RECOMBINANT ENVELOPE PROTEIN OF HIV-1 SUBTYPE E AS ANTIGEN IN HIV-1 ANTIBODY DETECTION ENZYME IMMUNOASSAY

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Abstract. In order to develop a reliable and inexpensive serodiagnostic method to be used for anti-HIV antibody detection in Thailand, recombinant envelope (TM or gp41 subunit) protein of HIV-1 subtype E was produced from prokaryotic cell (*Escherichia coli*) as the source of antigen in enzyme immunoassay (TE diagnostic EIA kit). HIV-1 gp41 subunit of subtype E was successfully expressed in *E. coli* in the form of polyhistidine-tagged proteins, comprising of rgp41A (601 bases N-terminal half of TM or 25kDa) and rgp41B (560 bases C-terminal half of TM or 24 kDa) by using an expression vector, pBAD/His C. The amount of protein, dilution of sera, and anti-human IgG labeled HRP used in the EIA test optimized by a checker board titration of the protein and seropositive or seronegative sera, were 5.0 μg/ml, 1:300, and 1:4,000, respectively. The blinded test evaluation of TE-diagnostic EIA in 500 seropositive and 500 seronegative sera which have been simultaneously tested by two available commercial kits and compared with our TE diagnostic EIA, gave 99.6% sensitivity and specificity. The other known genetic subtypes sera such as subtype A (n=5), B (n=9), C (n=4) and D (n=5) were also positive with this EIA. The estimated manufacturer cost per test of rgp41 based anti-HIV antibody detection EIA or TE-diagnostic EIA was about 15 baht. This recombinant envelope (gp41 or TM) protein from HIV-1, which can be produced in large quantities without any hazards from growing the virus and has lower cost to produce anti-HIV antibody serological diagnostic kit, should be considered as an HIV screening test in Thailand.

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1), formally known as HTLV-III or LAV-1, and a causative agent of acquired immunodeficiency syndrome (AIDS), was first reported in 1983 (Barre-Sinoussi *et al*, 1983). Soon thereafter, this virus was recognized as a member of the same group of retroviruses, *Lentivirinae* family. In 1986, the International Committee on Taxonomy of Viruses recommended the name of AIDS virus as human immunodeficiency virus, or HIV (Clavel *et al*, 1986; Coffin *et al*, 1986).

HIV-1 was first spread among IDUs in Thailand in 1988 (Ungchusak 1993; Vanichseni *et al*, 1989; Weniger *et al*, 1991; Wright *et al*, 1994). Two major HIV-1 subtypes are circulating in infected Thai population, B′ and E. HIV-1 subtype B′, which is quite different from the predominant HIV-1 circulating in North America and Europe, began spreading rapidly among IDUs (Ou *et al*, 1993). Until 1990, HIV-1 epidemic was observed among males who had sexual contacted with female sex workers in northern Thailand (Nopkesorn *et al*, 1993); the virus was later found to be subtype E which has rapidly expanded throughout the country and become the predominant subtype in Thailand (Weniger *et al*, 1994). Approximately 98% of heterosexually acquired cases in Thailand

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are HIV-1 subtype E (Subbarao *et al*, 1998). Although HIV-1 subtype B′ was first found to be the common subtype among IDUs group, however, increasing of HIV-1 subtype E infection (75%) (Subbarao *et al*, 2000) has been recently reported among these groups. In Thailand, more than 900,000 HIV-1 infected subjects were reported in 2000 and 100,000 cases develop AIDS (Division of Epidemiology, 2000).

Several laboratory methods are used for diagnosis of HIV-1 infection: the detection of anti HIV antibody by enzyme immunoassay (EIA), agglutination test, Western blot, indirect immunofluorescent assay; HIV-1 p24 antigen by enzyme immunoassay (EIA); HIV-1 gene detection by polymerase chain reaction (PCR). Although various assays for diagnosis of HIV-1 infection have been developed for the detection of antibodies to this virus, EIA is suitable for a large-scale screening by blood banks (*ie*, more than 100 samples per day) (Tamashiro *et al*, 1993). Commercial kit available for anti-HIV antibody detection in human sera is based on technique of using HIV-1 antigen; viral lysate, recombinant protein: gp120, gp41, p24, and synthetic peptide coated solid phase.

