.68</b><br>(0.20–0.18)<br>80; 20 | <b>1.14</b><br>(0.03–2.25)<br>89; 47 | <b>0.51</b><br>(0.09–0.92)<br>62; 29 | <b>2.02</b><br>(0.77–3.27)<br>96; 69  | <b>0.48</b><br>(0.20–0.76)<br>62; 22 |
| 8 to <12    | 66  | <b>70.5</b><br>(43.7–97.3)  | <b>24.4</b><br>(18.3–30.4) | <b>34.6</b>            | <b>7.56</b>       | <b>0.42</b><br>(0.25–0.58)<br>59; 11 | <b>1.15</b><br>(0.40–1.95)<br>94; 44 | <b>0.65</b><br>(0.38–0.92)<br>79; 32 | <b>0.90</b><br>(0.53–1.27)<br>88; 33 | <b>1.34</b><br>(0.50–3.18)<br>89; 56 | <b>0.64</b><br>(0.30–1.29)<br>71; 24 | <b>1.80</b><br>(0.20–4.58)<br>97; 74  | <b>0.66</b><br>(0.20–1.39)<br>67; 30 |
| 12 to <18   | 107 | <b>68.3</b><br>(50.5–86.2)  | <b>23.6</b><br>(18.1–29.0) | <b>34.5</b>            | <b>8.64</b>       | <b>0.52</b><br>(0.31–0.73)<br>59; 21 | <b>1.08</b><br>(0.63–1.53)<br>94; 42 | <b>0.81</b><br>(0.11–1.52)<br>76; 37 | <b>1.00</b><br>(0.62–1.39)<br>94; 43 | <b>1.11</b><br>(0.35–1.87)<br>89; 48 | <b>0.84</b><br>(0.43–1.25)<br>78; 39 | <b>2.51</b><br>(1.38–3.65)<br>100; 79 | <b>0.77</b><br>(0.33–1.21)<br>75; 39 |
| $\geq 18$   | 46  | <b>70.0</b><br>(39.4–100.5) | <b>25.3</b><br>(14.7–35.9) | <b>36.2</b>            | <b>7.28</b>       | <b>0.32</b><br>(0.20–0.86)<br>51; 11 | <b>0.86</b><br>(0.2–1.52)<br>89; 38  | <b>0.66</b><br>(0.30–1.70)<br>73; 36 | <b>0.83</b><br>(0.30–2.19)<br>89; 24 | <b>1.27</b><br>(0.05–2.50)<br>89; 51 | <b>0.98</b><br>(0.30–2.59)<br>80; 44 | <b>1.55</b><br>(0.30–3.16)<br>93; 60  | <b>0.81</b><br>(0.06–1.57)<br>73; 40 |

PnC-IgG<sub>2</sub> % = pneumococcal IgG<sub>2</sub> percentage of total global pneumococcal IgG>0.35; >1 = percentage of subjects reaching the protective threshold levels of at least 0.35 or 1.0  $\mu\text{g/ml}$  serotype-specific pneumococcal antibodies as determined by ELISA

**Table 3** Serum levels [ $\mu\text{g/ml}$ ] of pneumococcal global antibody assays (PnC-IgG, IgG<sub>2</sub>) and serotype-specific antibody concentrations in geometric mean concentrations (5–95 % confidence intervals) in subjects with any antecedent pneumococcal immunization (in bold) and percentage of subjects reaching threshold levels

