# ACCELERATING THE MANUFACTURE OF GLYCOCONJUGATE VACCINES FOR PNEUMOCOCCAL DISEASE

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*Streptococcus pneumoniae (S. pneumoniae)* is one of the leading causes of invasive bacterial disease in children. In 2000 it contributed to 11% of deaths in children aged 1-59 months (1). Invasive pneumococcal disease can result in septicemia, meningitis and pneumonia. Two pneumococcal glycoconjugate vaccines were introduced in 2000 and 2010 in the USA. Deaths due to the serotypes included in these vaccines in children under 5 have fallen from 183 cases per 100,000 in 1998, to 11 per 100,000 in 2015.

The current gold standard glycoconjugate vaccine for immunization is Prevenar 13. It is the leading global vaccine product generating over $6 billion dollars in revenue in 2016. The cost per dose in the USA is over $100 with a total of four doses required in young children. Due to the complex manufacturing process for the vaccine the expense of the dose is driven up. The process involves separate fermentations for the thirteen serotypes and for the carrier protein component. Subsequent to this are stripping, purification, activation and chemical conjugation steps to make the vaccine and another series of purifications to make the final formulation. The chemical conjugation step requires personnel with a high level of experience and intricate knowledge of the reaction and is a limiting factor for new low income country manufacturers in entering this market.

The advent of Protein Glycan Coupling Technology (PGCT) has been an important development. This plasmid-based technology is able to produce glycoconjugate vaccines intracellularly in *E. coli* (2). As a result, there is no need for the chemical conjugation steps, meaning personnel do not need to have the niche skills currently required. Furthermore, the number of purification steps during the process are also reduced.

*E. coli* cells have been engineered with PGCT to produce a glycoconjugate vaccine of Serotype 4 of *S. pneumoniae*. Using this cell line, transition of *E. coli* growth from shake flasks into small scale bioreactors has been performed. Results show cell biomass is increased in bioreactors and volumetric productivity of cells is improved. It has also become apparent that any changes to the system need to be carefully considered. For example, comparison of two different proteins in this system has found one is more amenable to glycosylation indicating that choice of protein will have an effect on glycoconjugate production. Overall these experiments have demonstrated the scalability of PGCT and has laid the foundation for future optimization of the system.

The work presented here is using *S. pneumoniae* in a platform process for the production of pneumococcal conjugate vaccines. Here a new vaccine production technology is being optimized and scaled to increase product yields, and with a long term aim of reducing cost per dose of the vaccine.

1. O'Brien, K.L. et al., 2009. Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. *The Lancet*, 374(9693), pp.893–902.
2. Cuccui J, Thomas RM, Moule MG, D'Elia R V, Laws TR, Mills DC, et al. Exploitation of bacterial N-linked glycosylation to develop a novel recombinant glycoconjugate vaccine against Francisella tularensis. Open Biol. England; 2013 May;3(5):130002.