UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR

DEVELOPMENT OF INTEGRATED MULTISENSOR ISOTHERMAL AMPLIFICATION WITH NURCLEIC ACID LATERAL FLOW SYSTEM FOR POINT OF CARE BIOSENSOR ASSAY

PENYELIDIK

ASSOC. PROF. DR. CHAN YEAN YEAN

PENYELIDIK BERSAMA

ASSOC. PROF. DR. MAIZAN BT MOHAMED

2016

KEMENTERIAN PENDIDIKAN MALAYSIA

FINAL REPORT
PROTOTYPE DEVELOPMENT RESEARCH GRANT SCHEME (PRGS)

Laporan Akhir Skim Geran Penyelidikan Pembangunan Prototaip (PRGS)
Pindaan 1/2015

ESEARCH TITLE: DEVELOPMENT OF INTEGRATED MULTISENSOR ISOTHERMAL AMPLIFICATION WITH UCLEIC ACID LATERAL FLOW SYSTEM FOR POINT-OF-CARE BIOSENSOR ASSAY

ROJECT MEMBERS: 1. ASSOCIATE PROFESSOR DR MAIZAN BT MOHAMED (UNIVERSITI MALAYSIA KELANTAN)

JECT ACHIEVEMENT *(Prestasi Projek)*

ACHIEVEMENT PERCENTAGE			
Project progress according to milestones achieved up to this period	**0 - 50%**	**51 - 75%**	**76 - 100%**
Percentage *(please specify the percentage #%)*			100%

RESEARCH OUTPUT	
Fully developed prototype *(Please specify the percentage of completion)*	**100%**
Intellectual Property *(Please specify)*	**Patent filing application was submitted to Intellectual Property & Commercialisation Management Unit, Innovations & Commercialisation Office (Ref no.: USM/ICO/PT/NAN/15060)**
Application for pre-commercialization funds *(Please specify)*	**In future (together with licensing commercializing company – funding for external evaluation and CE marking to be used as a diagnostic kit according to Medical Act for medical devices)**

)BLEMS / CONSTRAINTS IF ANY *(Masalah/ Kekangan sekiranya ada)*

ıt approval started from 1st August 2013 until 31st July 2015 according to Ministry of Education (MOE) offer letter. ever, grant approval letter (as attached) was received after 18th October 2013, which is almost 3 months after the of the offer. Since the money was officially loaded into the account was delayed, the labworks for this project only be started after the middle of October 2013. Thus, extension application was done by end of April 2015 through ;, and was approved at RMC level for 6 months from 1st August 2015 to 31st January 2016.

:OMMENDATION *(Cadangan Penambahbaikan)*

ernal evaluation by other agency is recommended, which was not conducted in this study.

SEARCH ABSTRACT – Not More Than 200 Words (*Abstrak Penyelidikan – Tidak Melebihi 200 patah perkataan*)

: developed prototype provides a method of definitive detection multiplex for loop-mediated isothermal amplification AMP). Method of amplicons detection relates to a label-based lateral flow dipstick device. The device comprises an orbent pad and a reaction pad. The reaction pad immobilized with more than two sorts of antibodies to detect tiple dual-labeled LAMP amplicons. A first line on the reaction pad, a chromatography control line, is an ıunological component that capable of binding to an enzyme-labeled antibody. The second and third lines, known as lines, are also an immunological component that capable of specifically binding to labeled site of LAMP amplicons ch are another labeled site are bound to enzyme-labeled antibody to form the sandwich complexes. The enzyme-eled antibody produces a visual red color by which it is pre-coated with gold nanoparticles. In specific, the present totype simplifies the multi-steps of mLAMP reagent preparation, reduces the risk of carry-over contamination, ease of AMP amplicons detection and improved the credibility of the study. The ready-to-use thermostabilized mLAMP gents can be potentially applied as a diagnostic kit for diagnosis of diseases on field or in clinical settings.

COMMENTS, IF ANY/ ENDORSEMENT BY RESEARCH MANAGEMENT CENTER (RMC)
(Komen, sekiranya ada/ Pengesahan oleh Pusat Pengurusan Penyelidikan)

.....

.....

.....

