APPLICATION OF ANALYTICAL CHARACTERIZATION TOOLS IN PROCESS AND FORMULATION DEVELOPMENT OF LOW COST VACCINES USING THE ULTRA MANUFACTURING PLATFORM

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In 2016, an estimated 19.5 million infants worldwide did not receive routine life-saving vaccinations according to the World Health Organization (WHO)[1]. Two major limitations in improving global vaccination coverage include the costs associated with vaccine manufacturing and the challenges associated with maintaining a consistent supply. The aim of the 'ULTRA' project (Ultra Low-cost TRansferable Automated Platform for Vaccine Manufacturing) is to standardize the development and production of new protein subunit vaccine candidates at globally affordable costs by creating a generic, low-cost, integrated, and automated vaccine manufacturing platform. In a collaborative effort between MIT, UCL, and KU (*Figure 1*), state-of-the-art analytical tools will be utilized to provide well-characterized vaccine bulk and drug product facilitating process changes and reduced QC costs. Additionally, to ensure the stability and potency of vaccines during production, storage, transport and administration to people in the developing world, vaccines will be formulated with stabilizers/adjuvants/preservatives in a multi-dose presentation to further assist in the development of low-cost vaccines. The initial candidate for the ULTRA platform is a subunit vaccine against rotavirus containing three recombinant non-replicating rotavirus (NRRV) protein antigens, P2-VP8-P[4], P2-VP8-P[6], and P2-VP8-P[8]. These protein antigens are currently being developed clinically by PATH with antigens expressed in *E. coli* [2]. Under ULTRA, these NRRV antigens are being produced in *Pichia pastoris*. Primary structure and post-translational modification analysis of the first generation *Pichia* NRRV strains using SDS-PAGE and LC-MS approaches (intact mass and peptide mapping) revealed the presence of intact protein antigen along with certain levels of N-terminal truncation variants, N-linked glycosylation, and protein aggregation. These analytical observations have guided a 'quality-by-design' approach to introduce site-specific modifications in NRRV antigens to minimize N-terminal truncations, glycosylation and reduce aggregation. Further, given the known compatibility issues of NRRV antigens with preservatives and limited binding to aluminum adjuvants (*see our collaborative poster with PATH*), a 'fast-to-formulate (F2F)' direction has been undertaken to engineer variants of NRRV antigens with potentially improved solubility and stability, especially in the presence of preservatives required for multi-dose formulations. High throughput analytical assays utilizing small amounts of material (~0.5 mg) to provide maximal information on antigen structure, relative solubility, and antibody binding have been established to assess developability of F2F NRRV variants for formulation development.

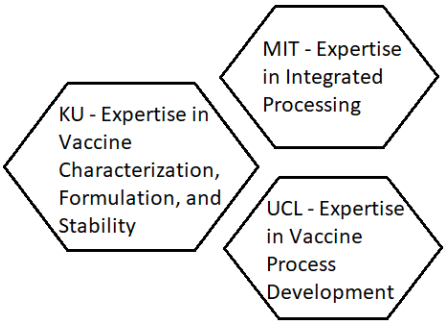

Figure 1 – The academic team of The ULTRA Collaborative

References:
(1) WHO, Immunization coverage http://www.who.int/mediacentre/factsheets/fs378/en/
(2) Fix, A.D. et al, Vaccine (2015), 33(31): 3766-72

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