| Age [years] | N  | PnC-IgG                      | PnC-IgG <sub>2</sub>       | PnC-IgG <sub>2</sub> % | $\Sigma$ PnC 4–23 | PnC4<br>>0.35; >1                     | PnC5<br>>0.35; >1                     | PnC6B<br>>0.35; >1                    | PnC7F<br>>0.35; >1                    | PnC14<br>>0.35; >1                    | PnC18C<br>>0.35; >1                   | PnC19F<br>>0.35; >1                   | PnC23F<br>>0.35; >1                   |
|-------------|----|------------------------------|----------------------------|------------------------|-------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| 0 to <1     | 11 | <b>30.9</b><br>(19.9–41.9)   | <b>4.51</b><br>(2.48–6.54) | <b>14.6</b>            | <b>10.7</b>       | <b>1.46</b><br>(0.30–2.63)<br>90; 70  | <b>0.40</b><br>(0.13–0.67)<br>60; 30  | <b>0.79</b><br>(0.19–1.40)<br>80; 60  | <b>0.49</b><br>(0.15–0.84)<br>80; 30  | <b>2.99</b><br>(1.82–4.17)<br>90; 90  | <b>1.51</b><br>(0.48–2.54)<br>90; 60  | <b>2.21</b><br>(0.35–4.07)<br>100; 80 | <b>0.80</b><br>(0.50–2.06)<br>70; 30  |
| 1 to <2     | 30 | <b>34.9</b><br>(27.7–42.1)   | <b>5.14</b><br>(3.25–7.02) | <b>14.7</b>            | <b>12.0</b>       | <b>1.56</b><br>(0.82–2.30)<br>97; 60  | <b>0.42</b><br>(0.32–0.53)<br>60; 3   | <b>1.42</b><br>(0.05–2.79)<br>93; 43  | <b>0.41</b><br>(0.29–0.53)<br>67; 3   | <b>4.52</b><br>(0.89–8.16)<br>100; 90 | <b>1.18</b><br>(0.56–1.81)<br>97; 50  | <b>1.19</b><br>(0.76–1.61)<br>97; 57  | <b>1.32</b><br>(0.50–3.12)<br>83; 50  |
| 2 to <3     | 12 | <b>46.8</b><br>(4.99–88.8)   | <b>10.6</b><br>(1.9–24.1)  | <b>22.6</b>            | <b>12.6</b>       | <b>1.46</b><br>(0.40–5.33)<br>92; 42  | <b>0.55</b><br>(0.30–0.80)<br>75; 17  | <b>1.34</b><br>(0.40–5.41)<br>92; 42  | <b>0.59</b><br>(0.28–0.91)<br>83; 25  | <b>3.78</b><br>(1.40–10.0)<br>100; 92 | <b>1.57</b><br>(0.40–4.67)<br>92; 67  | <b>1.82</b><br>(0.92–2.72)<br>100; 75 | <b>1.47</b><br>(0.30–5.53)<br>75; 58  |
| 3–<4        | 10 | <b>66.7</b><br>(27.2–106.2)  | <b>19.7</b><br>(4.26–35.1) | <b>29.5</b>            | <b>14.8</b>       | <b>1.79</b><br>(0.32–3.25)<br>100; 70 | <b>0.63</b><br>(0.35–0.90)<br>90; 10  | <b>1.33</b><br>(0.50–4.80)<br>100; 40 | <b>0.54</b><br>(0.40–0.69)<br>80; 33  | <b>4.26</b><br>(1.20–37.7)<br>100; 90 | <b>1.84</b><br>(0.15–3.52)<br>100; 70 | <b>2.41</b><br>(1.30–10.1)<br>100; 80 | <b>2.03</b><br>(0.46–3.61)<br>100; 70 |
| 4 to <8     | 15 | <b>107.2</b><br>(55.7–158.8) | <b>27.9</b><br>(14.5–41.4) | <b>26.1</b>            | <b>11.1</b>       | <b>1.06</b><br>(0.30–8.43)<br>67; 40  | <b>0.68</b><br>(0.47–0.90)<br>87; 40  | <b>1.11</b><br>(0.20–3.31)<br>87; 53  | <b>0.86</b><br>(0.40–1.87)<br>80; 33  | <b>2.38</b><br>(0.20–15.0)<br>87; 60  | <b>1.58</b><br>(0.30–4.30)<br>80; 60  | <b>2.54</b><br>(0.70–7.48)<br>100; 53 | <b>0.89</b><br>(0.30–2.39)<br>73; 27  |
| $\geq 8$    | 10 | <b>116.6</b><br>(28.2–205.0) | <b>31.1</b><br>(14.3–47.8) | <b>26.6</b>            | <b>19.9</b>       | <b>1.02</b><br>(0.28–1.76)<br>89; 67  | <b>2.45</b><br>(1.20–3.70)<br>100; 89 | <b>2.56</b><br>(1.38–3.74)<br>100; 89 | <b>1.18</b><br>(0.53–1.82)<br>100; 67 | <b>2.89</b><br>(0.12–5.66)<br>100; 89 | <b>1.24</b><br>(0.35–2.13)<br>100; 56 | <b>6.52</b><br>(0.90–17.9)<br>100; 89 | <b>2.02</b><br>(0.38–3.65)<br>100; 56 |

PnC-IgG<sub>2</sub> % = pneumococcal IgG<sub>2</sub> percentage of total global pneumococcal IgG>0.35; >1 = percentage of subjects reaching the protective threshold levels of at least 0.35 or 1.0  $\mu\text{g/ml}$  serotype-specific pneumococcal antibodies as determined by ELISA

As expected, antibodies against serotypes not included in PCV-7 (5, 7F) did not increase after pneumococcal conjugate vaccine immunization, but when our subjects grew older, representing naturally acquired immunity.