BIL	NO. RUJ.	FASA	NAMA KETUA PROJEK	TAJUK PROJEK	TEMPOH PROJEK			STATUS PROJEK (AKTIF / TAMAT)	JUM. PERUNTUKAN DILULUSKAN (RM)	PECAHAN VOT ASAL YANG DILULUSKAN (RM)								PECAHAN VOT (JIKA ADA PINDAAN) (RM)								PECAHAN PERBELANJAAN (RM)								BAKI BELUM BELANJA (RM)	PERATUS BELANJA (%)
					MULA (DD/MM/YYYY)	TAMAT (DD/MM/YYYY)	PELANJUTAN TEMPOH (DD/MM/YYYY) hingga (DD/MM/YYYY)			V11000	V21000	V24000	V27000	V26000	V29000	V35000	JUMLAH BELANJA (RM)	V11000	V21000	V24000	V27000	V26000	V29000	V35000	JUMLAH BELANJA (RM)	V11000	V21000	V23000	V27000	V25000	V29000	V28000	JUMLAH BELANJA (RM)		
1			ASSOCIATE PROFESSOR DR CHAN YEAN YEAN	DEVELOPMENT OF INTEGRATED MULTISENSOR ISOTHERMAL AMPLIFICATION WITH NUCLEIC ACID LATERAL FLOW SYSTEM FOR POINT-OF-CARE BIOSENSOR ASSAY	1/8/2013	31/7/2015	1/8/2015 HINGGA 31/1/2016	TAMAT	211,000.00	10,800.00	30,000.00		140,200.00		30,000.00		211,000.00									3,900.00	7,321.77	1,472.33	138,435.53	126.45	51,350.34	5,590.00	208,196.42	2,803.58	98.7
2																																			
3																																			
4																																			
5																																			
6																																			
7																																			
8																																			
9																																			
10																																			
JUMLAH KESELURUHAN (RM)									211,000.00	10,800.00	30,000.00		140,200.00		30,000.00		211,000.00									3,900.00	7,321.77	1,472.33	140,285.53	126.45	51,910.34	5,590.00	210,606.42	393.58	99.8

UNIVERSITI SAINS MALAYSIA
JABATAN BENDAHARI
KUMPULAN WANG PENYELIDIKAN FUNDAMENTAL
PENYATA PERBELANJAAN SEHINGGA 31 JANUARI 2016

Jumlah Geran	RM211,000.00	Ketua Projek	DR. CHAN YEAN YEAN
Peruntukan 2013 (Tahun 1)	RM117,200.00	Tajuk Projek	DEVELOPMENT OF INTEGRATED MULTISENSOR ISOTHERMAL AMPLICATION WITH NUCLEIC ACID LATERAL FLOW SYSTEM FOR POINT-OF-CARE BIOSENSOR ASSAY
Peruntukan 2014 (Tahun 2)	RM93,800.00		
		Tempoh	24 BULAN (01 OGOS 2013-31 JULAI 2015)
Peruntukan 2013 (Tahun 3)		No. Akaun	203/PPSP/6740018

Kwgan	Akaun	PTJ	Projek	Peruntukan Projek	Perbelanjaan Terkumpul sehingga Tahun lalu	Jumlah Peruntukan Terkumpul	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
203	11000	PPSP	6740018	10,800.00	3,900.00	6,900.00	-	-	-	6,900.00
203	14000	PPSP	6740018			-	-		-	-
203	15000	PPSP	6740018	-		-	-		-	-
203	21000	PPSP	6740018	30,000.00	7,321.77	22,678.23		-	-	22,678.23
203	22000	PPSP	6740018			-			-	-
203	23000	PPSP	6740018		1,472.33	(1,472.33)	-	-	-	(1,472.33)
203	25000	PPSP	6740018		126.45	(126.45)	-	-	-	(126.45)
203	26000	PPSP	6740018			-	-		-	-
203	27000	PPSP	6740018	140,200.00	135,885.97	4,314.03	4,209.56	190.00	4,399.56	(85.53)
203	28000	PPSP	6740018			-	-	5,590.00	5,590.00	(5,590.00)
203	29000	PPSP	6740018	30,000.00	51,350.34	(21,350.34)	-	560.00	560.00	121,910.34
				211,000.00	200,056.86	10,943.14	4,209.56	6,340.00	10,549.56	393.58

UNIVERSITI SAINS MALAYSIA

Pusat Pengajian Sains Perubatan
School of Medical Sciences

Rujukan : USM/PPSP/(Pent.)/K2/b Jld II

Tarikh : 31 Januari 2016

Memorandum

Kampus Kesihatan
Universiti Sains Malaysia,
16150 Kubang Kerian
Kelantan, Malaysia.
Tel: 609 767 3000
Fax: 609 765 3370
www.medic.usm.my

Pejabat Pengurusan dan Kreativiti Penyelidikan
Universiti Sains Malaysia
Aras 6, Bangunan Canselori
11800, USM Pulau Pinang.

