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Table 1 Monoclonal antibodies used in this study

No.	Clone	Specific With	Isotype	Protective activity
1	P1 (2C3D4)	*L .australis, L. bangkok, L .bratislava*	IgM	ND
2	P2 (8C6C4A12)	*L. australis, L. bangkok,*	IgMk	yes
3	P4 (2D2A12G3)	*L. autumnalis*	IgMk	ND
4	P5 (15BC2G1)	*L. biflexa*	IgMk	ND
5	P6 (1B4D3F10)	*L. bataviae*	ND	ND
6	P7 (16B1C9H7)	*L. Sejroe (serogroup)*	IgMk	ND
7	P9 (4A2D5D6)	*L. bratislava*	ND	ND
8	P10 (1A4B10E1)	*L. pyrogenes*	IgMk	ND
9	P17 (8C1E11)	*L. ranarum*	IgMk	ND
10	F11 (9B4C1D8)	*Leptospira*	ND	yes
11	F20 (9A4G1A8)	*Leptospira*	ND	yes
12	F21 (9A5A11A11)	*Leptospira*	ND	yes
13	LD5	*L. interrogan*	IgG1	ND
14	LF9	*L. biflexa*	IgG1	ND

Table 2 Deduced amino acid sequences of capsid fusion peptides of T7 phage that respectively bind to fourteen MAbs

T7/ MAb	display peptides
T7/P1 (7)	CSSKSYRPC (5), CNKPKNASC (1) , CPHLPNSTC (1)
T7/P2 (9)	CTPKKSGRC (2), CDSNKSGRC (2), CSKKSTRNC (1), CSKKDPRNC (1), CRKKNTNNC (1), CRKSKSASC (1), CTTNSKRKC (1)
T7/P4 (10)	CDPNTNSFC (5), CDPNTNNFC (1), CGIPGTPDC (3), CDINTNSFC (1)
T7/P5 (9)	CKSKKSSSC (5), CNKKDPSSC (1), CSKKNPGNC (1), CKSKLVRC (1), CESNKSSC (1),
T7/P6 (10)	CDAPPSGIC (9), CGRTRVTRC (1)
T7/P7 (9)	CFATVERAC (7), CLLTVERAC (1), CDSDWPVSC (1)
T7/P9 (8)	CPPPKKGNC (1), CDT* (1), CKSKKSSAC (1), CERILWVVC (1), CNIRNAYTC (1), CDRNTMVIC (1), CDPNTSNFC (1), CCTNILVVC (1),
T7/P10 (10)	CTKKKSSSC (1), CRKKISTIC (1), CRKKNPSNC (1), CDKKYYGCC (1), CDTNTYYCC (1), CRPNKKNAC (1), CDT* (1), CSPKKKGSC (1), CRKS* (1), CCDSKYYLRC (1)
T7/P17 (10)	CRTITNDKC (1), CSTIYNDDC (3), CNNIYNDDC (3), CPSIYNDDC (1), CTKKGPRNC (1), CDTRDLVVC (1),
T7/F11 (8)	CPKSKSSRC (1), CFNSTNDPC (2), CFNATNDPC (2), CTPKKNRAC (1), CSKKRSISC (1), CLTPLNDPC (1)
T7/F20 (6)	CSTLINIFC (3), CRTKKTGSC (1), CFK* (2)
T7/F21 (1)	CSPKRKANC (1)
T7/LD5 (5)	LTPCDNY* (5)
T7LF9 (5)	CVLKKNRPC (3), CLP* (2)

- Numbers in parentheses indicate the numbers of clones identified
- All Phages clones were ELISA positive with each monoclonal antibody (respectively)
- Bold letter mean the amino acid sequences that match between phage display peptide and Leptospira genome from gene bank amino acid sequence
- * Mean stop codon (TAG)

Table 3 Comparison of phages peptide sequence with gene bank protein sequences

Consensus sequence of five phages T7/P1

M L G D P N S L T P C S S K S Y R P C

S S K S Y R

Part of amino acid sequence from hypothetical protein LIC12572 of Leptospira interrogans serovar Copenhageni Strain Fiocruz L1-130 (genebank accession number YP 002496.1)

