Adherence of Pneumococcal Surface Adhesin A (PsaA)-coated particles to human nasopharyngeal (NP) epithelial cells for the evaluation of anti-PsaA functional antibodies
Pneumococcal Surface Adhesin A, PsaA

- Membrane associated, cell surface exposed, metal-binding lipoprotein, 37 KDa
- Part of a ABC-type manganese permease complex
- Common to all 90 serotypes
- Highly conserved
- Immunogenic (Johnson et al. 2002)
**S. pneumoniae Adhesins**

PsaA – Pneumococcal surface adhesin A  
CbpA – Choline binding protein A  
PavA – adherence and virulence protein A  
AdcA – Zn binding adhesion lipoprotein  
Lmb – Adhesion lipoprotein

*S. pneumoniae* binds to Detroit-562 (D-562) human nasopharyngeal epithelial cells. Because there is more than one adhesin present in *S. pneumoniae*, a ‘pure system’ was created to look at PsaA only.
Recombinant, purified, non-lipidated PsaA was covalently linked to fluorescent beads.
The rPsaA fluorescent beads bind to D-562 cells.
This detection system was used to analyze the inhibition of PsaA adherence to D-562 cells (Romero-Steiner et al. 2001).
**Beads adherence inhibition assay**  
(Romero-Steiner et al., ISPPD4, 2004)

- Round bottom 96 well titer plate
- Serum diluted with PBS (with 1% BSA) with the final volume of 80 µl/well
- Carboxylate-modified fluorospheres coated with P4 peptide (20 µl/well)
- Incubated for 15 min at 37°C, 5% CO₂
- Replica plated (45 µl/well) on to confluent monolayers of Detroit 562 cells in EMEM (45 µl/well). Final volume - 90 µl/well
- Incubated for 2 hrs at 37°C, 5% CO₂
- Washed 5X with PBS (with 0.5%BSA)
- Read in a flurometer (mean fluorescence unit (FU)/well)

**Bacterial adherence inhibition assay**  
(Romero-Steiner et al., CDLI, 2003; 10:246-257)

- Round bottom 96 well titer plate
- Serum diluted with PBS (with 1% BSA) with the final volume of 80 µl/well
- Pnc 4 (DS2341) inoculated - 20 µl/well (Dilutions in Hanks+0.2%BSA buffer)
- Incubated for 15 min at 37°C, 5% CO₂
- Replica plated (45 µl/well) on to confluent monolayers of Detroit 562 cells in EMEM (45 µl/well)
- Incubated for 2 hrs at 37°C, 5% CO₂
- Washed 5X with PBS (with 0.5%BSA)
- Overlaid with Todd-Hewitt yeast extract agar + 0.1% TTC (65 µl/well)
- Incubated at 37°C, 5% CO₂ overnight
- Colony forming unit (CFU)/well counted in AlphaImager retro-fitted with ELISPOT adaptor.
Bead Preparation

EDAC

COO⁻ fluorospheres

Specifically coated fluorospheres

rPsaA or Peptides
Adherence of rPsaA coated fluorescent beads to D-562 NP cells

Inhibition of adherence of rPsaA coated fluorescent beads to D-562 NP cells with human serum containing anti-PsaA antibodies
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**Data output:** Fluorescent units (FU)

**Inhibition titer = serum’s % inhibition of adherence as compared to controls**
Neutralization of Pnc adherence in sera from mice immunized with non-lipidated rPsaA

![Bar chart showing mean % inhibition (CFU/well) for different groups: rPsaA (AlOH3), rPsaA (PBS), and Placebo (PBS). The bars indicate the mean % inhibition at various dilutions (-7, -8, -9).]
Mouse Antibody inhibition of PsaA bead binding to D-562

Figure 1A. Log10 serum dilution

% Inhibition of adherence

Log10 serum dilution

Pre
Pab
Rabbit Antibody inhibition of PsaA bead binding to D-562

![Graph showing inhibition of PsaA bead binding with Rabbit Anti-PsaA at different dilutions (P1:8, P1:16, P1:32) with error bars indicating variability.]
Acknowledgements

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