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

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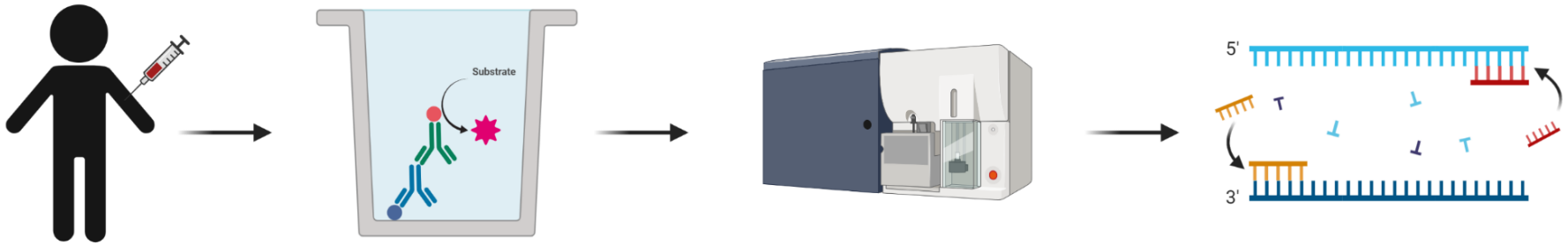
Neisseria meningitidis

- 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

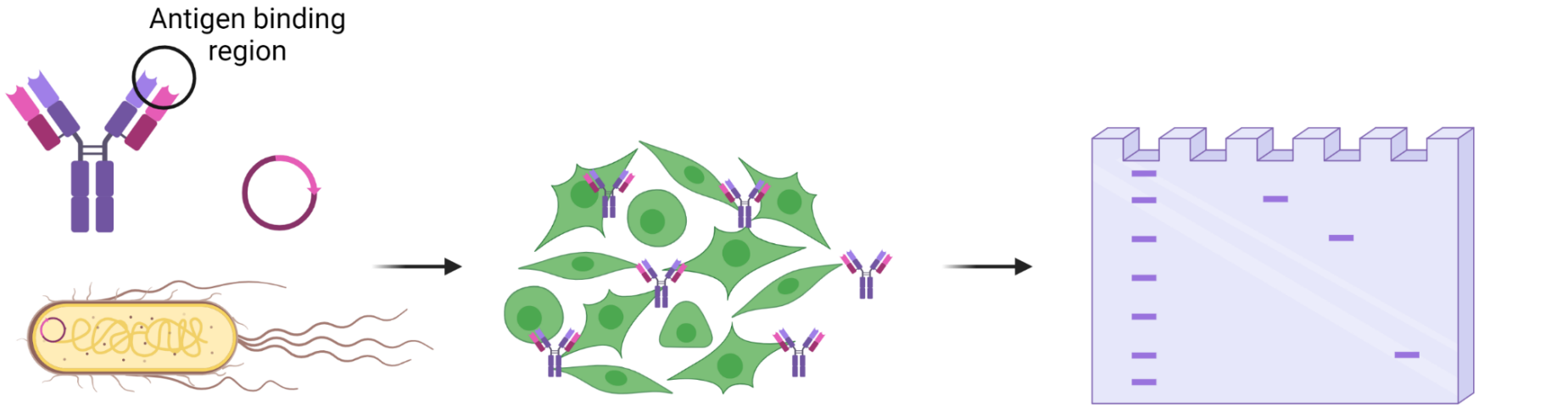


Patient blood sample

Test patient sera for functional response

FACS single cell sorting of patient B cells

RT and nested PCR of B cell variable regions



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

Created in biorender

Assays used to characterise hmAbs

- ELISA and flow cytometry
  - Test for binding of antibody to *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

Antibody	ELISA and flow cytometry	Western blot	Target size (kDa)	SBA	CDA: C3c	CDA: C5b-9
P02-1A1			30 - 40			
P02-5A2			ND			
P02-5E10			30 - 40			
P09-2F2			20-30			
P09-2F7			ND			

Future work:

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