In Thailand, more than one million anti-HIV antibody tests are conducted per year using imported test kits. HIV-1 antigens used in these test kits derived from subtype B that mainly found among HIV-1 infected Europeans and Americans. HIV-1 subtypes B and E are noticeably endemic in Thailand (McCutchan *et al*, 1992). Envelope nucleotide sequences of each subtype are about 20-30% different (Subbarao *et al*, 1996). The envelope gene product, SU or gp120 and TM or gp41 subunits, must be different and it might interfere with specificity of HIV-1 antibodies in early infection that specific for the same epitope. This together with Thai economic problem, this study has established a TE diagnostic EIA kit for anti HIV antibody detection using HIV-1 subtype E which is endemic in Thailand, to increase sensitivity and specificity test and to reduce the cost of the test.

MATERIALS AND METHODS

Virus and serum samples

HIV-1 subtype E isolate, 96THH10 (GeneBank Accession number AY005168), was isolated from a 25-year-old HIV-1 infected asymptomatic Thai male and the nucleotide sequence of envelope gene was identified as subtype E. HIV-1 proviral DNA of 96THH10 virus was used as target for gp41 region of *env* gene amplification. One thousand serum samples were collected from 225 workers (who were checked up for HIV infection before going aboard), 417 pregnant women from antenatal care clinic, and 358 outpatients and admitted patients from Siriraj Hospital. Random sampling was used by selection the sera that came on the odd date during March 1998 until March 1999. These sera consisted of 500 each seropositive and seronegative by using anti-HIV antibody detection from commercial kits of Vironostika HIV Uniform II plus O (Organon Teknika BV, Netherlands) and Serodia HIV (Fujirebio, Japan).

Cloning of HIV gp41 part A and part B

DNA lysate from HIV-1 infected cells (96THH10) was heated at 56ºC for 1 hour, and transferred to 95ºC for 10 minutes. Nested PCR was used to amplify env (gp41) part A and B with outer primer (DO1: CTT CAG ACC TGG AGG AGG, 7608-7625, and envM: TAG CCC TTC CAG TCC CCC CTT TTC TTT TA, 9069-9097) and two inner primer pairs; gp41A (41BFA: GGC *CTG* *CAG* GCA GTG GGA ATA GGA GCT and 41RA: GGC *GAA* *TTC* TCA TTA TCC CTG CCT AAC TCT ATT TAC) and gp41B (41BFB: GGC *CTG* *CAG* CTG TGG TAT ATA AAA ATA TTT and 41RB: GGC *GAA* *TTC* TCA TTA TAG CAA AGC CCT TTC T). PCR was performed by using the 2400 DNA Thermal Cycler (Perkin Elmer Cetus, USA) for 3 cycles, each consisted of 94ºC denaturation step for 1 minute (6 minutes for first cycle), 55ºC annealing step for 1 minute, and 72ºC extension step for 1 minute, followed by 31 cycles of 94ºC for 15 seconds, 55ºC for 45 seconds, 72ºC for 90

seconds and the final extension at 72ºC for 10 minutes in both of first round and second round PCR.

The specific amplified products of gp41 part A (601 bases) and gp-41 part B (560 bases) were cloned into vector pGEM-T (Promega, USA). Digested gp41A or gp41B DNA, *Pst* I / *Eco*R I cut was inserted into expression vector pBAD/His C vector and used to transform LMG194 competent *E. coli* cells. All colonies of *E. coli* containing pBAD/His C-gp41A and pBAD/His C-gp41B was checked for positive clones containing gp41A and gp41B insert fragment of 601 and 560 bp by digestion plasmid DNA with restriction enzyme, *Pst* I and *Eco*RI.