Consensus sequence of two phages T7/P2

M L G D P N S L T P C T P K K S G R C

P K K S

Part of amino acid sequence from Leptospira kirschneri serovar grippotyphosa strain RM52 ligC gene, complete sequence; and unknown gene (genebank accession number i|31322246|gb|AY190127.1)

Consensus sequence of four phages T7/P2

M L G D P N S L T P C D S N K S G R C

K S G R C

Part of amino acid sequence from hypothetical protein LIC10450 [Leptospira interrogans serovar Copenhageni str. Fiocruz L1-130] (genebank accession number gi|45656347|ref|YP_000433.1)

Consensus sequence of one phage T7/P2

M L G D P N S L T P C T T N S K R K C

T N S K R K

Part of amino acid sequence from deoxyribodipyrimidine photolyase [Leptospira interrogans serovar Copenhageni str. Fiocruz L1-130] (genebank accession number gi|45657217|ref|YP 001303.1)

Consensus sequence of five phages T7/P4

M L G D P N S L T P C D P N T N S F C

D P N V N S F

Part of amino acid sequence from Leptospira interrogans serovar lai str. 56601 chromosome I, section 325 of 397 of the complete sequence (genebank accession number gi|24197574|gb|AE011516.1)

Consensus sequence of four phages T7/P5 and one phage T7/P9

M L G D P N S L T P C K S K K S S S C

K S K K S S

Part of amino acid sequence from cysteine synthase [Leptospira interrogans serovar Copenhageni str. Fiocruz L1-130] (genebank accession number gi|45657685|ref|YP_001771)

Consensus sequence of one phage T7/P10

M L G D P N S L T P C T K K K S S S C

C T K K K W S

Part of amino acid sequence from hypothetical protein LIC12958 [Leptospira interrogans serovar Copenhageni str. Fiocruz L1-130](genebank accession number gi|45658785|ref|YP_002871.1|)

Consensus sequence of one phage T7/F11

M L G D P N S L T P C P K S K S S R C

S K S S R C

Part of amino acid sequence from hypothetical protein LIC12963 [Leptospira interrogans serovar Copenhageni str. Fiocruz L1-130](genebank accession number gi|45658790|ref|YP 002876.1|)

Consensus sequence of three phages T7/F20
M L G D P N S L T P C S T **L I N I F** C
T L I N I F
Part of amino acid sequence from unknown protein [Leptospira interrogans]
Length = 502 (genebank accession number gi|17940077|gb|AAL49467.1)

Consensus sequence of five phages T7/LD5
M L G D P N S **L T P C D** N Y
V G I P I L T **L T P C D N** Q N L
Part of amino acid sequences from hypothetical protein LIC11014 [Leptospira interrogans serovar Copenhageni str. Fiocruz L1-130] (genebank accession number gi|45656900|ref|YP_000986.1|)

Bold letter mean the display peptide of bound phage that match with the gene bank protein sequences

APPLICATION OF PHAGE DISPLAY AND POLYMERASE CHAIN REACTION (PCR) - BASED TECHNIQUE FOR MIMOTOPE SEARCHING AND STRAIN IDENTIFICATION OF ENTERIC BACTERIA

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APPLICATION OF PHAGE DISPLAY AND POLYMERASE CHAIN REACTION (PCR) - BASED TECHNIQUE FOR MIMOTOPE SEARCHING AND STRAIN IDENTIFICATION OF ENTERIC BACTERIA.

