

Opsonophagocytic Killing (OPK) 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, neutrophils and other phagocytic cells, like macrophages, are in the *N. gonorrhoeae* infection milieu and could be available for opsonophagocytic killing (OPK).
 - b. OPK assays have been used in vaccine development for the pneumococcus and streptococcus.
2. The OPK assay measures the ability of antibodies to opsonise bacteria for phagocytic killing.
3. OPK assays are performed by incubating serial dilutions of antibody with a bacterial target strain, complement, and phagocytic cells, resulting in bacterial killing (Fig. 1).
4. The OPK titre for each serum is the reciprocal of the serum dilution that results in $\geq 50\%$ killing of the bacteria.

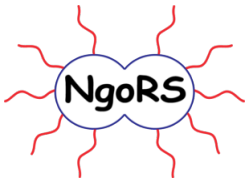
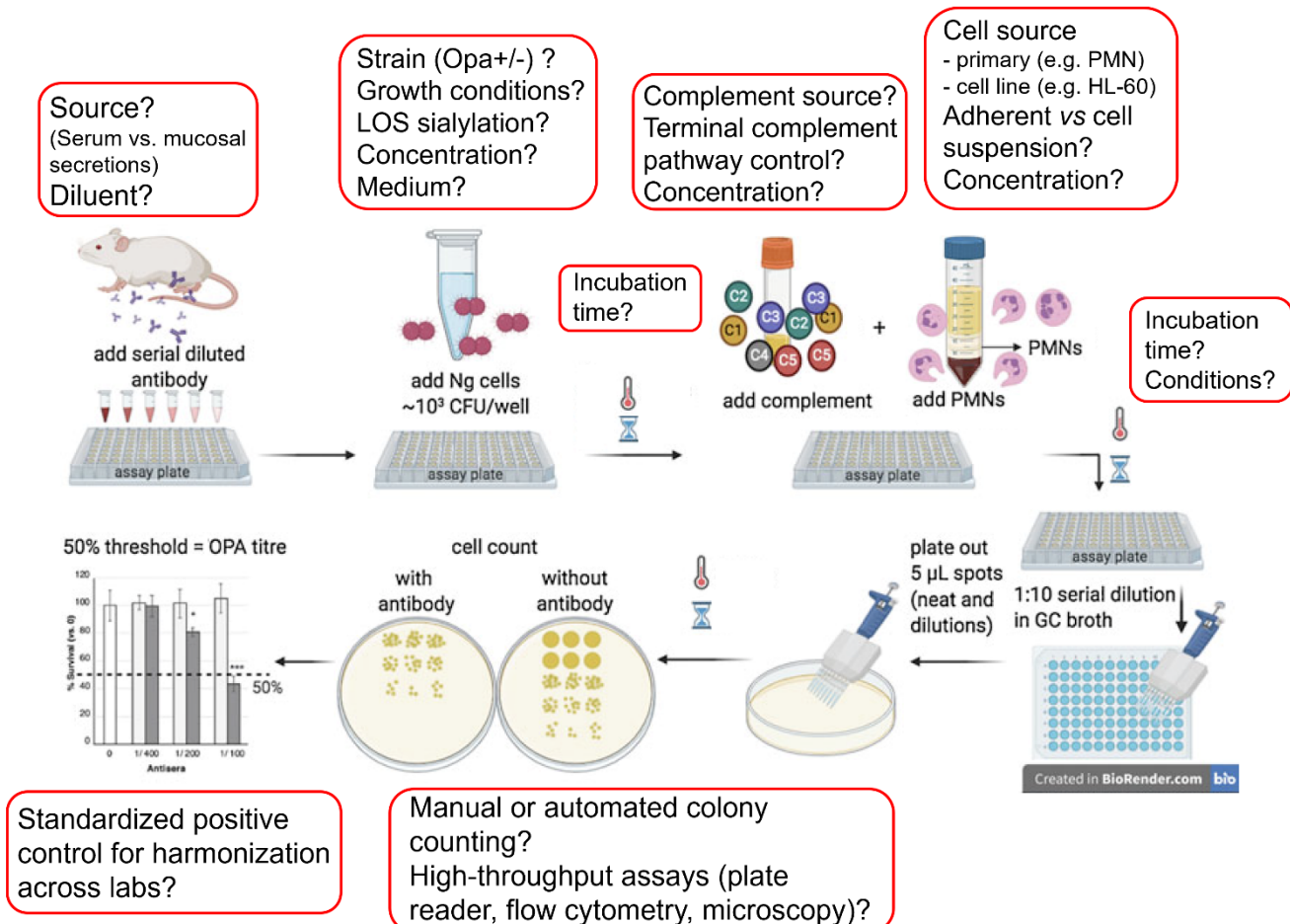


Figure 1. Schematic overview of the opsonophagocytic killing (OPK) assay.



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

- OPK assays are commonly used in vaccine development for gram-positive pathogens. However, there is currently no standardised method for *N. gonorrhoeae*, and there are many variables for each step of the assay (red boxes).

Recent published OPK assay protocols

- 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
- Phase-variable heptose I glycan extensions modulate efficacy of 2C7 vaccine antibody directed against *Neisseria gonorrhoeae* lipooligosaccharide. Chakraborti S, Lewis LA, Cox AD, St Michael F, Li J, Rice PA, Ram S. *J Immunol.* 2016 Jun 1;196(11):4576-86. doi: 10.4049/jimmunol.1600374. PMID: 27183633
- 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. PMID: 37991382