Induction of recombinant protein expression (Guzman *et al*, 1995)

Overnight culture of *E. coli* strain LMG containing pBAD/His C-gp41A or pBAD/His C-gp41B was diluted 1/10 in RM medium containing 50 μg/ml ampicillin and incubated at 37ºC with vigorous shaking until OD_{600} was 0.5-0.6. Arabinose was added to a final of 0.0002% after a variation of ten-fold dilution from 0.2% to 0.00002% to induce the production of polyhistidine tagged protein. The culture was incubated for 3 hours at 37ºC with shaking after varied induction time from 1-7 hours.

Inclusion bodies of recombinant proteins were collected by centrifugation of cells at 10,000 rpm for 20 minutes at 4ºC. The pellet was solubilized in 1/50 of the original volume in 6M guanidine lysis buffer (Fischer *et al*, 1993). The supernatant was applied to metal affinity column to purify the polyhistidine tagged protein.

Purification of recombinant proteins (Belew *et al*, 1987)

Nickel affinity resin (ProBond™) is commercially available from Invitrogen, Inc, USA. One ml resin in denaturing binding buffer pH 7.8 was mixed with 5 ml of protein extract in 6M guanidium lysis buffer and allowed to bind the resin for 20 minutes at room temperature by slightly shaking. The bound resin was transferred to the column, then the resin was eluted by denaturing elution buffer pH 4.0. Fractions were collected and measured for OD_{280}. The eluted fractions of gp41 at the peak of high absorbance (OD_{280}) were pooled and dialyzed against 50 mM NaH_2PO_4 and 100 mM NaCl pH 8.0 with slow decreasing of urea concentration (8M to 0M). Finally, the recombinant proteins were dialyzed against 50mM NaH_2PO_4 and 50 mM NaCl pH 8.0 overnight at 4ºC. After dialysis of polyhistidine tagged protein, protein quantity was determined by a colorimetric method, using BioRad Dc protein assay kit. The polyhistidine tagged protein was characterized by SDS-PAGE (3.85% stacking gel and 15% separating gel) by staining the gel with Coomassie blue for protein identification or blotted onto a nitrocellulose membrane for immunoassay. The proteins on the nitrocellulose membrane were assayed by incubation with mouse anti-Xpress serum (QIAGEN, USA) or HIV seropositive and seronegative sera.

Enzyme immunoassay (EIA)

Polyvinyl microtiter plates were coated with 100 μl/well of recombinant gp41A and gp41B proteins diluted in 0.1 M bicarbonate buffer and kept at room temperature overnight. After washing three times, the rgp41-coated plates were blocked with 100 μl/well of milk buffer at 37ºC for 1 hour. One hundred microliter per well of diluted sera, varied from 1:50 to 1:500 to identify the suitable dilution, were added and the plates were incubated at 37ºC for 1 hour. After five times washing, 100 μl/well of varied concentrations from 1:2,000 to 1:4,000 of horseradish peroxidase (HRP) (Zymed, USA) conjugated goat anti-human IgG in milk buffer were added into each plate. The plates were put in an incubator at 37ºC for 1 hour and washed five times. Finally, 100 μl/well of TMB chromogen were added and the plates were left at room temperature (in the dark) for 10 minutes. The reaction was then stopped with 100 μl/well of 1 M H_2SO_4. The color intensity was measured at 450 nm with

microtiter plate reader. The cut-off value of absorbance was calculated as: cut-off = 0.124 [(X+3SD) x 2], X = mean of all negative samples absorbance +3 standard deviation) X 2. ELISA index (EI = Absorbance /cut off) is a ratio of absorbance value of any serum and cut-off value. EI of any area is less than 1, interpretation is negative, and EI ≥ 1 means a positive result.