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ABSTRACT

A phage display technique was applied for the searching of the mimotopes *Shigella sonnei* and Shiga-toxin producing- *Escherichia coli*, using established specific monoclonal antibodies (MAbs). The PCR-based techniques, namely the touch-down (TD)-PCR and ERIC-PCR were applied for the study of the virulence genes of *Vibrio parahaemolyticus* isolated from patients with diarrhea (namely *tdh* and *trh*). By using the random peptide T7 phage display libraries, a partial consensus sequence of T7 phages which bound to *S. sonnei* MAb was RxxxNSN. Using TD-PCR, 13 of 22 (59.1 %) *V. parahaemolyticus* clinical isolates were tdh^+, trh^-, 2 other isolates were tdh^+, trh^+. All of the 22 *V. parahaemolyticus* isolates were sensitive to trimethoprim/sulfamethoxazole, nalidixic acid, chloramphenicol, gentamycin and tetracycline but were resistant to cephalothin and ampicillin. O3:K6 was the most predominant serotype among the isolates. Using ERIC-PCR, the *V. parahaemolyticus* isolates could be classified into three different ERIC profiles, *i.e.* P1 (90.9%) which consisted of DNA bands 200, 300, 500, 600, 1031 bp, P2 (4.5%) which consisted of DNA bands 200, 250, 300, 480, 500, 650, 1031 bp and P3 (4.5%) which consisted of DNA bands 200, 250, 300, 500, 650, 700 bp. The conclusion that can be drawn from the study is the most (90.9%) of the *V. parahaemolyticus* clinical strains were closely related genetically.

KEY WORDS : PHAGE DISPLAY / TOUCH DOWN PCR / ERIC-PCR / SHIGA-TOXIN PRODUCING - *E. COLI / SHIGELLA SONNEI / V. PARAHAEMOLYTICUS*

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MOLECULAR SUBTYPING OF *AEROMONAS* SPP. USING ENTEROBACTERIAL REPETITIVE INTERGENIC CONSENSUS (ERIC)-PCR

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ABSTRACT

An Enterobacterial repetitive intergenic consensus (ERIC)-PCR technique was used for subtyping of 106 *Aeromonas* strains from Thailand and Italy. All 106 strains fall into 80 genomic ERIC patterns (P1-P80), where ERIC pattern P1 represent by 6 strains (5.7 %) were predominant, followed by ERIC pattern P76 (4 strains), then ERIC pattern P30, P70, P71 (3 strains each), ERIC pattern P2, P3, P4, P11, P32, P50, P55, P58, P68 and P75 (2 strains each), and the rest of the strains have one strain each. Twenty clinical *Aeromonas* strains from Thailand, were fall into 11 ERIC patterns (pattern P1-P11), for eighty five environmental strains and one reference strain from Italy, were fall into 69 ERIC patterns (P12-P80). All *Aeromonas* strains could be grouped based on their genetic distance and geographic distribution of the strains, into 3 major clusters, cluster A, cluster B and cluster C, that contained 9, 30 and 41 ERIC PCR patterns, with 18, 38 and 50 strains respectively. There appears to be geographically restricted of ERIC patterns among *Aeromonas* strains from Thailand, in that 85% of clinical strains from Thailand that fall in to ERIC pattern P1-P5, P8, P9 and P11, have closed genetic similarity and fall in to the same cluster of cluster A. Except three Thai strains that fall in to ERIC pattern P6, P7 and P10, were in cluster C. Cluster B contained 38 environmental strains from Italy, which 52% came from river sediment of F. Basento river and 48% came from brackish water of L. Miseno lagoon. Cluster C contained 47 environmental strains from Italy, which 77% came from river sediment of F. Basento river, only 19% came from brackish water from L. Miseno lake, this cluster also contained 3 clinical strains from Thailand. There was no correlation between ERIC patterns and host, collection time, antibiotic resistant profiles, also no correlation between ERIC patterns and biochemical profiles of the strains.

KEY WORDS: MOLECULAR SUBTYPING, *AEROMONAS*, ERIC-PCR

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MUTATIONS IN THE GYRA AND GYRB GENES OF FLUOROQUINOLONE-RESISTANT *MYCOBACTERIUM TUBERCULOSIS* FROM TB PATIENTS IN THAILAND

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MUTATIONS IN THE *GYRA* AND *GYRB* GENES OF FLUOROQUINOLONE-RESISTANT *MYCOBACTERIUM TUBERCULOSIS* FROM TB PATIENTS IN THAILAND