Anti-PnC antibodies of the IgG<sub>2</sub> isotype also increased with age. Plateau levels were also reached by 8–12 years of

age (24.4 µg/ml) and maintained thereafter. In young children, only about 20 % of the anti-pneumococcal antibodies belonged to the IgG<sub>2</sub> subclass, whereas thereafter the proportion of IgG<sub>2</sub> anti-pneumococcal antibodies increased steadily, reaching adult proportions (>30 %) beyond age 4. For all specific antibodies measured, there were no differences between male and female participants. As expected, levels of global as well as specific pneumococcal antibodies were consistently higher in immunized young subjects (Table 3).

Comparing both assays, we found an acceptable correlation between the pneumococcal IgG and IgG<sub>2</sub> screening EIA and our specific pneumococcal antibody levels as determined by ELISA (Pearson's coefficient  $r = 0.447$  or  $0.364$ , respectively;  $p = 0.001$  each; Fig. 1).

#### Receiver operating characteristic (ROC)

##### *Subjects with no previous pneumococcal immunization*

In pneumococcal vaccine-naïve subjects, logistic regression revealed an impact of the age of our study population, resulting in three different putatively protective global IgG-thresholds ( $p = 0.002$  and  $0.018$ , respectively). The area under the ROC (AUROC) confirmed these results for non-immunized individuals: AUROC = 84.0 %, 95 % confidence interval (CI) 79.8–88.2. When correlating the estimated threshold geometric mean concentration of >0.35 and >1.0 µg/ml with the pneumococcal IgG<sub>2</sub> levels, logistic regression excluded an impact of the age of our study population ( $p = 0.053$  and  $p = 0.15$ , respectively). The AUROC confirmed these results for non-immunized individuals (83.1 %, 95 % CI 78.8–87.4; Fig. 2a).

##### *Subjects vaccinated against pneumococci*

In subjects with previous pneumococcal immunization, logistic regression revealed that the age of our study

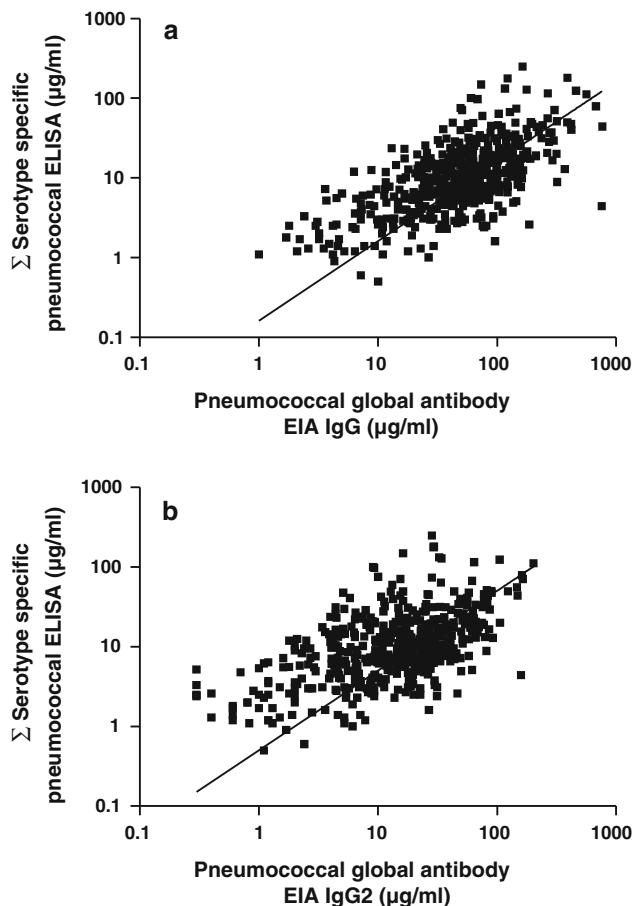


**Fig. 1** Correlation between pneumococcal serotype-specific ELISA and **a** global pneumococcal IgG EIA and **b** global pneumococcal IgG<sub>2</sub> EIA (Pearson's correlation test)

**Fig. 2** Receiver operating characteristic (ROC) of sensitivity versus specificity of a global pneumococcal IgG- and IgG<sub>2</sub> assay **a** for subjects without and **b** with previous pneumococcal immunization. The area under the ROC curve was used as a measure of effectiveness