RESULTS

Recombinant protein expression

The size of polyhistidine-tagged rgp41A protein was shown to be about 25 kDa, whereas polyhistidine-tagged grp41B protein was about 24 kDa (Fig 1). Although, Western blot analysis against anti-Xpress antibodies (data not shown) and anti-HIV seropositive pooled sera were able to identify 25 kDa rgp41A and 24 kDa rgp41B protein products in both pellet and supernatant, both polyhistidine-tagged rgp41A and rgp41B proteins were found more in pellet part of cell lysate than in supernatant part of cell lysate in 15% SDS-PAGE as showed in Fig 1.

Optimization of rgp41 protein expression

The optimization of protein expression in *E. coli* was performed to obtain maximum level of protein induction by arabinose. Two factors were found, *ie*, concentration of arabinose and duration of arabinose induction. The varied final concentrations of arabinose to induce protein expression for 4 hours were 0.2%, 0.02%, 0.002%, 0.0002% and 0.00002%. The result showed the difference in level of protein expression among these conditions with the highest in 0.0002% and lowest in 0.00002% arabinose concentration (Fig 2). The arabinose concentration of 0.0002% was selected for induction in the further experiments because it was the lowest concentration of arabinose that can induce protein products.

The result of Western blot showed that after induction with 0.0002% arabinose, all

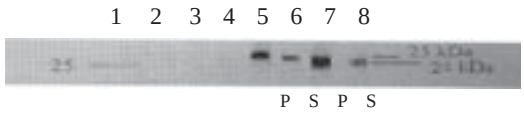

Fig 1–Western blot analysis of polyhistidine-tagged rgp41A and rgp41B expressed in *E. coli* LMG 194 strain. Protein from SDS-PAGE was transferred to nitrocellulose membrane and reacted with anti-HIV seropositive sera. P and S denoted as pellet and supernatant fractions respectively.
Lane 1: pre-stain broad-range protein molecular weight markers, 16-175 kDa.
Lanes 2-4: protein produced by *E. coli* cells containing only pBAD/His C vector.
Lanes 5-6: protein produced by recombinant clone containing rgp41A.
Lanes 7-8: protein produced by recombinant clone containing rgp41B.

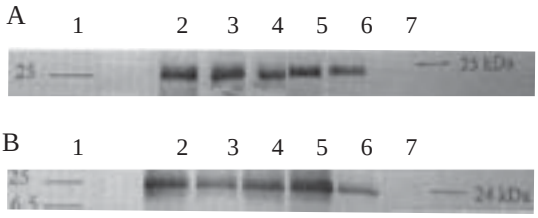

Fig 2–Western blot analysis of polyhistidine-tagged rgp41A (A), and rgp41B (B) expressed in various concentrations of arabinose induction for 4 hours. Bacterial cells were collected and solubilized in sample buffer before separation by 15% SDS-PAGE and Western blot onto nitrocellulose membrane.
Lane 1: pre stain broad-range protein molecular weight markers, 16-175 kDa.
Lanes 2-6: protein from the induction of 0.2%, 0.02%, 0.002%, 0.0002%, 0.00002 % arabinose, respectively.
Lane 7: uninduced protein.

samples (at 1 hour to 7 hours induction) contained the polyhistidine-tagged rgp 41 protein (Fig 3). Although, the highest level of protein expression of rgp41A and rgp41B were demonstrated at 5 and 7 hours induction, there was slightly different in protein expression comparing with 3 or 4 hours induction. Because of

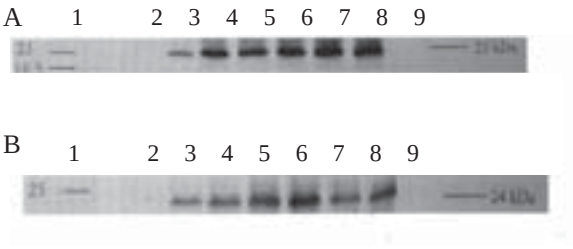

Fig 3–Western blot analysis of polyhistidine-tagged rgp41A (A) and rgp41B (B) expressed in various incubation times after induction with 0.0002% arabinose. Bacterial cells were collected and solubilized in sample buffer before separation by 15% SDS-PAGE and Western blot onto nitrocellulose membrane.
Lane 1: pre-stain broad-range protein molecular weight markers, 16-175 kDa.
Lanes 2-8: protein from various times; 1-7 hours of induction with 0.0002% arabinose respectively.
Lane 9: uninduced protein.

