

Serum Bactericidal Activity (SBA) Assays for *Neisseria gonorrhoeae*

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Introduction

1. A key aspect of bacterial vaccine development is characterisation of the functional activity of antibodies raised to the vaccine targets under investigation.
 - a. There are no established correlates or surrogates of protection for *N. gonorrhoeae*; however, serum bactericidal activity (SBA) is an established correlate of protection for the closely related bacterium *Neisseria meningitidis*.
 - b. SBA assays for *N. meningitidis* have been standardised and used extensively for the development and licensing of meningococcal vaccines.
2. The SBA assay measures antibody-mediated, complement-dependent killing of bacteria.
3. SBA assays are performed by incubating serial dilutions of antibody with a bacterial target strain and a complement source.
4. The antibody binds to the bacterial surface and activates the classical complement pathway, resulting in bacterial lysis (Fig. 1).
5. The SBA titre for each serum is the reciprocal of the serum dilution that results in $\geq 50\%$ killing of the bacteria, relative to the number of bacteria present before incubation with serum and complement.

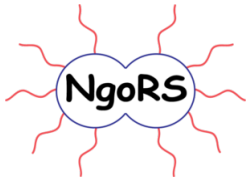
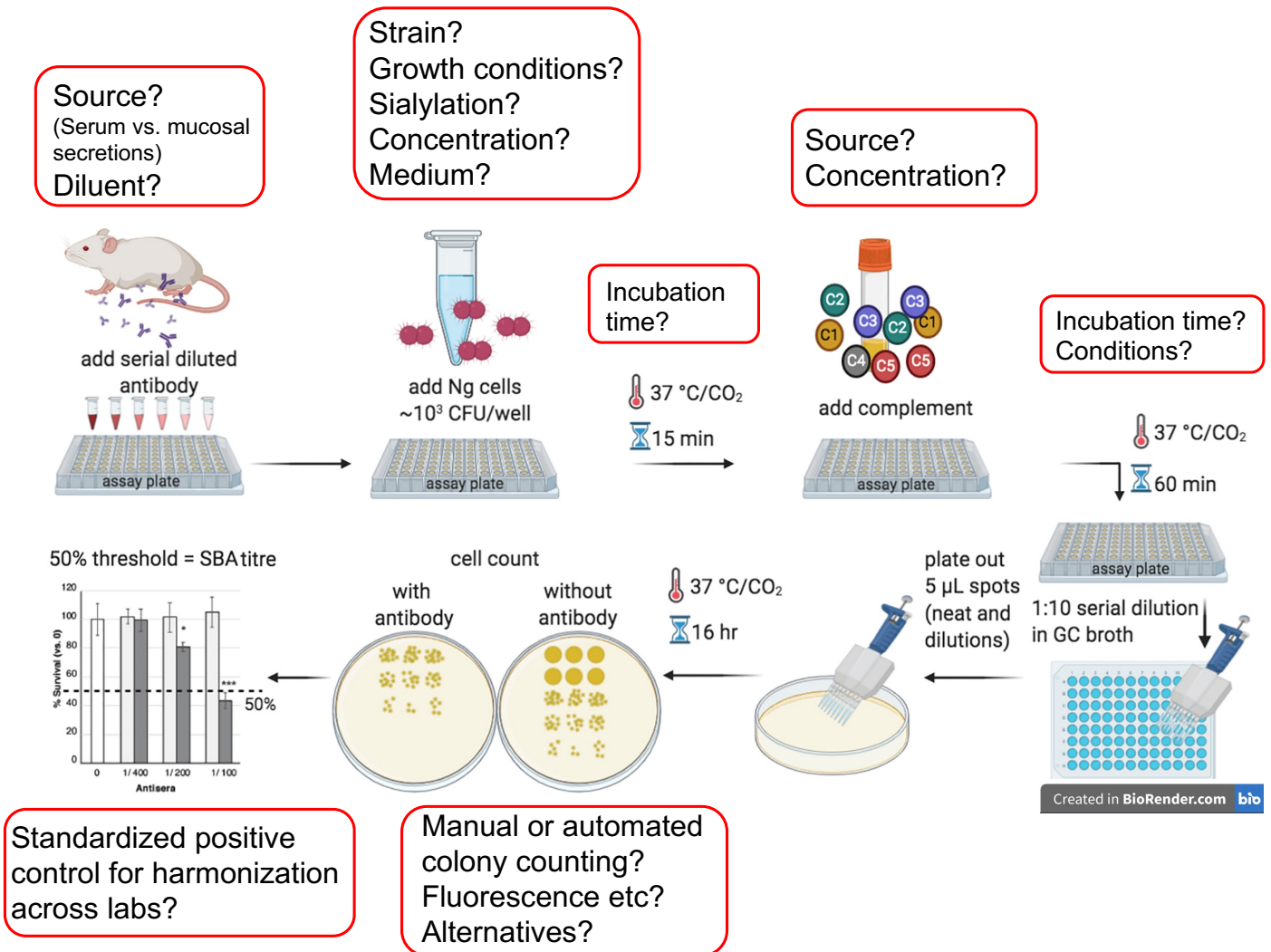
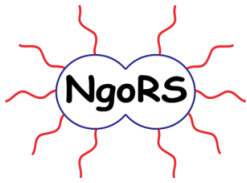