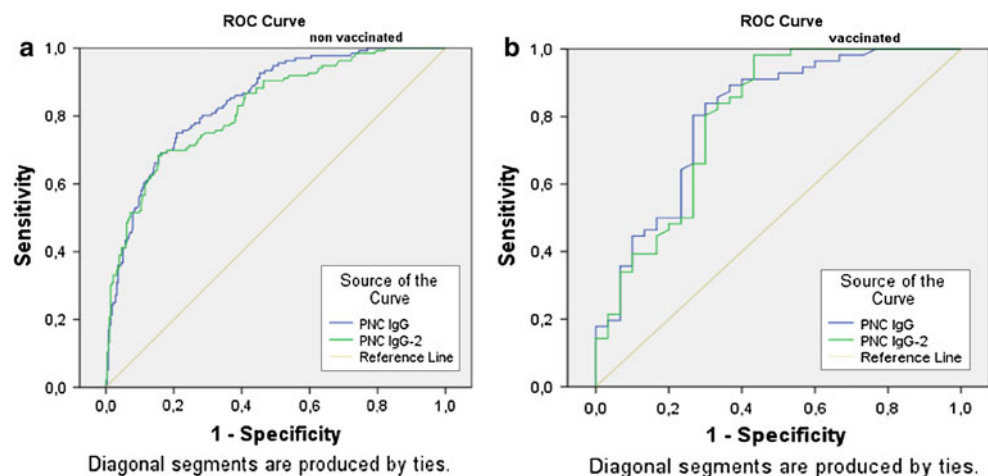
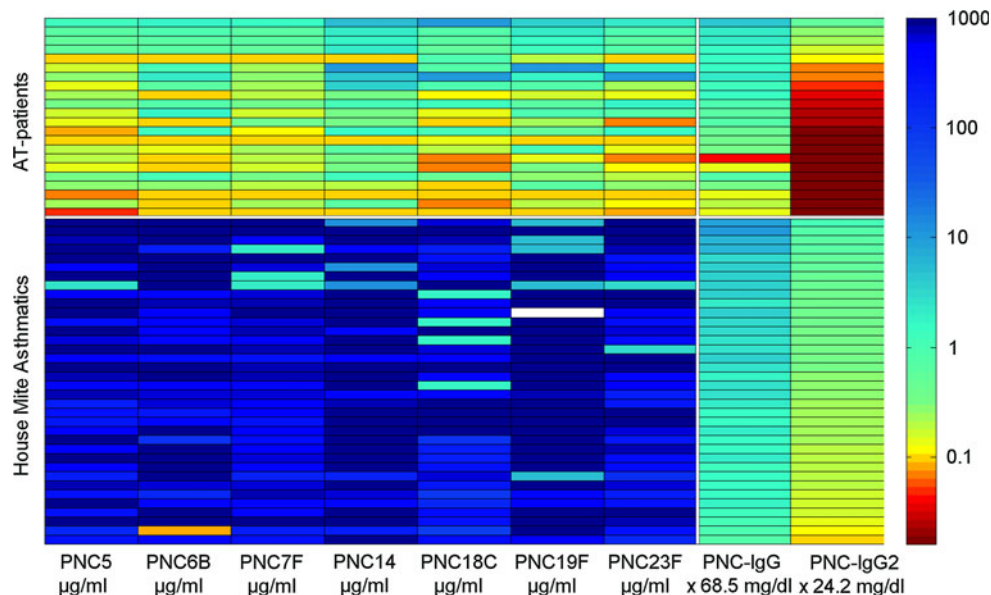


**Table 4** Pneumococcal global assay—cutoff values from correlations with protective ELISA antibody levels

|                    | PnC-IgG [ $\mu\text{g/ml}$ ] cutoff levels (specificity, sensitivity [%]) |                   | PnC-IgG <sub>2</sub> [ $\mu\text{g/ml}$ ] cutoff levels (specificity, sensitivity [%]) |                   |
|--------------------|---------------------------------------------------------------------------|-------------------|----------------------------------------------------------------------------------------|-------------------|
|                    | >0.35                                                                     | >1.0              | >0.35                                                                                  | >1.0              |
| PnC vacc +         | 27.5 (80.5, 75.0)                                                         | 55 (64.3, 76.7)   | 3.0 (89.0, 75.0)                                                                       | 13.9 (13.9, 76.7) |
| PnC vacc –         |                                                                           |                   |                                                                                        |                   |
| Age <2 years       | 8.0 (93.5, 79.1)                                                          | 23.3 (91.7, 76.7) | 2.1 (90.3, 75)                                                                         | 5.5 (75.0, 76.7)  |
| Age 2–7 years      | 42.9 (68.1, 76.5)                                                         | 60.7 (74.3, 75.2) | 14.9 (68.4, 76.5)                                                                      | 22.6 (69.7, 75.7) |
| Age $\geq$ 8 years | 51.5 (51.4, 78.3)                                                         | 68.5 (78.6, 75.6) | 19.5 (65.7, 78.3)                                                                      | 24.2 (80.6, 75.6) |

