Development of a serum bactericidal antibody (SBA) assay for *Haemophilus influenzae* type b

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BACKGROUND AND OBJECTIVES

▪Prior to routine immunisation, *Haemophilus influenzae* serotype b (Hib) was a major cause of serious bacterial infections, particularly in young children.

▪Evaluation of the immune response to Hib conjugate vaccines includes the measurement of serum antibodies to the Hib capsular polysaccharide (polyribosyl-ribitol-phosphate (PRP)) by ELISA, with accepted short term and long term levels of ≥0.15 µg/mL and ≥1.0 µg/mL, respectively (Käyhty *et al*., 1983).

▪The relevance for protection in children who have been primed with glycoconjugate vaccines remains unclear, as these levels were derived by passive immunisation, or immunisation with pure polysaccharide.

▪Antibody function can be measured by a serum bactericidal antibody (SBA) assay which measures the titre of antibodies that bind to the specific target strain initiating complement mediated lysis.

▪We developed , optimised and evaluated a Hib SBA assay, which was tested on sera from vaccinated adults and from infants under the current UK 2-3-4-12 month schedule.

METHODS

▪The developed Hib SBA was a modification of a method described by Romero-Steiner *et al.* (2001).

▪Two fold serial dilutions of heat inactivated serum and bactericidal buffer (BB) were prepared on a U-bottomed 96 well microtitre plate. Target cells grown to log phase (2.4- 3.4 x 10^8 cfu/mL), BB, and 33% 3-4 week old baby rabbit complement were added to appropriate wells, bringing the total well volume to 60 µL. Prior to incubation, 10 µL was removed from the appropriate control wells for T0 count. Microtitre plates were incubated for 50 min at 37˚C. Following incubation, 10µL of the reaction mixture was plated out using the tilt method onto chocolate agar and incubated overnight. The following morning colonies were counted and SBA titres expressed as the reciprocal of the final dilution giving ≥50% killing.

▪ Adult serum samples collected prior to vaccination and 4-6 weeks following vaccination with a combined Hib/Men C vaccine (Menitorix ,GSK), were used to validate the optimised Hib SBA assay. The assay was validated in terms of inter and intra assay precision, specificity, and linearity. Following validation, SBA titres were compared to previously determined anti-PRP IgG concentrations.

▪ The Hib SBA assay was used to evaluate the immune response of UK infants under the current UK immunisation schedule. For primary vaccination, three doses of DTa_5P/IPV/Hib-TT(Pediacel, Sanofi Pasteur) were given at 2, 3, and 4 months of age. A booster dose of Menitorix was given in the second year of life. SBA titres from post primary and post booster responses were correlated to previously determined IgG concentrations. Using the power function $y=a\ x^b$ (y=log transformed IgG concentration, log a=y intercept, x= log transformed SBA titre, b= slope) , the predictive SBA titre that corresponds to the accepted short term and long term levels of ≥0.15 µg/mL and ≥1.0 µg/mL, was calculated.

Table 1. Microtitre plate layout for the Hib SBA assay

Column	1	2	3	4	5	6	7	8	9	10	11	12
Bactericidal buffer (µL)	15	15	15	15	15	15	15	15	15	15	15	
Patient serum (µL)	15 µL 9 TWOFOLD SERIAL SERUM DILUTIONS Transfer 15 µL from column ≠ 1 – 9, mix 6 times and discard 15 µL from column # 9									0	0	See below
Complement (µL)	20	20	20	20	20	20	20	20	20	20	20*	
Cells (µL)	10	10	10	10	10	10	10	10	10	10	10	
Bactericidal buffer (µL)	15	15	15	15	15	15	15	15	15	15	15	
Final volume (µL)	60	60	60	60	60	60	60	60	60	60	60	
Rreciprocal final serum dilution	8	16	32	64	128	256	512	1024	2048	n/a	n/a	

Column 12 contains 22.5 µL of BB, 7.5 µL of heat inactivated serum, 20 µL of heat inactivated complement, and 10 µL of working solution of organisms. * Column 11: heat inactivated complement.

RESULTS

•Validation of the Hib SBA assay was deemed acceptable in all assay parameters tested. In vaccinated adults, a strong correlation (*r*= 0.81) between anti-PRP IgG concentrations and SBA titres was shown (figure1).

▪ SBA titres measured by the Hib SBA assay were compared to previously determined anti-PRP IgG concentrations. Correlation analysis was performed for post primary and post booster responses. A good correlation coefficient (*r*) of 0.635 was shown following primary vaccination. A stronger correlation was shown for post booster responses (*r*= 0.746). In both correlations, sera with low SBA titres and high anti-PRP IgG concentrations were observed. Sera with high SBA titres and low anti-PRP IgG concentrations were also observed (figures 2 and 3).

▪ The predictive protective SBA titre that corresponds to the established long term protective antibody concentration of 1.0 µg/mL was calculated. As a good correlation was seen in post booster responses, only these data were used. The predictive SBA titre was calculated to be 8. The SBA titre was not determinable when calculating using the short term correlate of 0.15 µg/mL as it gave a value smaller than the lowest starting dilution.

Figure 1. Correlation of SBA titres of validation panel sera with previously determined anti-PRP IgG concentrations.

