IgG Avidity Assay

1. Coat a flat bottom ELISA plate (Nunc) with 100ul of either BPI (10ug/ml), tetanus toxoid (0.1LF/ml) in PBS or PBS alone
2. Incubate covered plate for 2 hours at 37^{o}C
3. Wash plate 3x with PBS + 0.05% Tween-20
4. Add 300ul PBS + 1% BSA to each well and incubate covered plate overnight at room temperature
5. Wash plate 3x with PBS + 0.05% Tween-20
6. Add 100ul of patient serum diluted accordingly in PBS + 1% BSA
    a. For anti-BPI IgG
        i. CF and BE: 1:500
        ii. Septicemia: 1:100
    b. For anti-TT IgG 1:250 for all cohorts
7. Incubate plate for 1hour at room temperature
8. Wash plate 3x with PBS + 0.05% Tween-20
9. Add 200ul of either 3M NaSCN in PBS or PBS alone for 15min at room temperature
    a. 6M NaSCN stock = 97.28g NaSCN in 200ml PBS
10. Wash plate 3x with PBS + 0.05% Tween-20
11. add 100ul of goat anti-human IgG HRP labelled antibody (1:50,000 in PBS + 1% BSA) and incubate for 1 hour at room temperature
12. Wash plate 3x with PBS + 0.05% Tween-20
13. Add 100ul substrate (A:B, 1:1, R&D Systems) to each well and incubate in the dark for ~20min
14. Add 50ul stop solution (R&D Systems) to each well
15. Read absorbance at 450nm and 570nm
16. Calculate % residual binding (after subtracting the OD from the respective PBS coated control well for each treatment)

% Residual binding = (OD of 3M NaSCN wash / OD of PBS wash)* 100