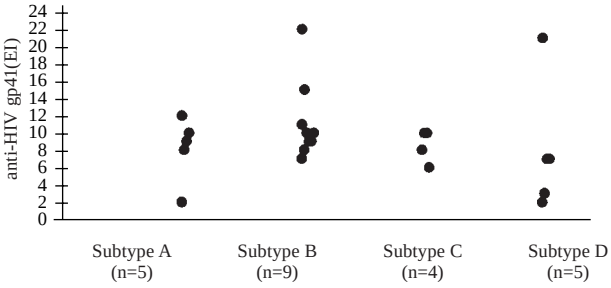

Fig 4–Anti-HIV antibody detection by rgp 41 based EIA in known genetic subtypes sera including subtype A (n=5), B (n=9), C (n=4) and D (n=5).

the lowest background appeared in 3 hours induction; thus 3-hour incubation was selected.

Purification of polyhistidines-tagged rgp41 protein

Sixteen fractions from rgp41A and thirteen fractions from gp41B were collected from acid elution of bound polyhistidine-tagged rgp41 protein. The purity of protein was determined by 15% SDS-PAGE followed by Western blot against anti-HIV seropositive pooled sera (data not shown). The concentration of rgp41A and rgp41B products that was purified by ProBond affinity chromatography per liter cell culture was 0.2 mg/ml.

Enzyme immunoassay

In order to find optimal amount of antigen (rgp41) that would give the lowest background in the EIA test. A checker board titration of the rgp41 protein from 2.5 μg/ml, 5.0 μg/ml and 10 μg/ml dilutions of the tested sera from 1:50, 1:250 and 1:500, and concentration of peroxidase-goat anti-human immunoglobulin G (Gamma Chain) from 1:2000 to 1:4000 were set up (Table 1). The concentrations of the 5.0 μg/ml antigen, 1:300 diluted sera and 1:4000 of peroxidase-goat anti-human immunoglobulin G that gave strong positive in anti-HIV seropositive sera with low background in anti HIV seronegative sera were chosen.

After the optimal condition of rgp41 based anti-HIV antibody detection, EIA or TE diagnostic EIA kit was set up using known 100 seropositive and 126 seronegative sera to test sensitivity and specificity. Each serum was tested both with one commercial ELISA test and gel particle agglutination. The 100% sensitivity and 99.2% specificity were detected as shown in Table 2. This rgp41 based anti-HIV antibody detection EIA was also used to test sera from other HIV-1 subtypes including subtypes A (5), D (9), C (4), and D (5) and all these sera were positive as shown in Fig 4. We also performed blind test of 500 seropositive sera and 500 known seronegative sera by using Vironostika HIV Uniform II (Organon Teknika BV, Netherlands) and serodia HIV (Fujirebio Inc, Japan) compared with our rgp41 based anti-HIV antibody detection EIA. Both sensitivity and specificity of rgp41 based anti-HIV antibody detection EIA in these 1,000 sera were 99.6% (two each out of 500) (Table 3).

DISCUSSION

The transmembrane protein of the HIV-1, gp41 or TM subunit, contains complex sugar

Table 1
The checkerboard titration of the gp41 protein in enzyme immunoassay. The protein was varied from 2.5 μg/ml, 5.0 μg/ml and 10 μg/ml, dilution of the tested sera from 1:50, 1:250 and 1:500 and concentration of peroxidase-goat anti-human immunoglobulin G (Gamma Chain) from 1:2,000 to 1:4,000 were set. The concentration of the 5.0 μg/ml antigen, 1:300 diluted sera and 1:4000 of peroxidase-goat anti-human immunoglobulin G that gave strong positive in anti-HIV seropositive sera with low background in anti-HIV seronegative sera were chosen.

