Identification of *Neisseria meningitidis* specific patient derived antibodies using reverse vaccinology 2.0

Millie Gladstone  
Imperial College London  
Department of Infectious Disease
**Neisseria meningitidis**

- The most common cause of bacterial meningitis and septicaemia in the UK
- 10% of infections are fatal
- 25% of survivors have long term effects
- Six serogroups cause the majority of disease – A, B, C, W, X and Y
  - Recently MenB has been the dominant serogroup in the UK

**Current vaccines**

- Conjugate vaccines against *N. meningitidis* serogroups A, C, W and Y
- Recombinant protein vaccine against serogroup B
  - Strain coverage
  - Impact on carriage
Patient blood sample → Test patient sera for functional response → FACS single cell sorting of patient B cells → RT and nested PCR of B cell variable regions

Antigen binding region

Cloning of antibody IgH and IgL variable regions into E. coli → Transfection of HEK293 cells - production of IgG antibodies into supernatant → hmAb characterisation and identification of epitope i.e. ELISA, flow cytometry, western blot, hSBA, LC-MS/MS
Assays used to characterise hmAbs

- ELISA and flow cytometry
  - Test for binding of antibody to \textit{N. meningitidis}
- Western blot
  - Identification of antigen (target protein) size
- Serum bactericidal assay (SBA)
  - Test for bacterial killing by antibody in combination with human complement
- Complement deposition assay (CDA)
  - Assess ability of antibody to recruit human complement factors C3c and C5b-9
  - C3c = opsonisation
  - C5b-9 = membrane attack complex
<table>
<thead>
<tr>
<th>Antibody</th>
<th>ELISA and flow cytometry</th>
<th>Western blot</th>
<th>Target size (kDa)</th>
<th>SBA</th>
<th>CDA: C3c</th>
<th>CDA: C5b-9</th>
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Future work:

- Identify target antigen
  - Immunoprecipitation and mass spectrometry
- Further characterise remaining antibodies