PnC vacc +(-) = subjects with(-out) antecedent pneumococcal immunization

**Fig. 3** Color plot of specific and global pneumococcal antibody levels in immunodeficient A-T-patients and house dust mite asthmatics. Patterns of single pneumococcal serotype-specific ELISA and pneumococcal global IgG- and IgG<sub>2</sub> EIA values in patients with house mite allergy and A-T. Data are displayed as a two-dimensional pseudo-color representation. The *x*-axis shows the analyzed parameter. The *color bar* along the right *y*-axis shows the antibody concentration increasing from dark red to dark blue



population was of no relevance for a putatively protective global IgG threshold ( $p = 0.92$  and  $0.916$ , respectively). The AUROC confirmed these results for immunized individuals (87.0 %, 95 % CI 67.0–100). When correlating the estimated threshold geometric mean concentration of  $>0.35$  and  $>1.0$   $\mu\text{g/ml}$  with the pneumococcal IgG<sub>2</sub> levels, logistic regression also excluded an impact of the age of our study population ( $p = 0.87$  and  $p = 0.72$ , respectively). The area under the ROC confirmed these results for immunized individuals: AUROC = 87.5 %, 95 % CI 67.4–100 (Fig. 2b).

#### Determination of threshold antibody levels for the global EIA

We correlated the estimated cutoff GMCs for pneumococcal antibody concentrations of  $>0.35$   $\mu\text{g/ml}$  (surrogate for protection from invasive disease) and  $>1.0$   $\mu\text{g/ml}$  (surrogate for protection from non-invasive disease) as determined by ELISA with the pneumococcal IgG and IgG<sub>2</sub> levels as determined by EIA (see Table 4). Cutoff levels for subjects without previous pneumococcal

immunization are shown in Fig. 2a, for individuals with pneumococcal vaccination in Fig. 2b. To evaluate sensitivity and specificity of the determined cutoff levels for clinical practice, we used serum samples from patients suffering from A-T as subjects without protection (negative control) and a group of healthy allergic asthmatics as positive controls. The two-dimensional color plot displayed the patterns of the serotype-specific pneumococcal ELISA (serotype 5-23F) and global IgG and IgG<sub>2</sub> EIA of the two groups (Fig. 3). Color pattern analysis clearly indicated the insufficient immune response against pneumococcal capsular polysaccharide of A-T patients predominantly in the IgG<sub>2</sub> subclass.

An evaluation of the global pneumococcal assay's cutoff values and their individual sensitivity and specificity was done by confusion matrix analysis. In subjects with previous pneumococcal immunization, the global IgG assay performed well with cutoff levels of 27.5  $\mu\text{g/ml}$  (for ELISA values  $\geq 0.35$   $\mu\text{g/ml}$ ) and 55  $\mu\text{g/ml}$  (for ELISA values  $\geq 1$   $\mu\text{g/ml}$ , respectively). In contrast, in the group immunized against pneumococci, results for pneumococcal global IgG-cutoff values varied with age, from 8.0/23.3  $\mu\text{g/ml}$

(for ELISA values  $\geq 0.35$  or  $\geq 1$   $\mu\text{g/ml}$ , respectively) in subjects younger than 2 years, over 42.9/60.7  $\mu\text{g/ml}$  in 2–7 year olds, to 51.5/68.5  $\mu\text{g/ml}$  above the age of 8 years. For detailed specificity and sensitivity results and IgG<sub>2</sub> values see Table 3).

## Discussion

Measurement of specific antibodies is an indispensable part of the immunological assessment of patients with suspected immunodeficiency. The putatively protective concentration of  $>0.35$   $\mu\text{g/ml}$  for pneumococcal anti-capsular antibodies to the seven serotypes in the heptavalent pneumococcal conjugate vaccine (PCV-7) has been recommended by a WHO Working Group as applicable on a global basis for assessing the efficacy of future pneumococcal conjugate vaccines. These principles were applied to an improvement in the ELISA for anti-capsular antibodies, that is, absorption with serotype 22F pneumococcal polysaccharide to neutralize antibody binding to C-polysaccharide and other common contaminants present in the pneumococcal polysaccharide coating antigens, particularly with sera from adults [4]. The present study was designed to establish reference values for this modified ELISA protocol in a large cohort of age-stratified healthy subjects.

The results have to be interpreted with regard to the use of the antigen as vaccines for primary prophylaxis and considering the nature of the immunological response elicited by the antigen. The virulence of *Pneumococcus* spp. is determined by the polysaccharide capsule, impairing opsonization and phagocytosis [10, 11]. The ability to produce antibodies to capsule polysaccharide antigens has been correlated with the appearance of a subset of CD21<sup>+</sup>B lymphocytes in the splenic marginal zone, coming up in the third year of life [12]. This physiological immaturity during infancy is associated with an increased risk of infection with encapsulated bacteria (e.g., pneumococci).