		1	2	3	4	5	6	7	8	9	10	11	12
		conj	1:2000	conj	1:4,000	conj	1:2,000	**Conj**	**1:4,000**	Conj	1:2,000	conj	1:4,000
	serum dilution	serum +	serum -	serum +	serum -	serum +	Seru m-	serum +	serum -	serum +	serum -	serum +	serum -
A	1:50	3.348	0.344	3.172	0.195	3.474	0.366	2.908	0.029	3.065	0.407	2.364	0.255
B	1:250	3.498	0.130	2.819	0.085	3.254	0.137	**2.555**	**0.094**	2.764	0.156	1.916	0.109
C	1:500	3.138	0.076	2.507	0.062	2.954	0.084	2.146	0.069	2.350	0.109	1.474	0.125
D	Bank	0.006	0.003	0.008	0.006	0.011	0.011	0.009	0.006	0.009	0.003	0.006	0.005
Protein concentration		10 μg/ml				5.0 μg/ml				2.5 μg/ml			

Table 2
Sensitivity and specificity of rgp41 based anti-HIV antibody detection EIA in 100 seropositive and 126 seronegative sera.

Specimen tested	No. positive[a]/total	Sensitivity (%)	Specificity (%)
HIV-l positive sera based on commercially tests[b]	100/100	100	
HIV-l negative sera based on commercially tests[b]	1/126		99.2

[a]Evaluation as positive or negative is based on an OD_{450} greater or less than the established cut-off point of OD_{450} = 0.183 for each assay, respectively.
[b]Commercially tests are Genelavia Mixt (Sanofi Diagnostics Pasteur Ltd, France); Behring (Dade Behring Marburg GmbH, Germany), Vironostika HIV Uniform II (Organon Teknika BV, Netherlands), and Serodia (Fujirebio Inc, Japan).

Table 3
Sensitivity and specificity of rgp41 based anti-HIV antibody EIA in blinded test of 500 seropositive and 500 seronegative sera.

Specimen tested	No. positive[a]/total	Sensitivity (%)	Specificity (%)
HIV-l positive sera based on commercially tests[b]	498/500	99.6	
HIV-l negative sera based on commercially tests[b]	2/500		99.6

[a]Evaluation as positive or negative is based on an OD_{450} greater or less than the established cut-off point of OD_{450} = 0.124 for each assay, respectively.
[b]Commercially tests are Vironostika HIV Uniform II (Organon Teknika BV, Netherlands) and Serodia (Fujirebio Inc, Japan).

side chains and a stretch of hydrophobic amino acids that serve to anchor the protein in the cell membrane and catalyze fusion between the virus membrane and the membrane of the target cell (Veronese *et al*, 1985). The carboxyl-terminal side of the probable membrane-spanning region consists of a hydrophilic region (residues 724-745) and a terminal region (residues 745-856) of alternating hydrophilic and hydrophobic character. The N-terminal sequences of gp41 are largely hydrophilic although small stretches of hydrophobic amino acid do occur. It is known that the hydrophobic sequences can inhibit recombinant protein synthesis in bacteria, part of this region may normally be held within the lipid membrane thereby limit its immunogenicity (Windheuser and Wood, 1988). N-terminal region (548-736) of gp41 clone demonstrating very strong immunoreactivity with the immunodominant epitope (s), as well as the C-terminal region (732-863) of gp41 clone, reacted well with most or all of the HIV-positive sera. Because of the transmembrane domain (647-758) of gp41 molecule traverses the cell membrane, the immunoreactive epitopes do not appear in this region. (Samuel *et al*, 1988). HIV-1 nucleotide sequencing of *env* gene from 96THH10 isolate was compared with other subtype E isolates from Thailand and other subtypes (data not shown) and TM or gp41 region is the least genetic diversity among *env* gene.