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ABSTRACT

Among fluoroquinolone-resistant *Mycobacterium tuberculosis* (FQ^r-MTB) isolates, mutation at positions 90, 91, and 94 in *gyrA* gene and at positions 495, 516, and 533 in *gyrB* gene have been frequently reported. In this study, 22 isolates of FQ^r-MTB were collected from Siriraj Hospital and the Chest Disease Institute. The quinolone resistance-determining regions (QRDR) of *gyrA* and *gyrB* genes in all 22 FQ^r-MTB isolates and from the H37Ra MTB strain were amplified using polymerase chain reaction (PCR). DNA-sequencing and single strand conformation polymorphism (SSCP) were further utilized for characterization of the mutation in the QRDR of *gyrA* and *gyrB* genes and mutation screening, respectively. From DNA-sequencing, 10 of 22 (45.5%) exhibited single-point mutation at different positions, at Ala90Val, Ser91Pro, and Asp94(Gly / Ala) in the *gyrA* gene and Asp495Asn in the *gyrB* gene. These positions were previously frequently reported to be responsible for FQ^r-MTB. The other 12 FQ^r-MTB isolates (54.5%) had no mutation. This study is the first report of mutation occurring only in the QRDR of the *gyrB* gene, without prior mutation in the QRDR of the *gyrA* gene, among FQ^r-MTB isolates. By SSCP analysis, the SSCP patterns of mutated FQ^r-MTB isolates were differentiated from the SSCP patterns of FQ^s-MTB.

KEY WORDS : MUTATION / *GYRASE A* / *GYRASE B* / FLUOROQUINOLONE RESISTANCE / *MYCOBACTERIUM TUBERCULOSIS* / THAILAND

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CHARACTERIZATION OF RIFAMPICIN RESISTANT *MYCOBACTERIUM AVIUM* COMPLEX FROM THAILAND USING MOLECULAR TECHNIQUES

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ABSTRACT

Forms of mutation never before described in the *rpoB* gene are reported for a sample of 20 rifampicin-resistant *Mycobacterium avium* complex (MAC) strains isolated from AIDS patients in Thailand. All strains were analyzed by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and polymerase chain reaction-DNA (PCR-DNA) sequencing. The sequence analysis of these strains revealed that only one strain (5%) has missense mutation at Lys–626 (Thr) and the rest of the strains had 15 different silent mutations within the 542 bps region of the *rpoB* gene. Five strains (25%) had a silent mutation at only one position, 7 (35%) at two, 7 (35%) at three, and 1 (5%) at seven positions. The silent mutation at the Ala-630 codon occurred in the largest number of the strains (15, 75%), followed by the Val-581 in 8 strains (40%), Tyr-578 and Thr-600 in 4 strains (20%), and Gly-597 in 3 strains (15%). This investigation demonstrated that mutations in the *rpoB* gene of MAC strains from Thailand are more varied than previously reported for RIF^r MAC strains. Screening by means of PCR-SSCP clearly separated RIF^r strains from rifampicin-susceptible (RIF^s) strains.

KEY WORDS : *MYCOBACTERIUM AVIUM COMPLEX* / RIFAMPICIN-RESISTANT / *RPOB* / GENE MUTATION / THAILAND

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บทคัดย่อ การประชุม

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จังหวัดกาญจนบุรี

สกว

TRF

Epitope mapping of monoclonal antibodies specific to serovars of Leptospira, using phage display technique

Pongrama Ramasoota [a,*], Rongdej tungtrakarnpoung [a], Pannamthip Pitaksajjakul[a], Pattama Ekpo [b], and Wanpen Chaicumpa [c]

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[b] *Faculty of Medicine Siriraj, Mahidol University,* [c] *Faculty of Alliences health Sciences, Thammasart University*