In our study, the very young subgroup of unimmunized infants displayed a low but detectable concentration of anti-pneumococcal polysaccharide antibodies. These antibodies found in unimmunized infants might be explained by persisting maternal antibodies or cross-reactive protective antibodies [13, 14]. However, a value in the normal range does not necessarily prove an effective protection against any pneumococci, of which 94 serotypes have been identified so far. It is also known from vaccination studies that the immune response does not only depend on the individual age and immunity, but even varies between individuals of the same age group [15–17]. Last but not least, pneumococcal antibody assays might also detect non-opsonic antibodies which are directed against the pneumococcal cell

wall. Our results clearly show that even young subjects with previous pneumococcal immunization had comparable antibodies against PCV-7-included serotypes as immunological mature individuals, underlining the importance of pneumococcal conjugate vaccine immunization.

For clinical routine, a standardized, validated, reproducible, and preferably inexpensive assay is desired which can be conveniently applied to large numbers of patients. Such a screening assay exists in form of a global pneumococcal EIA, which detects the 23 most prevalent serotypes of pneumococcal capsular polysaccharides, reflecting the natural acquisition of anti-pneumococcal immunity. However, so far, no threshold levels could be established for this assay. Our work presents cutoff levels for this universal pneumococcal antibody EIA as calculated from the assumed protective antibody levels determined by the serotype-specific pneumococcal antibody ELISA, which are directly linked to clinical protection observed in young children via controlled efficacy trials [7]. In determining such cutoff values, it is important to avoid choosing levels that are either too high or too low. If the assumed level is too high, an unnecessary amount of patients might undergo additional testing, resulting in costs and waste of medical resources. If the estimated threshold is too low, individuals at risk for severe infections might not be detected by the assay. In order to transfer results from one laboratory method to another (“bridging”), it is of critical importance that the study population for bridging should be the same or equivalent to the one in which clinical efficacy was demonstrated. We fulfilled this criterion by investigating the same study population with the EIA and the ELISA in parallel.

One must keep in mind that the assumed threshold concentration of  $>0.35$   $\mu\text{g/ml}$  applies only to the prevention of invasive pneumococcal disease (IPD) in young children and after complete pneumococcal conjugate vaccine immunization. Circulating anti-capsular antibody concentrations required in immunocompromised conditions or for prevention of pneumonia, otitis media, or colonization are likely to be higher [18–21] and have not been established. In a particular individual, the outcome of an encounter with the pneumococcus may vary depending on multiple host and pathogen factors, in addition to the circulating antibody level. In order to be on the safe side, we calculated EIA-threshold concentrations correlating with ELISA antibody concentrations of  $>0.35$   $\mu\text{g/ml}$  and of  $>1.0$   $\mu\text{g/ml}$  (which is an even more conservative value and can be considered as correlate for a protection also from non-invasive disease such as otitis media). As a negative control, we determined pneumococcal antibodies with the screening EIA in a group of AT -patients, who are definitely immunodeficient. Their values can be safely considered as lower limits of the pneumococcal EIA, where no

protection can be expected at all, and our color plot nicely demonstrates their good differentiation from immunological healthy individuals such as allergic asthmatics.

Potential functional differences between the subclasses of IgG antibodies have renewed interest in determining the subclass distribution of IgG antibodies to pneumococcal polysaccharide antigens in adults and children. Pneumococcal conjugate vaccines induce primarily T cell-dependent IgG<sub>1</sub> antibody responses in infants, while pneumococcal polysaccharide vaccines induce predominantly IgG<sub>2</sub> responses. This distribution is also reflected by our data, with IgG<sub>2</sub> being relatively low in subjects with previous pneumococcal immunization, which was mainly conjugate vaccine, while total pneumococcal IgG was constantly higher in vaccinated individuals.

The acquisition of natural immunity is expressed by pneumococcal IgG<sub>2</sub> antibodies. Our reference values confirm the observation that natural IgG pneumococcal capsular polysaccharide-specific antibody levels show a slight decline during the first year of life, presumably due to the loss of maternal antibodies. They remained low until 3–4 years and reach adult levels at age eight to twelve. There is a controversy about the natural course of pneumococcal IgG<sub>2</sub> antibodies. In line with other published data, in our study, the proportion of IgG<sub>2</sub> anti-pneumococcal antibodies increased from 15 to 20 % in young infants to adult levels (>30 %) beyond age 4. Specific pneumococcal antibodies as determined by ELISA demonstrated clearly higher levels in immunized young subjects, where protection from invasive disease due to capsulated bacteria is critical.