Figure 1. Schematic overview of the serum bactericidal activity (SBA) assay.



Serial dilutions of antibody are prepared and incubated with *Neisseria gonorrhoeae* and a complement source, then cell viability is measured.

SBA assays are commonly used in gonococcal vaccine development. There is currently no standardised methodology, and there are many variables for each step of the assay (red boxes).



Recent published SBA assay protocols

- Development and validation of a standardized human complement serum bactericidal activity assay to measure functional antibody responses to *Neisseria gonorrhoeae*. Matthias KA, Reveille A, Dhara K, Lyle CS, Natuk RJ, Bonk B, Bash MC. Vaccine. 2025 Jan 1;43(Pt 2):126508. doi: 10.1016/j.vaccine.2024.126508. Epub 2024 Nov 15. PMID: 39549368
- High-throughput bactericidal assays for monoclonal antibody screening against antimicrobial resistant *Neisseria gonorrhoeae*. Stazzoni S, Troisi M, Abbiento V, Sala C, Andreano E, Rappuoli R. Front Microbiol. 2023 Aug 16;14:1243427. doi: 10.3389/fmicb.2023.1243427. eCollection 2023. PMID: 37655342
- Evaluating vaccine-elicited antibody activities against *Neisseria gonorrhoeae*: cross-protective responses elicited by the 4CMenB meningococcal vaccine. Gray MC, Thomas KS, Lamb ER, Werner LM, Connolly KL, Jerse AE, Criss AK. Infect Immun. 2023 91:e0030923. doi: 10.1128/iai.00309-23.
- Assessment of Serum Bactericidal and Opsonophagocytic Activity of Antibodies to Gonococcal Vaccine Targets. Semchenko EA, Jen FE, Jennings MP, Seib KL. Methods Mol Biol. 2022; 2414:363-372. doi: 10.1007/978-1-0716-1900-1_19. PMID: 34784046
- Feasibility of Using a Luminescence-Based Method to Determine Serum Bactericidal Activity against *Neisseria gonorrhoeae*. Clow F, O'Hanlon CJ, Christodoulides M, Radcliff FJ. Vaccines (Basel). 2019 Nov 21;7(4):191. doi: 10.3390/vaccines7040191.
- Complement-Dependent Serum Bactericidal Assays for *Neisseria gonorrhoeae*. Gulati S, Rice PA, Ram S. Methods Mol Biol. 2019; 1997:267-280. doi: 10.1007/978-1-4939-9496-0_16. PMID: 31119629

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Lucidarme J, Louth J, Townsend-Payne K, Borrow R (2019) Meningococcal serogroup A, B, C, W, X, and Y serum bactericidal antibody assays. *Methods Mol Biol.* 1969:169–179. https://doi.org/10.1007/978-1-4939-9202-7_12