

## Terrestrial Paradise: Multiplex Immunoassay for Specific Pneumococcal Polysaccharide Antibodies

Ger T. Rijkers<sup>1,2\*</sup>

<sup>1</sup>Department of Science, University College Roosevelt, Middelburg, The Netherlands

<sup>2</sup>Laboratory for Medical Microbiology and Immunology, St Antonius Hospital, Nieuwegein, The Netherlands

**\*Corresponding author:** Ger T. Rijkers, Department of Sciences, University College Roosevelt, P.O. Box 944330 AB Middelburg, The Netherlands. Tel: +31118655500; Fax: +31118655508; Email: g.rijkers@ucr.nl

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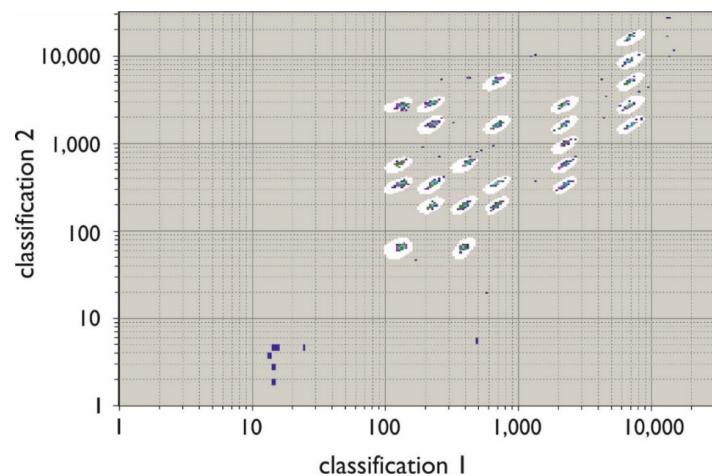
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### Commentary

*Streptococcus pneumoniae* is an encapsulated Gram-positive, facultative anaerobic bacterium. It is a cause for major mucosal as well as invasive diseases, including otitis media, pneumonia, bacteremia, meningitis [1]. The WHO estimates that annually half a million children under five years of age die due to pneumococcal infections [2]. In elderly, a similar high burden of invasive pneumococcal disease is found [3]. A variety of diagnostic tests exists to identify the causative pneumococcal serotype in case of a suspected infection [4]. An indirect, but specific, method to investigate the involvement of *S. pneumoniae* in a given infectious disease is analysis of the serological response, i.e. the increase in serotype specific antibodies during the course of disease [5]. *Streptococcus pneumoniae* comes in 93 different serotypes. This is a challenge for the immune system to defend against, as antibodies which have been produced during an infection with a given serotype will not protect against an infection with a different serotype. This also presents a challenge for vaccine development, to select the most prevalent serotypes to be included into a vaccine and a challenge to medical immunologists, to measure the antibodies against all these different serotypes. The latter aspect is the topic of this Commentary. Before multiplex immunoassays were available, the only way to determine serotype specific pneumococcal antibodies was by Enzyme-Linked Immunosorbent Assay (ELISA) [6,7]. Ideally, 93 different ELISAs would be required to detect specific antibodies to all serotypes. In practice, the best that could be achieved was to test for antibodies to eight of the most common serotypes within the IgG, IgA, IgG1 and IgG2 (sub)class. This meant that every blood sample had to be tested on 32 ELISA plates, which was extremely time-consuming and also required quite a lot of material. Furthermore, because of the

restricted dynamic range of ELISA, each sample had to be tested by serial dilution, limiting the number of samples to 8 per ELISA plate.

The development of bead-based, multiplex immunoassays has allowed for high throughput analysis of complex sets of biomarkers, including cytokines [8] and also antibodies [9]. For pneumococcal polysaccharide antibodies it meant that it became possible to measure multiple serotypes in a single assay [10]. We have developed the assay to include a total of 25 different serotypes (Figure 1) but the ambition is to come to an all-inclusive assay of 93 serotypes.



**Figure 1:** Multiplex immunoassay for determination of serotype specific pneumococcal polysaccharide antibodies. Screenshot of Bio-Plex analyzer 200 (IS 2.3; Luminex corporation, Austin, TX) showing 25 different bead sets (xMAP Technology<sup>®</sup>), coupled with 25 pneumococcal polysaccharides of serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12A, 12F, 14, 15B, 18C, 19A, 19F, 20A, 22F, 23F, 33F, and 45. Further details of the technique can be found in reference [11].

The need for a serotype specific pneumococcal antibody assay is evident. A specific measurement is needed for diagnosis of specific pneumococcal antibody deficiency [12], and for detection of vaccine failures and serotype replacement diseases [13,14]. Research into pneumococcal immunity and infections has a global impact and will continue all over the world. Currently, the best vaccines only cover 13 serotypes, so there are 80 pneumococcal serotypes not included in the vaccine. This vaccine is optimal for the US and Europe, where these 13 serotypes are the most common, but not for Asia and Africa where different serotypes dominate [15]. Preliminary data from our own laboratory suggest that out of the pneumococcal serotypes which dominate in childhood pneumonia in Bangladesh, only 1 from the top 10 is represented in current vaccines (Vestjens, et al., manuscript in preparation). In order to design vaccines with a better coverage in these areas, further research into serotype distribution, and vaccine driven serotype drift, is needed. Measurement of serotype specific antibody levels and responses is a crucial instrument in this respect. Apart from multiplex immunoassays for antibodies against pneumococcal antibodies, similar assays are available for meningococcal polysaccharides [16] and viral proteins [17].

The development of immunoassays is symbolized in the Jheronimus Bosch quadriptych painting *Visions of the Hereafter*. One of the panels of this painting is named Hell. In the past this could have been the nick-name of the part of the laboratory where the pneumococcal ELISAs were being processed. With the full implementation of multiplex technologies this part of medical immunological laboratory diagnostics will become a Terrestrial Paradise (Figure 2).



**Figure 2:** Detail of the panel *Terrestrial Paradise* which is part of the quadriptych *Visions of the Hereafter* (1482-1486) painted by Jheronimus Bosch. Museo di Palazzo Grimani, Venice, Italy. [http://boschproject.org/view.html?pointer=0.406,0.000&i=32\\_34\\_36\\_38\\_MCPVIS](http://boschproject.org/view.html?pointer=0.406,0.000&i=32_34_36_38_MCPVIS)

Multiplex immunassays have not yet overtaken the ELISA technology. A search in PubMed (<https://www.ncbi.nlm.nih.gov/pubmed/>; accessed December 23, 2017) on “pneumococcal

polysaccharide antibody Elisa” finds 479 publications, with 19 from 2017 (4% of total). A similar search for “pneumococcal polysaccharide multiplex” finds 30 publications, of which 5 are from 2017. The data indicate that apparently ELISA still is the method of choice for measurement of pneumococcal polysaccharide antibodies. The Terrestrial Paradise still may have to be discovered in many laboratories.

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