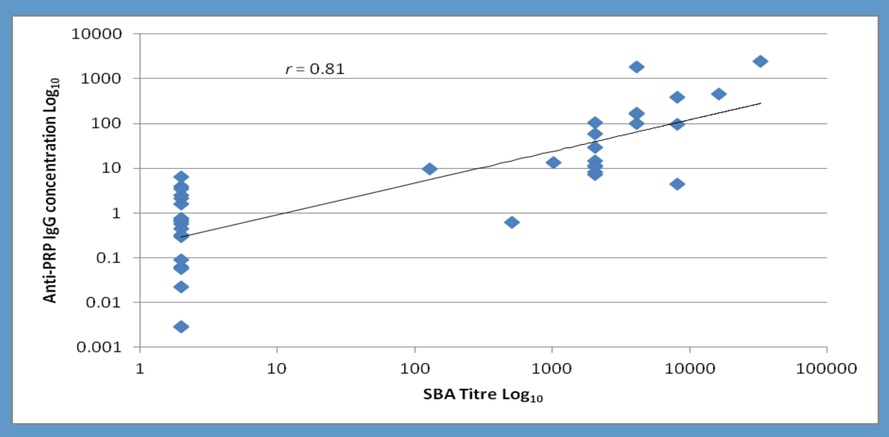


Figure 2. Correlation of anti-PRP IgG concentrations and SBA titres for post primary responses (T50)

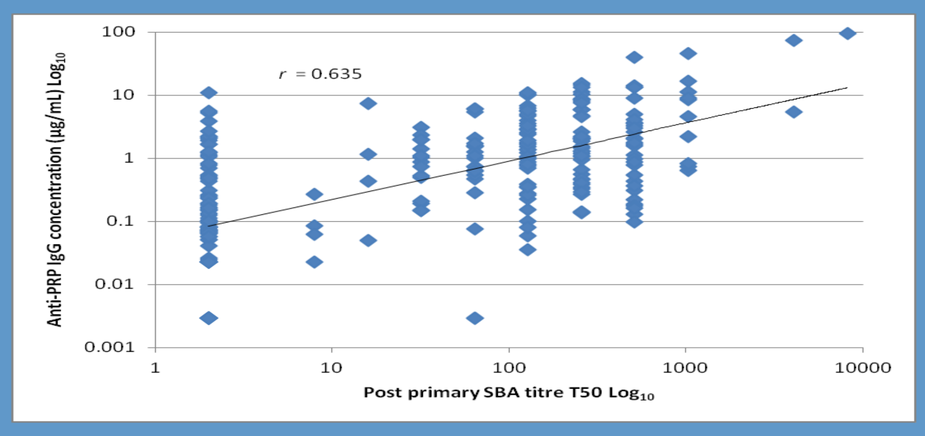
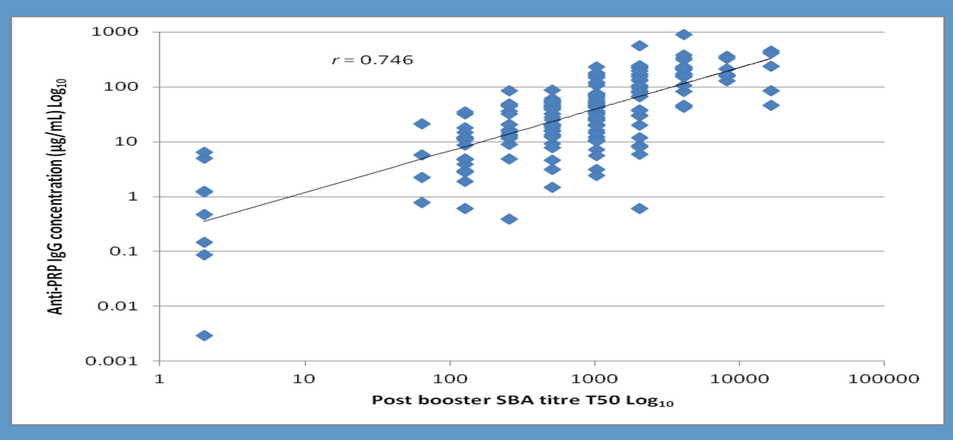


Figure 3. Correlation of anti-PRP IgG concentrations and SBA titres for post booster responses (T50)



DISCUSSION

▪The Hib SBA assay was deemed highly specific, and reproducible which highly correlated to IgG concentrations.

▪The SBA GMT enhancement calculated for infant post primary and post booster responses support the anti-PRP GMC enhancement in UK infants reported by Borrow *et al*. (2010).

▪The predictive SBA titre of 8 must be interpreted with caution as it was calculated from a correlation coefficient of *r*= 0.746.

▪The presence of low avidity antibodies may explain the presence of high IgG concentrations and low SBA titres.

▪The high SBA titres and low IgG concentrations may be due to the presence of IgM or antibodies raised to other surface antigens. These discrepancies accord with Findlow *et al*. (2009) who reported moderate correlations between IgG correlation measured by ELISA and SBA titres against Men A.

CONCLUSIONS

The SBA assay is reliable and reproducible when evaluating the humoral response following Hib vaccination.

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