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KEY WORD : HAEMOPHILUS INFLUENZAE / POLYMERASE CHAIN REACTION / ENZYME-LINKED IMMUNOSORBENT ASSAY
DUANGPORN PHULSUKSOMBATI: DEVELOPMENT OF PCR/ELISA FOR RAPID DETECTION OF *HAEMOPHILUS INFLUENZAE* TYPE B. THESIS ADVISOR: UNCHALEE TANSUPHASIRI, M.Sc., KANDA VATHANOPHAS, M.D., M.Sc., SURANG DEJSIRILERT, M.Sc. 178 p. ISBN 974-588-925-3

The polymerase chain reaction (PCR) and simplification of PCR product detection of *H. influenzae* type b (Hib) are developed to increase the sensitivity and specificity of test and for use as an alternative method in clinical microbiology laboratory. Two DNA extraction methods are compared. The DNA extraction by a commercial product was more rapid and convenient than conventional method, phenol-chloroform extraction. DNA yield from both methods was not statistically significantly different. The newly designed PCR primers, 21 bp in length were designed from capsule type-specific DNA sequences from the capsular gene cluster of Hib. It was found that the newly designed primers had more sensitivity which could detect 1 pg of standard Hib DNA. There was a perfect agreement between the newly designed primers and a pair of published primers with K = 1. The PCR product detection was developed by using the ELISA principle. The sense strand of primer was biotinylated and the antisense strand was incorporated with fluorescein at 5′termini. After the optimization of ELISA, the agreement rate of PCR products detection between ELISA and 3 conventional methods, agarose gel electrophoresis, Southern blot hybridization and dot blot hybridization were 1.0, 1.0 and 1.0, respectively by Kappa analysis. A total of 311 blind bacterial meningitis isolates were tested for the validity of PCR/ELISA using culture and serotyping as standard methods. With the OD 405 nm value 0.15 was used as the cut off point, the PCR/ELISA showed high sensitivity, specificity and efficiency with 100%, 99.63% and 99.67%, respectively. The PCR/ELISA developed in this study shows rapidity, simplicity, high sensitivity and specificity for detection of Hib.