The Clinical Application of a Chemiluminescent Multiplex ELISA for the detection of 23 Human Pneumococcal Serotypes

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INTRODUCTION

Streptococcus pneumonia causes severe bacterial infections and can manifest as invasive pneumococcal disease. Type-specific IgG antibodies to capsular polysaccharides protect against invasive disease by opsonizing the organism and preventing the acquisition and carriage of pneumococci.

Some patients may have normal serum immunoglobulins but are still unable to mount an effective response to polysaccharide capsule pathogens. Investigation of specific antibody deficiency involves checking response to vaccines.

Pneumococcal vaccines may provide protection for up to 23 different serotypes (PPV23), however most centres only measure up to 12 serotypes. We evaluated the Quansys (Qplex) multiplex assay which measures 23 pneumococcal serotypes to determine if the additional serotype responses add clinical value.

AIMS & OBJECTIVE

The aim was to determine if the Quansys assay can detect vaccine responses comparable to an established Luminex method and determine if the additional serotypes measures are beneficial when interpreting responses to the different pneumococcal vaccines available.

METHOD

121 patient samples (63 paediatric and 58 adult) were tested on both the Luminex bead-based assay and the new Quansys Chemiluminescent multiplex ELISA assay (figure 1). Protective and not protective values were established for patients using both assays using the following criteria:

Putative protective antibody level ≥0.35 mg/L.

- **Protective - Luminex Assay: 8/12 serotypes**
- **Protective - Quansys: 15/23 serotypes.**

Luminex serotypes measured: 1, 2, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F

Quansys serotypes measured : All Luminex serotypes + 3, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, 33F.

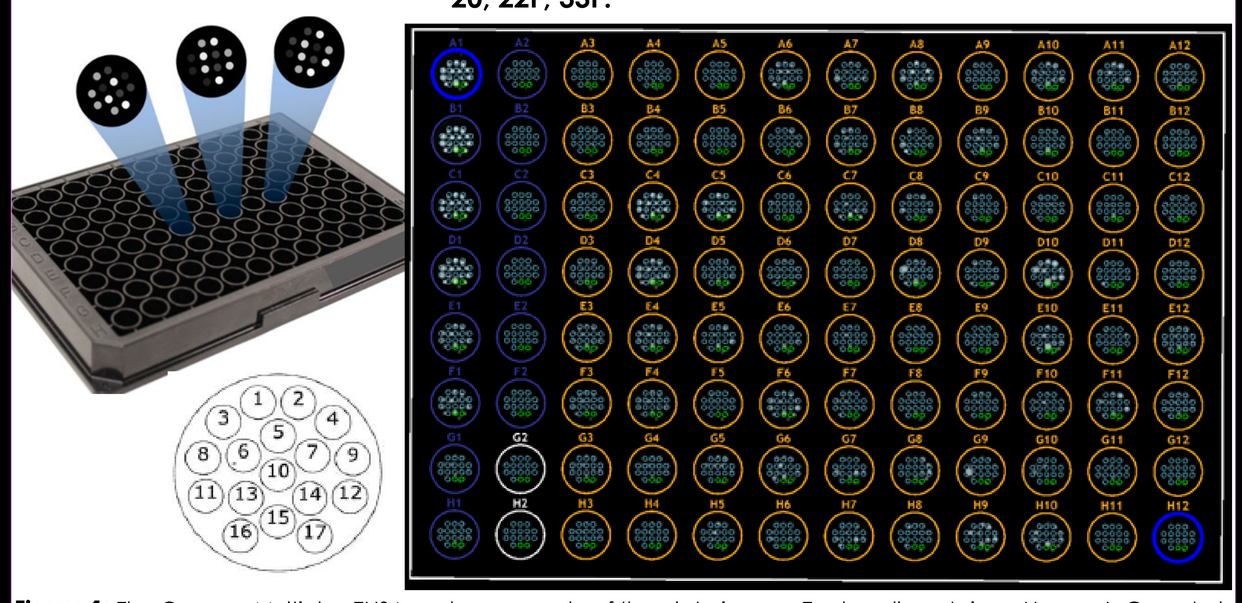

Figure 1: The Quansys Multiplex ELISA and an example of the plate image. Each well contains a Human IgG control spot, CWPS negative control spot (to rule out non-specific binding) and a reference spot.

Vaccination history and total pneumococcal level was available for the majority of patients. Serotype specific response was matched against the vaccine given to determine if the additional serotypes on Quansys added clinical value to the interpretation.

RESULTS

Data when directly comparing protective status for the 12 serotypes which are tested by both methods showed agreement in 103/121 cases (85%).

18 patients showed discrepancy in interpretation.

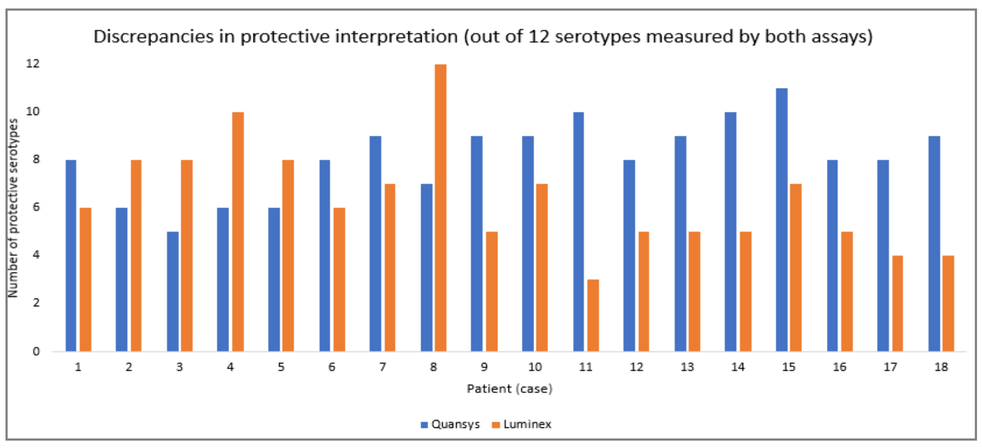

Figure 2: Diagram illustrating the 18 samples that were out of consensus between methods. Majority were borderline results within +/-30% CV of 0.35mg/L.