The ability to quantitate IgG<sub>1</sub> and IgG<sub>2</sub> subclasses is informative in understanding and evaluating the immune response(s) to *S. pneumoniae* polysaccharide conjugate and non-conjugate vaccines. When comparing the immune serologic status in adult and pediatric sera obtained from individuals following pneumococcal conjugate vaccine immunization, a predominance of IgG<sub>1</sub> can be found, while in adults IgG<sub>2</sub> antibodies predominate. Soininen and coworkers have shown that adults have preexisting serotype-specific IgG<sub>2</sub> antibody, due to prior natural exposure to pneumococci, and that both IgG<sub>1</sub> and IgG<sub>2</sub> responses may follow pneumococcal vaccine immunization [22, 23]. This goes with our observation of an increase in pneumococcal IgG<sub>2</sub> with age also in unimmunized subjects. Different vaccine formulations may elicit preferentially different subclass responses, or responses of different magnitudes.

In naive immune systems, such as in infants, IgG<sub>1</sub> antibodies are elicited by effective pneumococcal conjugate vaccines, as expected for T cell-dependent antigens. The ability to measure and characterize the anti-pneumococcal antibody response in all age groups and in

immunocompromised individuals provides a useful tool to assess immune responses relative to protection from invasive pneumococcal disease. Akikusa and Kemp found that a low response to serotype 3, post-PPV-23 vaccination, in combination with a low titer to serotype 4/6B, was suggestive of a severe immune deficiency state in children being investigated following recurrent upper or lower respiratory tract infections. Thus, a low IgG response to serotype 3 significantly correlated with pneumonia [10]. In children with recurrent respiratory infections, an unsatisfactory IgG response to more than 50 % of 10 pneumococcal serotypes (1, 3, 4, 5, 6B, 7F, 9V, 14, 15B, 18C, 19F, 23F) following PPV-23 vaccination defined polysaccharide-specific immunodeficiency (PSI). Especially, a failure to respond to serotypes 4, 9V, 15B, or 23F significantly increased the risk of PSI, suggesting that these serotypes might be favorable to assess specific antibody defects [23].

Our calculated EIA screening reference values and ELISA normal ranges allow identifying individuals who have very low levels for further study. In patients with recurrent airway infections, the global pneumococcal EIA is a valid instrument in search of immunodeficiencies. Results below our calculated cutoff values should be re-evaluated by pneumococcal serotype-specific ELISA testing. In case of this confirmation diagnostics being positive, pneumococcal conjugate immunization should be considered irrespective of the patient's age [24–27]. The acceptable correlation between the global EIA and the serotype-specific ELISA underlines the value of the global pneumococcal EIA as a screening instrument in search of immunodeficiency. With new and even broader pneumococcal conjugate vaccines being introduced in the future, the value of these tests especially compared to functional opsonophagocytosis testing will have to be re-evaluated.

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## References

1. de Vries E (2005) Patient-centred screening for primary immunodeficiency: a multi-stage diagnostic protocol designed for non-immunologists. Clin Exp Immunol 145:204–214
2. Schauer U, Stemberg F, Rieger CHL, Büttner W, Borte M, Schubert S et al (2003) Levels of antibodies specific to tetanus toxoid, Haemophilus influenzae type B, and pneumococcal capsular polysaccharide in healthy children and adults. Clin Diagn Lab Immunol 10(2):202–207

