Obtaining of Vi Polysaccharide Conjugate Batches Using Tetanus and Diphtheria Toxoids at Pilot Scale

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Background: A Vi polysaccharide conjugate demonstrated high effectiveness in 2-5 year old children. It was safe, elicited protective levels of IgG anti-Vi in infants and was compatible with routine vaccines at this age. Based on this success, several vaccine candidates are been developed. Finlay Institute of Vaccines adapted the general conjugation method to a procedure to obtain Vi polysaccharide conjugates using tetanus and diphtheria toxoids as carrier proteins in compliance with WHO guidelines. This procedure was brought to pilot scale. In this work is reported the results of lots produced by the established technology.

Methods: In these processes were used the tetanus (TT) and diphtheria (DT) toxoid batches TET-2014 and DIF2011 respectively, and Vi polysaccharide batches DF-PCST-301, 302 and 303, produced in GMP conditions. Modification of proteins started in 2-3 g and conjugation in 0.5-1 g of polysaccharide. Physicochemical characteristics of the conjugates were evaluated and preliminary stability studies were performed.

Results: Recoveries were above 70%. The protein had very low aggregation, K_D remained unchanged and the average active groups introduced were between 6 and 8 per molecule for tetanus and 3 for diphtheria. The crude reactions were transparent solutions that easily filtrated by 0.2 µm. Polysaccharide recoveries were above 85%. The purified conjugates had consistent K_D and polysaccharide/protein ratios about 0.4-0.5 and 0.5-0.6 for Vi-TT and Vi-DT, respectively. The ^{1}H-NMR analysis showed that polysaccharide identity was maintained and the *O*-acetylation percentages were over 90%. Conjugates were immunogenic in BALB/c mice after one and two dose. Key conjugate features were maintained for 18 months.

Conclusions: Three Vi-TT and Vi-DT conjugates batches at pilot scale were obtained in compliance with WHO guidelines.