Abstract— Random heptapeptide library displayed by bacteriophage T7 was used to characterized epitopes of the monoclonal antibodies (mAb) clone F11, F20, F21, 2C3D4, and 8C6C4A12, which specific to serovar L. australis, L. bratislava, and L. bangkok respectively. Phage selected by biopanning was cloned by plaque isolation, and the binding specificity of individual clones was confirmed by enzyme-linked immunosorbent assay before sending for PCR and DNA sequencing. Interestingly, in phage reacting with the mAb clone F11, F20, 2C3D4, and 8C6C4A12, the deduced amino acid sequence of the displayed peptides was corresponded to a segment of hypothetical protein of Leptospira genome (L. interrogans serovar Lai and Copenhageni). Considering the deduced amino acid sequences of phage reacting with the mAb clone F11, F20, 2C3D4, and 8C6C4A12, the consensus motif - SKSSRC-, - TLINIF-, -SSKSYR- and –CTPKKSGRC- appeared respectively. No similarity was observed among phage reacting with the mAb clone F21. The results demonstrate that T7 phage display technique has potential for display of peptides and for rapid analysis of the interactions between these peptides with the mAb antibodies.

Keywords—Epitope mapping, monoclonal antibody, Serovars, Leptospira, phage display

Output

1. P. Ramasoota , Tungtrakarnpoung R. [a], Pitaksajjakul P., EakPotii P., and W. Chaicumpa , *Joint International Tropical Medicine Meeting* 2004, in press.

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สำนักงานคณะกรรมการการอุดมศึกษา (สกอ.)
กระทรวงศึกษาธิการ

สำนักงานกองทุนสนับสนุนการวิจัย (สกว.)

การหาเอพิโทปของโมโนโคลนอลแอนติบอดีที่จำเพาะกับเชื้อเลปโตสไปราโดยใช้เทคนิคฟาจดิสเพลย์

EPITOPE MAPPING OF MONOCLONAL ANTIBODIES SPECIFIC TO LEPTOSPIRA SPP. USING PHAGE DISPLAY TECHNIQUE

พงศ์ราม รามสูต [1], รองเดช ตั้งตระการพงศ์ [1], พัชรินทร์ แสงจารึก [2], กุนนาร์ โฟรมัน [3] และวันเพ็ญ ชัยคำภา [4]

[1]Faculty of Tropical Medicine, Mahidol University, [2] Faculty of Medicine, Srinakarintarawirot University; [3] Department of Medical Biochemistry, Uppsala University, Uppsala, Sweden; [4]Faculty of Allied Health Sciences, Thammasart University,

Abstract: Random heptapeptide library displayed by bacteriophage T7 was used to characterized epitopes of the monoclonal antibodies clone LF9 and LD5 which specific to all members of the genus *Leptospira*, and specific only to the pathogenic species respectively. Bound phages were selected, followed by PCR and sequencing of inserted peptide sequences Binding specificity of bound phages were confirmed by ELISA. In phage reacting with the LD5 monoclonal antibody the deduced amino acid sequence of the displayed peptides corresponded to a segment of *Clostridium acetobutylicum*. enome. Considering all the deduced amino acid sequences of phage reacting with the LD5 antibody, the consensus motif -DNY-PA- appeared. Considering all the deduced amino acid sequences of phage reacting with the LF9 antibody, the consensus motif - VLKKNRP- and - LXKNCS- appeared. No similarity was observed among phage reacting with the antibody clone LF9. The results demonstrate that T7 phage display technique has potential for display of peptides and for rapid analysis of the interactions between these peptides with monoclonal antibodies.

