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CHINDA KANOKSINSOMBAT: PRODUCTION OF RECOMBINANT ENVELOPE GLYCOPROTEIN GP41 FROM HIV-1 SUBTYPE E. THESIS ADVISOR: RUENGPUNG SUTTHENT, M.D., Ph.D., SUDA LOUISIRIROTCHANAKUL, Ph.D., PRASERT AUEWARAKUL, Dr.Med., WANNEE KANTAKAMALAKUL, Ph.D. 163 p. ISBN 974-663-369-4

Approximately 900,000 HIV-1 infected subjects and 100,000 cases of AIDS were reported in year 1998 by Ministry of Public Health. One million cases of anti-HIV testing have been imported every year. An HIV-1 antigen used in anti-HIV antibody detection tests for diagnosis and screening of HIV-1 infection is derived from HIV-1 subtype B. This subtype has mainly spread in Europe and North America, while HIV-1 subtype E is predominant in Thailand. Enzyme-linked immunosorbent assay (ELISA) used with HIV-1 antigen is the most widely technique used in anti-HIV antibody detection testing because it is suitable for a large-scale screening by blood banks (i.e., more than 100 samples per day). In addition, its sensitivity and specificity are more than 99% and the cost per test is low.

In order to develop a reliable and inexpensive serodiagnostic method, the gp41 transmembrane glycoprotein of HIV-1 *env* gene which contained B cell epitope and conserved sequence, was produced by using two HIV-1 subtype E isolates from Thailand, M46SC and C05, as template DNA. The primers used for the amplification of two parts of *env* gene (gp41), gp41A (N-terminal) and gp41B (C-terminal), with amplified products size 601 bp and 560 bp, respectively, were designed to create unique *Pst* I and *Eco*R I restriction sites. The amplified products were ligated with pGEM-T Easy plasmid and transformed into *E.coli*. The recombinant plasmid DNA (pGEM-T Easy/gp41A and pGEM-T Easy/gp41B) were digested by designed restriction enzymes and then ligated to the pBAD/His C expression vector which contained hexahistidine peptide for easy purification by immobilized metal affinity chromatography (IMAC). After arabinose induction, the recombinant polyhistidine-tagged gp41A and gp41B proteins (25 and 24 kDa) were expressed. The gp41A and gp41B proteins were then purified by immobilized Ni<sup>2+</sup> affinity chromatography (ProBond). The concentration of purified gp41 protein is about 0.2 mg/l of bacterial culture.

Purified recombinant gp41A and gp41B proteins were used as antigen in in-house anti-HIV antibody detection ELISA (indirect method) by coating the protein of concentration 5 µg/ml. The sensitivity and specificity of 99.6 % of this in-house rgp41A and rgp41B based anti HIV-1 ELISA were assessed against 500 anti-HIV seronegative and 500 anti-HIV seropositive sera. The estimated manufacturer cost of in-house rgp41 based anti-HIV ELISA is about 26 Baht/test. These results suggested that this simple and rapid purification method is reliable for obtaining large quantities of purified HIV-1 gp41 recombinant protein without any hazard of growing the virus and the advantage of lower cost. This assay should be considered as a HIV screening test in Thailand especially during this economic crisis.