Correlation of high-throughput flow cytometry assays with live killing assays in the assessment of meningococcal vaccines

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INTRODUCTION

The use of functional assays to assess the efficacy of meningococcal vaccines is hampered by the low-throughput of traditional killing assays and the requirement for large volumes of serum. We have developed high-throughput, flow cytometric assays to measure total IgG binding onto whole meningococci; antibody-dependent opsonophagocytosis and antibody-dependent C3c (C3b/iC3b) and C5b-9 (membrane attack complex) deposition. These assays have been shown to be highly reproducible (Coefficients of Variance <30%) and suitable for large clinical trials. The correlations of these high-throughput assays with killing opsonophagocytosis and serum bactericidal assays have been assessed using a panel of human sera. All of these assays require the availability of human complement which contains low titres of cross reactive antibody, and this has lead to the investigation of using IgG-depleted human plasma as a complement source.

METHODS

Opsonophagocytosis Assay (OPA)

The granulocytic cell line HL60 was used as the phagocytic cells. Azide-killed meningococci labelled internally with fluorescent dye BCECF were used as the target bacteria; IgG-depleted human plasma was used as the complement source and a single-point determination of OP activity was made at a serum dilution of 1:20; all assays were performed in duplicate. Flow cytometry was used to determine the percentage of HL60 cells taking up the labelled target meningococci and the intensity of fluorescence uptake; the data were expressed as the signal of test antibody minus the signal from the no antibody, complement-only control (FI-C').

Opsonophagocytosis Killing Assay

Method adapted from Plested & Granoff (2008) to include the use of HL60 cells and IgG- and C7-depleted human complement. Assay endpoint expressed as the reciprocal of the dilution giving ≥50% killing, compared with the control.

IgG Binding to Meningococci

Goat anti-human IgG(FITC) was used to probe the binding of IgG to whole meningococci, with data expressed as signal of test antibody minus conjugate-only control (FI-Conj).

C3c and C5b-9 Deposition Assay (CDA)

Azide-killed meningococci were incubated with test antibody and human plasma, which had been IgG depleted using protein G Sepharose. Then sheep-anti-human C3c (FITC) and mouse-anti-human C5b-9 (AlexaFluor 647) were used to measure deposition by incubating for 20min at 4^{0}C. Flow cytometry was used to detect the percentage of meningococci showing fluorescence (complement binding) and the intensity of that fluorescence. The data was expressed as the signal of test antibody minus the no-antibody, complement-only control (FI-C').

Assay correlations

Assay correlations were determined using Pearson's Product Moment Correlation Coefficient (R value). For regression analysis, all data was expressed as a log10 value.

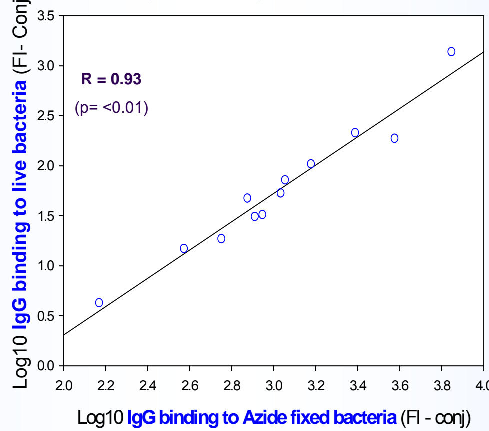
Serum Bactericidal Assay (SBA)

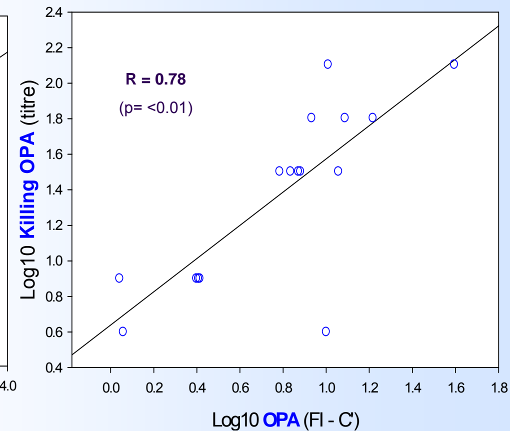
A standard SBA method was used.

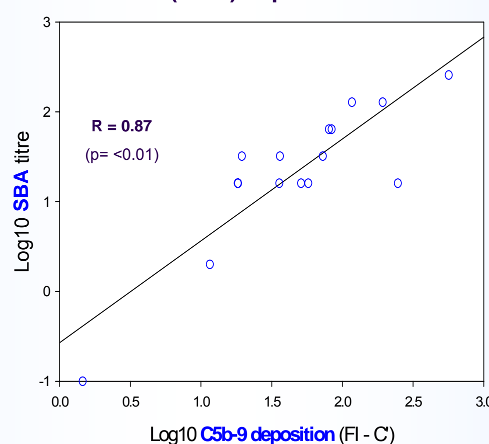
Total haemolytic complement assay

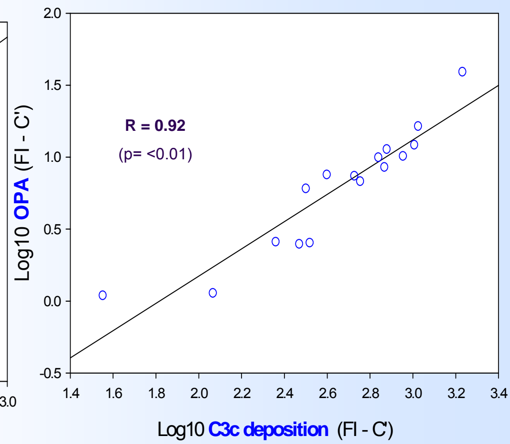
5µl of complement was applied to agar containing sensitised sheep erythrocytes, incubated at 4°C overnight. The plate was then incubated for 30 minutes at 37°C and then the radius of the lysis circles were measured and compared to a standard.

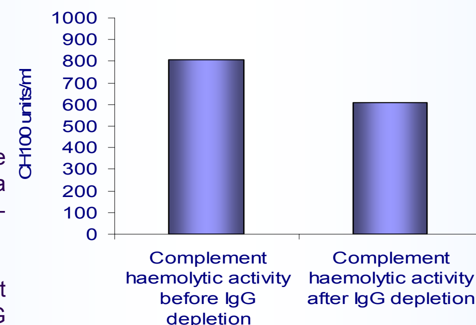
RESULTS



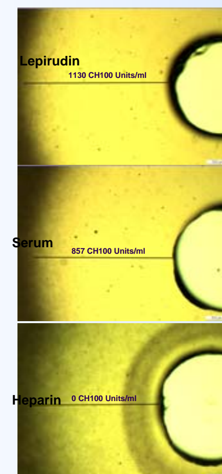








- Following IgG depletion the complement haemolytic activity is maintained.
- The small fall in activity is compensated for by the removal of cross-reactive antibody.



- The use of Lepirudin as an anticoagulant left the complement with the greatest haemolytic activity detectable when compared with Heparin and Serum.

SUMMARY

- Good correlations were observed between Killing OPA and flow-cytometry OPA with fixed bacteria, C5b-9 deposition and SBA, and C3b/iC3b deposition and flow cytometry OPA.
- These assays are high throughput flow-cytometry using azide-killed bacteria and are suitable for large scale serology.
- The use of killed bacteria means that no containment is required and a single stock of bacteria can be used for a study reducing assay variation.
- The requirement for small serum volumes means large panels of sera and strains can be assessed quickly.
- These assays use a IgG depleted human complement source, which has been isolated using an anticoagulant which does not interfere with the complement cascade.