Results, Discussion and Conclusion: The collection of T7/LD5 phages displayed a set of related peptides. In 2 of the 12 analyzed clones, the deduced amino acid sequence -CDNY-PAIC- was fused to the capsid protein, and other 2 of the 12 analyzed clones has deduced amino acid sequence -CDNY-PASC- fused to the capsid protein. Considering all the deduced amino acid sequences, the consensus motif -DNY-PA- appeared. When we compared the T7/LD5 phage sequences with the gene bank sequence. It was found that four T7/LD5 Phages with amino acid sequence ATA ATT ATT AGC CTG (that encode for consensus sequence peptide -DNY-PA –) were match with the sequence of *Clostridium* acetobutylicum ATCC824 Complete genome. One phage T7/LD5 with amino acid sequence GAT AAT TAT TAG CTT (that encode for peptide –CDLMHPGNC- was match with the sequence of Bacillus anthracis virulence plasmid PX01, complete sequence. One phage T7/LD5 with amino acid sequence GAT GCA TCC TGG TAA T (that encode for peptide – CDLMHPGNC- was match with the sequence of Legionella pneumophila gene enhA, enhB and enhanced entry protein enhC, complete Cdna In the T7/LF9 phages selected by MAb clone LF9 3 of 12 clones analyzed by DNA sequencing displayed the peptide – CVLKKNRPC -, the peptides were flanked by the original cysteine residues and were probably structurally constrained by an S-S bridge. Considering all the deduced amino acid sequences, the consensus motif - VLKKNRP- and - LXKNCS- appeared. There was no match of any sequence in the Gene bank among phage T7/LF9.The binding specificity of peptides included in the capsid protein of the isolated T7 phage clones was test by ELISA. The result of the analysis is shown that two phages T7/LD5 with peptide -CDNY-PAIC-, followed by two phage T7/LD5 with peptide -CDNY-PASC-, and one phages T7/LD5 with peptide CDNY-PASC, were positive binding specificity with MAb LD5. Among phage T7/LF9, three phages with peptide -CVLKKNRPC-, and two phage with peptide - CLP-KNCSC – were positive binding specificity with MAb LF9. In the ELISA some phage clones did not give a significant signal, even though they displayed peptides related to those with a positive reaction. Thus the ELISA appeared to have low sensitivity. We are planning to test the binding specificity of these peptides with MAb clone LD5 and LF9, using the higher sensitivity and specificity ELISA of the newly develop dot blot ELISA system (Lepto-dot test kit 2000; SDM Thailand)

Key Words: phage display; random peptide library; epitope mapping; monoclonal antibodies, Leptospirosis

No. JITMM 2004/SC 195

3 November 2004

Assist. Prof. Pongrama Ramasoota
Department of Social and Environmental Medicine
Faculty of Tropical Medicine
Mahidol University

Acknowledgement of Receipt of Abstract & Confirmation of your Presentation at the JITMM 2004, Bangkok, Thailand

Dear Assist. Prof. Pongrama Ramasoota,

We are pleased to acknowledge receipt of your abstract entitled **"Epitope mapping of monoclonal antibodies specific to serovars of Leptospira, using phage display technique".** It has been accepted for oral presentation at the upcoming Joint International Tropical Medicine Meeting 2004, 29 November – 1 December 2004 at the Ambassador Hotel, Bangkok, Thailand.

The Session is scheduled for:

Date: **Tuesday 30 November 2004**
Session: **Free Papers: Laboratory Diagnosis and Evaluation**
Chairpersons: George Watt
Varee Wongchotigul
Between: **11:00-12:30;** you have been allocated 15 minutes for your presentation, including questions and discussion.
Place: **Room C**, Ambassador Hotel, Bangkok, Thailand
Speakers and their topics:

- Molecular diagnosis of scrub typhus in patients presenting with acute febrile illness
  Piengchan Sonthayanon
- Quantitative real time polymerase chain reaction for detecting *Orientia tsutsugamushi* in human specimens
  Tasawan Singhsilarak
- Epitope mapping of monoclonal antibodies specific to serovars of Leptospira, using phage display technique
  Pongrama Ramasoota
- Mapping the immune response to *Burkholderia mallei* during experimental equine glanders infection
  Rachaneeporn Tiyawisutsri

Secretary of the JITMM Organising Committee
Faculty of Tropical Medicine, Mahidol University, 420/6 Rajavithi Road, Bangkok 10400, Thailand
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E-mail: jitmm@mahidol.ac.th Website: http://www.tm.mahidol.ac.th

- Diagnostic accuracy of rapid immunochromatographic assays for the detection of dengue virus IGM antibodies during the acute phase of infection: a systematic review and meta-analysis
  Stuart Blacksell
- How do ensure quality during drug analysis?
  Niklas Lindegardh

You are requested to send your short CV form and Audio Visual Form (attached) to us no later than **7 November 2004.** (If you have already sent them by email, there is no need to send them again.)

to e-mail: jitmm@mahidol.ac.th
Fax: 66 (0) 2643-5578 or 66 (0) 2643-5582
Mailing address: Scientific Programme Committee JITMM 2004
Faculty of Tropical Medicine
Mahidol University
420/6 Ratchawithi Road, Bangkok 10400, THAILAND

Looking forward to your presentation at the Meeting.

