

FORMULATION DEVELOPMENT OF A RECOMBINANT PROTEIN BASED NON-REPLICATING ROTAVIRUS (NRRV) VACCINE CANDIDATE: ANTIGEN-ADJUVANT-PRESERVATIVE INTERACTIONS

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Rotavirus is the leading cause of acute diarrhea and gastroenteritis among infants and young children worldwide. Over 215,000 children under five years of age die from rotavirus infection each year, mostly in developing world¹. Currently two live attenuated oral rotavirus vaccines are available globally (Rotarix® and RotaTeq®) to reduce the burden of this disease with an efficacy of >90% in developed countries². Vaccine efficacy is lower, however, in developing countries due to a variety of factors³. To this end, a non-replicating rotavirus (NRRV) vaccine candidate, containing three recombinant protein antigens (P2-VP8-P[4], P2-VP8-P[6] and P2-VP8-P[8]), is being developed by PATH and its partners as a trivalent vaccine for use in the developing world⁴. This trivalent rotavirus vaccine candidate includes the three antigens from the most prevalent serotypes associated with >90% of rotavirus gastroenteritis worldwide.

In the present study, the following formulation development issues were examined: (1) establish stability-indicating physicochemical assays for a NRRV protein antigen (P[8]) bound to an aluminum hydroxide adjuvant (Alhydrogel®), which include primary and higher-order structures, chemical and conformational stability of the protein on Alhydrogel, and the ability to desorb the antigen from Alhydrogel; (2) examine the adsorptive capacity and coefficients of Alhydrogel® for the P[8] antigen in several candidate drug product formulations; (3) investigate the effects of binding to Alhydrogel® and the addition of two antimicrobial preservatives (2-phenoxyethanol or thimerosal) on the structural integrity and conformational stability of P[8], the latter of which were found to be potent destabilizers of the antigen; and (4) monitor the real-time and accelerated storage stability over 3 months of P[8] bound to Alhydrogel® in several candidate formulations with and without thimerosal at different temperatures. In the absence of preservative, the P[8] protein antigen was overall stable with only a small amount of Asn deamidation observed in samples stored under real-time (4°C) or accelerated (25°C) temperatures. Similarly, little (if any) changes were observed in the real-time stability of the antigen on Alhydrogel® in the presence of thimerosal. Under accelerated storage temperatures (25 or 37°C) however, the preservative caused an increase in inter-molecular disulfide bonding, decrease of apparent enthalpy as measured by DSC, and a decrease in *in-vitro* antigenicity. Similar stability studies are currently ongoing with the P[4] and P[6] protein antigens.

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References:

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