3. Zielen S, Bröker M, Strnad N, Schwenen L, Schön P, Gottwald G, Hofmann D (1996) Simple determination of polysaccharide specific antibodies by means of chemically modified ELISA-Plates. *J Immunol Methods* 193(1):1–7
4. Concepcion NF, Frasch CE (2001) Pneumococcal type 22F polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide enzyme-linked immunosorbent assay. *Clin Diagn Lab Immunol* 8:266–272
5. The Binding Site. Human IgG subclass liquid reagent kits, U.K., insert code SIN 027; 2004: 17
6. Madore DV, Quataert SA, Mariani M, Berbers GAM (1999) Interlaboratory reproducibility of an enzyme-linked immunosorbent assay for quantitation of antibodies for Haemophilus influenzae type b polysaccharide. *Clin Diagn Lab Immunol* 6:446
7. Siber GR, Chang I, Baker S, Fernsten P, O'Brien KL, Santosham M, Klugman KP et al (2007) Estimating the protective concentration of anti-pneumococcal capsular polysaccharide antibodies. *Vaccine* 25(19):3816–3826
8. Sanders EAM, Rijkers GT, Kuis W et al (1993) Defective anti-pneumococcal polysaccharide antibody response in children with recurrent respiratory tract infections. *J Allergy Clin Immunol* 91:110–119
9. Schubert R, Reichenbach J, Rose MA, Zielen S (2004) Immunogenicity of the 7-valent pneumococcal conjugate vaccine in patients with ataxia telangiectasia. *Pediatr Infect Dis J* 23(3):269–270
10. Akikusa JD, Kemp AS (2001) Clinical correlates of response to pneumococcal immunization. *J Paediatr Child Health* 37:382–387
11. Ochsenbein AF, Pinschewer DD, Sierro S, Horvath E, Hengartner H, Zinkernagel RM (2000) Protective long-term antibody memory by antigen-driven and T help-dependent differentiation of long-lived memory B cells to short-lived plasma cells independent of secondary lymphoid organs. *Proc Natl Acad Sci USA* 97:13263–13268
12. Peset Llopis MJ, Harms G, Hardonk MJ, Timens W (1996) Human immune response to pneumococcal polysaccharides: complement-mediated localization preferentially on CD21-positive splenic marginal zone B cells and follicular dendritic cells. *J Allergy Clin Immunol* 97:1015–1024
13. Sorensen RU, Leiva LE, Javier FC, Sacerdote DM, Bradford N, Butler B, Giangrosso PA, Moore C (1998) Influence of age on the response to *Streptococcus pneumoniae* vaccine in patients with recurrent infections and normal immunoglobulin concentrations. *J Allergy Clin Immunol* 102:215–221
14. Soininen A, Karpala M, Wahlman SL, Lehtonen H, Kayhty H (2002) Specificities and opsonophagocytic activities of antibodies to pneumococcal capsular polysaccharides in sera of unimmunized young children. *Clin Diagn Lab Immunol* 9:1032–1038
15. Lau RC, Bettelheim KA, Patel AC (1988) The 1985 national immunization survey: diphtheria, tetanus, and pertussis. *NZ Med J* 101:797–800
16. Hey C, Rose MA, Kujumdshiev S, Gstoettner W, Schubert R, Zielen S (2005) Does the 23-valent pneumococcal vaccine protect cochlear implant recipients? *Laryngoscope* 115(9):1586–1590
17. Rose MA, Hey C, Kujumdshiev S, Gall V, Schubert R, Zielen S (2004) Immunogenicity of pneumococcal vaccination in patients with cochlear implants. *J Infect Dis* 190:551–557
18. Balmer P, North J, Baxter D, Stanford E, Melegaro A, Kaczmarek EB, Miller E, Borrow R (2003) Measurement and interpretation of pneumococcal IgG levels for clinical management. *Clin Exp Immunol* 133(3):364–369
19. Jokinen JT, Ahman H, Kilpi TM, Makela PH, Kayhty MH (2004) Concentration of antipneumococcal antibodies as a serological correlate of protection: an application to acute otitis media. *J Infect Dis* 190:545–550
20. Dagan R, Givon-Lavi N, Fraser D, Lipsitch M, Siber GR, Kohberger R (2005) Serum serotype-specific pneumococcal anticapsular immunoglobulin G concentrations after immunization with a 9-valent conjugate pneumococcal vaccine correlate with nasopharyngeal acquisition of pneumococcus. *J Infect Dis* 192(3):367–376
21. Millar EV, O'Brien KL, Bronsdon MA, Madore D, Hackell JG, Reid R, Santosham M (2007) Anticapsular serum antibody concentration and protection against pneumococcal colonization among children vaccinated with 7-valent pneumococcal conjugate vaccine. *Clin Infect Dis* 44(9):1173–1179
22. Parkkali T, Kayhty H, Anttila M, Ruutu T, Wuorimaa T, Soininen A et al (1999) IgG subclasses and avidity of antibodies to polysaccharide antigens in allogeneic BMT recipients after vaccination with pneumococcal polysaccharide and Haemophilus influenzae type b conjugate vaccines. *Bone Marrow Transplant* 24:671–678
23. Soininen A, Seppala I, Nieminen T, Eskola J, Kayhty H (1999) IgG subclass distribution of antibodies after vaccination of adults with pneumococcal conjugate vaccines. *Vaccine* 17:1889–1897
24. Boyle RJ, Le C, Balloch A, Tang MLK (2006) The clinical syndrome of specific antibody deficiency in children. *Clin Exp Immunol* 146:486–492
25. Rose MA, Schubert R, Strnad N, Zielen S (2005) Priming of immunological memory by pneumococcal conjugate vaccine in children unresponsive to 23-valent polysaccharide pneumococcal vaccine. *Clin Diagn Lab Immunol* 12(10):1216–1222
26. Lehrnbecher T, Schubert R, Behl M, Koenig M, Rose MA, Koehl U et al (2009) Impaired pneumococcal immunity in children after treatment for acute lymphoblastic leukaemia. *Br J Haematol* 147(5):700–705
27. Rose MA, Gruendler M, Schubert R, Kitz R, Schulze J, Rosewich M, Zielen S (2009) Safety and immunogenicity of sequential pneumococcal immunization in preschool asthmatics. *Vaccine* 27(38):5259–5264

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