Yours sincerely,

Assoc. Prof. Jitra Waikagul
Chairperson, Scientific Committee, JITMM 2004
E-mail: tmjwk@mahidol.ac.th
Tel: 66 (0) 2354 9100 ext. 1331
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JOINT INTERNATIONAL TROPICAL MEDICINE MEETING 2004

Abstracts

Ambassador Hotel, Bangkok, Thailand
29 November-1 December 2004

EPITOPE MAPPING OF MONOCLONAL ANTIBODIES SPECIFIC TO SEROVARS OF *LEPTOSPIRA*, USING PHAGE DISPLAY TECHNIQUE

Pongrama Ramasoota[1]*, Rongdej tungtrakarnpoung [1], Pannamthip Pitaksajjakul[1], Pattama Ekpo[2], Wanpen Chaicumpa[3]

[1]Faculty of Tropical Medicine, Mahidol University 420/6 Rajwithii Road, Bangkok 10400; [2]Faculty of Medicine, Siriraj hospital, Mahidol University, Faculty of Alliences health Sciences, Thammasart University

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Cold Spring Harbor Laboratory

Primus Inter Pares

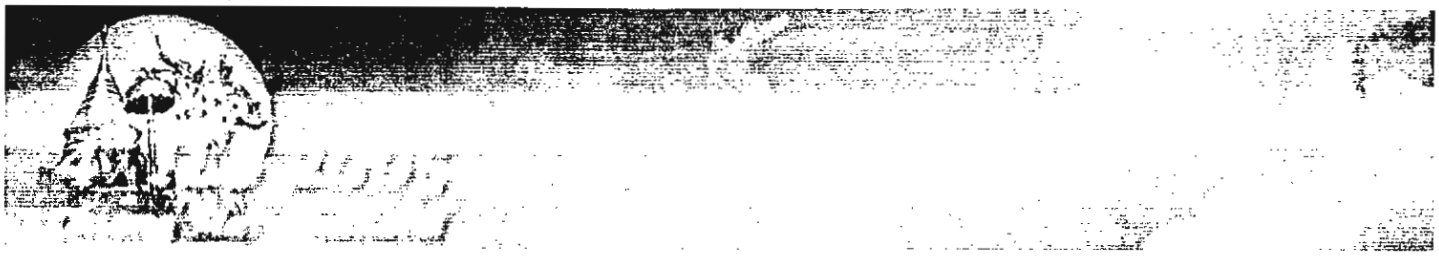
This Is To Certify That ....Pongrama...Ramasoota.....
Attended And Survived The Cold Spring Harbor Course On

Phage Display of Proteins & Peptides

Between The Dates Of November 9th And 22nd

In The Year Two Thousand And Four

Carlos Barbas *Don Siegel* *Gregg Silverman*



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1 June 2005

Dear Dr. Pongrama Ramasoota,

Subject: Notification of Abstract Acceptance

The Scientific Committee of the 4th International Leptospirosis Society Scientific Meeting (the 4th ILS 2005 Meeting), are pleased to inform you that your abstract under the title **"Mimotope identification from Monoclonal Antibodies specific to serovar of *Leptospira*, using phage-displayed random peptide library"** was selected to be presented as **"poster presentation"** in the 4th ILS Meeting 2005 during 14-16 November 2005. This Meeting serves as a forum for members of the society and leptospirophils to exchange information and experience, to update knowledge, to gain and renew friendship.

On behalf of the Scientific Committee, I would like to extend to you my warm welcome and invite you to join the meeting. I am sure the event will be a memorable and inspiring one.

Sincerely yours,

Visith Sitprija, M.D., Ph.D., F.A.C.P.
Chairman Scientific Committee

Dr. Pongrama Ramasoota
Department of Social and Environmental Medicine, Faculty of Tropical Medicine
Mahidol University, THAILAND

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