RESULTS

Data when taking into account <u>all serotypes</u> on both assays (23 on Quansys and 12 on Luminex):

Out of 121 patients:

- **Protective with both assays = 39 patients**
- **Non-protective with both assays = 48 patients**
- **Discrepant results = 34 patients** (15 cases Luminex protective/Quansys non-protective & 19 cases Quansys protective/Luminex non-protective).

Of the discrepancies:

- **Luminex protective/Quansys non-protective:** 10 of the 15 patients showed analytical agreement when assessing Luminex serotypes only. Difference mostly due to Quansys only serotypes giving overall non-protective interpretation.
- **Quansys protective/Luminex non-protective:** All of the 19 cases had 8 out of 11 of the serotypes measured by Quansys only showing protective interpretation.
  - 16/19 had received PPV23 vaccine, 1 had no vaccine and 2 PPV23 & PCV13 (figure 3).
  - Majority of these cases had no PID, normal Igs and total pneumococcal Abs >30 U/mL.

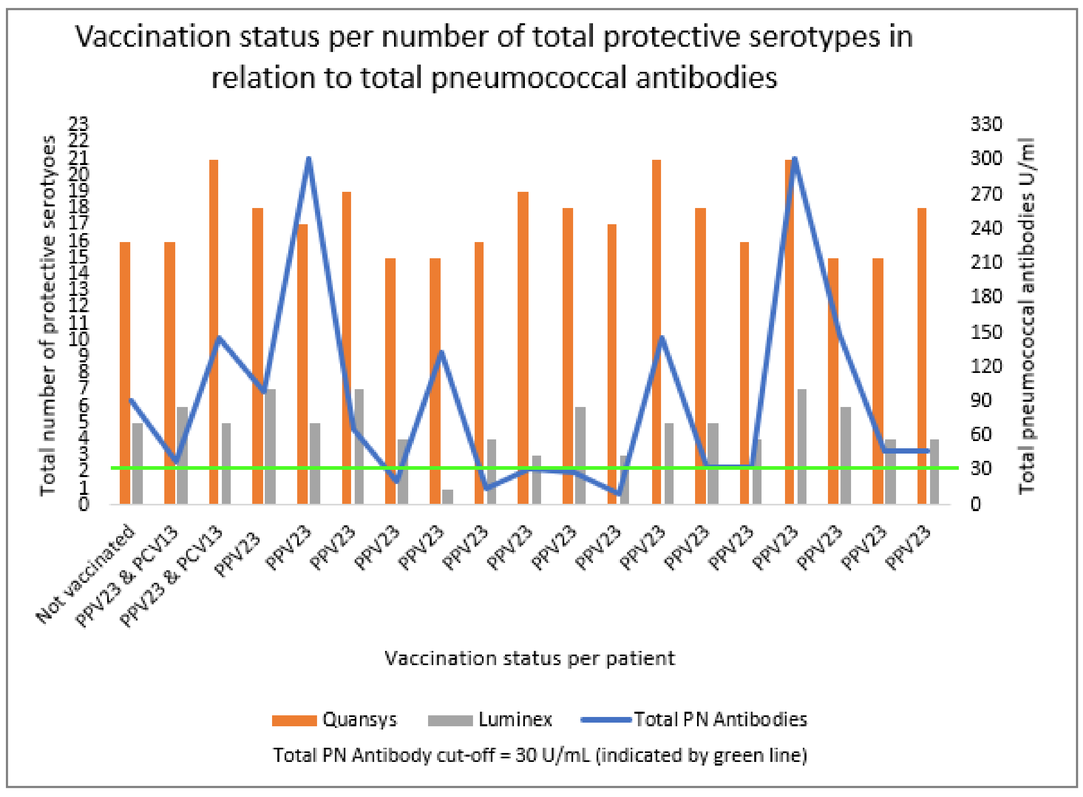

Figure 3: Chart illustrating the vaccination status, total pneumococcal Abs and number of protective serotypes for each of the 19 discrepant Quansys protective/Luminex non-protective samples.

CONCLUSION

Comparison between Luminex and Quansys methods showed good correlation between the 12 serotypes that were measured on both assays. The Quansys method was shown to be suitable for routine use following completion of a full in-house validation process.

The Quansys method identified more patients with protective levels after being given PPV23 (Pneumovax) than the Luminex assay. The Quansys method also showed fewer non-protective responses in patients with total pneumococcal Ab > 30 U/mL when compared with the Luminex. Thus, indicating that the Quansys method provides additional clinical benefit with additional intervention, such as repeat vaccination, not being required in these cases.

Patients that were given Prevnar should be interpreted with consideration given to the serotypes in the vaccine. Incorrect non-protective responses may be interpreted if all 23 serotypes are assessed. Interpretive advice will be given on reports.

Overall assessment of all 23 serotypes showed that they provide additional clinical use in the evaluation of vaccine response across all age ranges when compared to the alternative Luminex assay.

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