DEVELOPMENT OF A FLOW CYTOMETRIC COMPLEMENT DEPOSITION ASSAY FOR GROUP B *STREPTOCOCCUS*

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INTRODUCTION

Group B Streptococcus (GBS) is the leading cause of severe bacterial sepsis in newborns. Specific and functional antibody to GBS antigens, together with opsonising complement components, has a key role in the opsonisation of GBS. Therefore, sero-epidemiological and vaccine assessment studies have measured functional immunity to GBS using the opsonophagocytic killing assay (OPKA). Here we have developed an antibody-mediated complement deposition assay (CDA) as a surrogate assay for the more technically challenging OPKA, allowing the testing of large numbers of sera and at very small volume (<20µL).

METHODS

Bacteria:
Streptococcus agalactiae strains, representing the five prevalent serotypes in the UK, were grown in Todd Hewitt broth at 37°C with shaking until an OD_{600nm} of 1.0 (mid-late log phase) was reached. Washed cultures were killed using 2% formaldehyde at room temperature for 1h and stored at 4°C.

Sera:
The sera used for the development of this assay were pooled human reference sera (SHRS) and rabbit polyclonal sera (SSI) raised against serotypes Ia, Ib, II, III & V, and normal human donor sera.

Measurement of C3b/iC3b deposition:
35µL fixed bacteria (~$5x10^7$ ml^{-1} in PBS+ 1%BSA) were added to wells of a 96-well plate containing 10µL IgG-depleted human plasma and 5µL of each test serum. Plates were incubated for 7.5min at 37°C with shaking, centrifuged at 3060g and washed once in PBS+1%BSA. Samples were resuspended in 200µl PBS+1%BSA containing 1:500 sheep anti-human C3c FITC (Abcam) and incubated at 4°C for 20min. Samples were washed twice in PBS+1%BSA and analysed by flow cytometry.

Data Analysis:
Gates were drawn to include 10% of the population of the complement-only control. Assay readout was achieved by multiplying the percentage of bacteria moving into the gate by the average fluorescence of that defined population (X-mean), to give a Fluorescence Index (FI). The FI of the complement-only control was subtracted from the FI of the test sera to give a final measure of FI-C'.

RESULTS

Figure 1 compares CDA using live and formaldehyde-fixed bacteria and cultures that were harvested at OD_{600nm} 0.3 and 1.0. Correlations were strong, with R values of 0.96 (p=<0.01) and 0.74 (p=<0.01) for fixed versus live bacteria and OD_{600nm} 0.1 verses 0.3 respectively.

Figure 1. Comparison of live and fixed bacterial targets

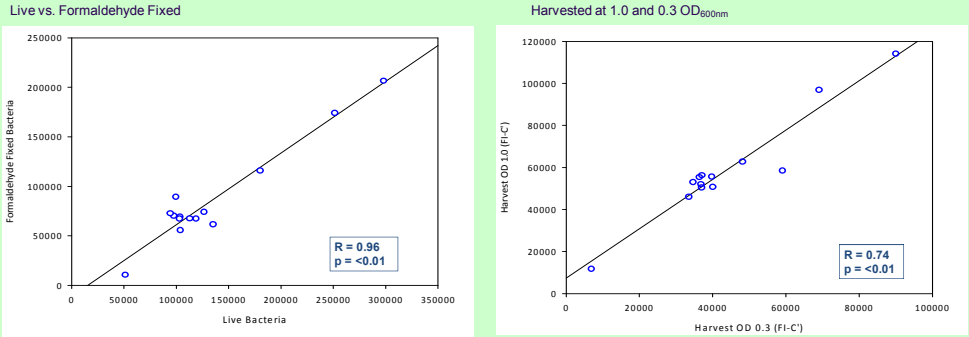


Figure 2 shows complement binding using SHRS human reference sera against homologous and heterologous GBS serotype targets. As has been observed in ELISA studies by others, all GBS serotypes exhibited cross-reactivity with heterologous sera

Figure 2. CDA of serotype specific human reference sera against all GBS serotypes

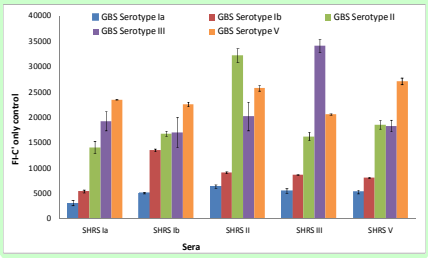
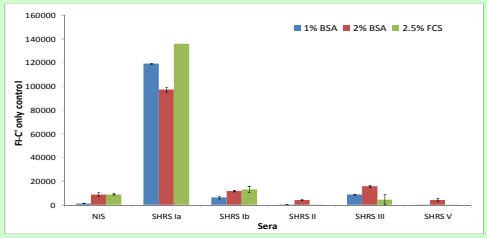


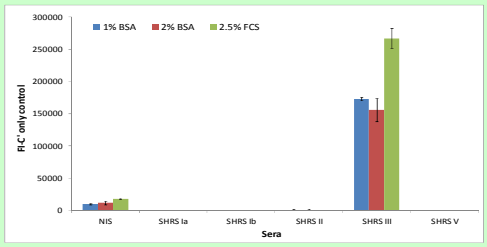
Figure 3 shows FI-C' responses to GBS serotypes Ia and III following optimisation of blocking buffers, complement concentration and incubation times. Strong serotype-specific responses against GBS were observed, with low cross-reactivity in response to heterologous serotypes.

Figure 3. CDA of serotype specific human reference sera post optimisation

GBS Serotype Ia



GBS Serotype III



Assay Qualification

Well-to-well variation across a 96 well microtitre plate was assessed using Zymosan as an inter-assay control

Well-to-Well variation CV **12.3%**

Day-to Day variation CV **7.3%**

Intra- and inter-assay precision of the CDA was performed for GBS serotype II against a selection of 16 donor sera along with homologous SHRS and SSI sera:

Intra-assay precision CV **22%**

(1 operator 3 assays 1day)

Inter-assay precision CV **26%**

(1 operator 3 assays 3 days)

SUMMARY

- **A C3b and iC3b complement deposition assay was developed and optimised for the 5 UK prevalent serotypes of GBS**
- **The assay is sensitive and reproducible, with intra-assay CV of 22% and inter-assay CV of 26%.**
- **The optimised assay showed good serotype specificity**

FURTHER WORK

- **A comparison of the CDA and the 'gold standard' plate killing assay (OPKA) is underway**
- **The CDA assay will be used for functional immune assessment in seroprevalence and vaccine studies**

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