Both polyhistidine-tagged HIV rgp41A and rgp41B proteins expressed in this study were accumulated in *E. coli* in the discrete form of the inclusion bodies. This is the greatest drawback of bacterial expression system (Marston, 1986). The advantage of expression in the form of fusion protein is that recombinant proteins can be purified using an affinity chromatography either with antibodies or ligand binding specific to carrier protein. The vector used throughout this work, pBAD/HisC vector, has a sequence that encodes an N-terminal peptide containing a hexahistidine tag which is a metal binding domain attached to the recombinant protein, and an enterokinase recognition sequence that can be used to cleave the protein from the hexahistidine if required. The advantages of using polyhistidine tag as carrier peptide are that the 6xHis tag is far smaller than most other affinity tags, poorly immunogenic in most species, rarely interferes with protein structure or function, and does not require removal by protease cleavage.

The level of the recombinant protein synthesis varied from an estimated 5-20% of the total *E. coli* protein, which was enough to show a distinct band on stained gel (Windheuser *et al*, 1988). The rgp41A and rgp41B fusion protein could be detected immunologically by Western blot with an anti-HIV positive serum but obvious fusion band was little visible on a Coomassie blue-stained SDS-PAGE. The protein from the 6xHis tag should not be cleaved by the enterokinase recognition sequence because it was not interfered with specificity of TE diagnostic EIA, which was demonstrated as no bands presented in Western blot assay against anti-HIV seropositive sera of non-inserted pBAD/His vector control lane.

The rgp41 protein yield, about 0.1 mg/ml of bacterial culture, was quite low. Although, the usage of pBAD/His vector can avoid toxicity caused by over-expression of some proteins. We primarily used both of pTrcHis and pET vectors as expression vectors and none of the proteins product was founded (data not shown). In this study, the rgp41A and rgp41B also has the transmembrane domain, which is toxic to bacterial cells. It was found that the HIV-1 transmembrane fragment with the highest lytic activity and that most drastically altered the membrane barrier was residue 684 to 705 (Arroyo *et al*, 1995). The above study was the reason for this study that the proteins expressed from both regions including transmembrane domain may be toxic to bacterial cells and gave the low yield of protein. Fragment A gave lower amount of expressed protein than the fragment B, which contains the transmembrane domain and the amphipathic helices.

The sensitivity and specificity of rgp41 based anti-HIV antibody detection EIA in blinded test trial were 99.6% (n=500) and 99.6% (n=500) (two each out of 500). The discordant

results were retested by both assays and also retested by Western blot (Genelabs) to confirm the result. There was a report showing 100% sensitivity (36/36) and 99.6% specificity (265/266) when purified gp41 (1μg/well) was used in EIA (Sohn *et al*, 1993). However, cross reaction of this EIA system can be demonstrated in tested sera because most false positive reactions may be derived from contaminated bacterial proteins which are often recognized by antibodies in human sera.

The commercially available tests have 3 to 5 times higher cost than our rgp41 based anti-HIV antibody detection EIA. The cost of EIA tests and agglutination tests that available in the HIV testing market are about 40-60 baht, while the automated HIV testing is about 120-150 baht. The cost of our rgp41 based anti-HIV antibody detection EIA test is calculated, based on reagent, equipment, maintenance, labor, and management, to be about 11-15 Baht/test.

Theoretically, a recombinant protein derived from genetic engineering of prokaryotic or eukaryotic system is a source of a superb antigen for initially screening of HIV antibodies due to high sensitivity and specificity. High sensitivity (99.6%) and specificity (99.6%) were found in this rgp41 based anti-HIV antibody detection EIA. Thus, this EIA which used HIV-1 recombinant gp41 proteins as antigen, can be produced in large quantities without any hazards from growing the virus and the cost is lower for HIV screening test in Thailand especially in this economic crisis. Moreover, this study will be the starting point for producing other HIV-1 recombinant proteins in order to develop a test kit with more sensitivity for the detection of anti HIV-1 antibodies in Thailand.

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