Optimization of an Indirect ELISA Methods for Detecting Haemophilus influenzae Type B Infections in Children

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Background & Objectives: *Haemophilus influenzae* type (Hib) is one of the most important causes of meningitis and other bacterial infections, especially in children less than 6 years of age. Among Heamophilus influenzae serotypes, serotype b is the most pathogen that can be isolate from patient's samples. It is essential to screen patients by a simple, fast, low cost and specific Methods that can assay the antibody's titration especially in children and infants. Despite the introduction of newdetecting Methods, further studies are being continued in order to modify past Methods. We tried to setup an ELISA system for detecting the anti-capsular antibodies against Hib to solve the problem of poor binding of polysaccharide antigens to solid phase that made the measurement of antibodies difficult.

Methods: Optimized ELISA system was developed to detect antibodies against capsular polysaccharide of Hib 'Polyribosyl ribitol phosphate (PRP)' in serum samples by use of Bovine Serum Albumin (BSA). The PRP was coupled to BSA by use of sodium periodate[3], then appropriate time and temperature of incubation, dilute for antigen and enzyme-conjugate antibody, was optimized. For each of them different modes were examined. Eighty clinical serum samples were collected from children less than 6 years of age and examined by this system to identify their natural immunity to Hib. Sensitivity and specificity of this system compared with a commercial kit produced by The Binding Site (VaccZyme TM Human Anti Haemophilus influenzae EIA Kit).

Results: The best result of each exmined mode was chosen. Anti capsular antibody were detected by optimized methods. Optical densities were similar to commercial kit. False-negative or false-positive didn't observe.

Conclusion: Comparison between results of optimized ELISA procedure and the commercial kit's results suggest that the methods is reliable. Usage of BSA for binding polysaccharide antigens was satisfactory.

Keywords: Haemophilus influenzae; ELISA; PRP