## R13 - DEVELOPMENT AND VALIDATION OF MULTIPLEX FOR MEASUREMENT OF ANTIBODIES AGAINST *C. diphteriae, C. tetani* AND *H. influenzae* TIPE B

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**Objectives**: Liquid microarray, a microsphere-based Multiplex assay is a method that is replacing immunosorbent assay (ELISA) in assessing the immunogenicity of multicomponent vaccines in preclinical and clinical trials. This technology utilizes fluorescent distinct microspheres as carriers for different molecules allowing the simultaneous detection of multiple reactions in a small volume of sample with high reproducibility and sensitivity. Therein, the aim of this study was to develop and validate a monoplex and subsequently the Multiplex assay to quantify IgG antibody against diphtheria, tetanus, and *H.influenzae* tipe B (Hib).

**Methods:** Purified diphtheria toxin, tetanus toxin and phosphoribosylribitol phosphate (PRRP) from Hib capsule, DTHib antigens, were coupled to microspheres according to manufactures instructions with different concentrations of those. To determine the best conditions for each antigen, standard curves were prepared using International Reference Serum from NIBSC. The concentration for tetanus and diphtheria ranged from 0.13UI/mL to 0.0002 and for PRRP ranged from 0.4 μg/mL to 0.0005μg/mL. Twenty sera from human pre and post immunized with DTP/Hib vaccine were analyzed by both ELISA and monoplex assays under conditions established. Concentrations of IgG antibodies were determined by 4-parameter logistic standard curve using the SoftMax® program. The values in Mean Fluorescence Intensity (MFI) were converted to IU/mL or μg/mL, also by interpolation for standard curve (4-parameter) for every microesphere region/standard. Values obtained by this assay were compared to those found by ELISA with Nonparametric Spearman analysis using the Graphpad Prisma®.

**Results:** By optimizing of the DTHib monoplexes the best results were obtained with coupled concentrations of 10μg/mL for diphtheria, 1μg/mL for tetanus and 400μg/mL for PRRP. Nonparametric Spearman analysis showed values statistical significant and good correlation between methods with coefficient of 0.608 (p=0.0045) for diphtheria, 0.775 (p=0.001) for tetanus. PRRP results are still being concluded.

**Conclusion**: The present study show promising results between ELISA and monoplex methods. From these results we proceed with the validation of Multiplex assay to evaluate the immunogenicity of the DTP/Hib vaccine.