Up: Puan Suzilah Mustapa

Puan,

PENYERAHAN ASET PENYELIDIKAN

Dengan hormatnya perkara di atas adalah dirujuk dan berkaitan.

2. Bersama-sama ini disertakan salinan Borang Penyerahan Aset/ Inventori yang telah siap diisi oleh staf berkenaan untuk tindakan pihak tuan/puan selanjutnya.

Bil	Nama	Projek	Kod Geran
1	Prof. Madya Dr. Chan Yean Yean	"Development Of integrated Multisensor Isothermal Amplication With Nucleic Acid Lateral Flow System For Point-Of-Care Biosensor Assay"	203/PPSP/6740018

3. Sukacita saya merakamkan ribuan terima kasih di atas kerjasama puan di dalam hal ini.

Sekian, terima kasih.

s.k. Dekan
Pusat Pengajian Sains Perubatan

Timbalan Dekan Penyelidikan
Pusat Pengajian Sains Perubatan

Ketua Penolong Pendaftar
Pusat Pengajian Sains Perubatan

Jabatan Bendahari
Kampus Kesihatan

Bahagian Penyelidikan
Pusat Pengajian Sains Perubatan

Pegawai Penerima Aset
Pusat Pengajian Sains Perubatan

Prof. Madya Dr. Chan Yean Yean
Jabatan Mikrobiologi & Parasitologi Perubatan

UNIVERSITI SAINS MALAYSIA

Pejabat Pengurusan dan Kreativiti Penyelidikan
Research Creativity and Management Office

Universiti Sains Malaysia

Ruj. Kami : V0018
Tarikh : 6 Mei 2015

Prof. Madya Dr. Chan Yean Yean
Pusat Pengajian Sains Perubatan
Universiti Sains Malaysia
Kampus Kesihatan
16150 Kubang Kerian
KELANTAN DARUL NAIM

Puan,

KEPUTUSAN PERMOHONAN PERLANJUTAN TEMPOH BAGI GERAN PENYELIDIKAN PEMBANGUNAN PROTOTAIP (PRGS) FASA 1/2013

TAJUK PROJEK : *DEVELOPMENT OF INTEGRATED MULTISENSOR ISOTHERMAL AMPLICATION WITH NUCLEIC ACID LATERAL FLOW SYSTEM FOR POINT-OF-CARE BIOSENSOR ASSAY*
NO. AKAUN : 203 / PPSP / 6740018 (GERAN PRGS)

Dengan segala hormatnya saya merujuk kepada perkara di atas dan Borang Perlanjutan Tempoh puan yang telah diterima pada 5 Mei 2015 adalah berkaitan..

2. Sukacita dimaklumkan bahawa pihak universiti telah meluluskan lanjutan tempoh selama **enam (6) bulan mulai 1 Ogos 2015 sehingga 31 Januari 2016** untuk projek penyelidikan Puan seperti tajuk di atas.

3. Diharapkan pihak puan dapat menyelesaikan penyelidikan ini dalam tempoh yang ditetapkan dan mohon kerjasama puan untuk <u>menghantar laporan akhir ke Pejabat RCMO pada akhir tempoh geran.</u>

Sekian, terima kasih.

(ADRIL ELLMI BIN MOHD ADNAN)
Pegawai Sains
Pejabat Pengurusan dan Kreativiti Penyelidikan

s.k. Pengarah
Pejabat Pengurusan dan Kreativiti Penyelidikan

Dekan
Pusat Pengajian Sains Perubatan
Universiti Sains Malaysia
Kampus Kesihatan
16150 Kubang Kerian
KELANTAN DARUL NAIM

Timbalan Bendahari
Jabatan Bendahari
Universiti Sains Malaysia
Kampus Kesihatan

UNIVERSITI SAINS MALAYSIA

Pejabat Inovasi dan Pengkomersilan
Innovations and Commercialisation Office
Bahagian Penyelidikan dan Inovasi
Division of Research and Innovation

Building J06
Universiti Sains Malaysia
11800 USM, Penang, Malaysia
T : 604-653 2616 / 3166 / 3038
F : 604-653 4399
W : http://innovations.usm.my

Our Ref. : USM/ICO/PT/NAN/15060

16 December 2015

ASSOC. PROF. DR. CHAN YEAN YEAN
School of Medical Sciences
Depatment of Medical Microbiology & Parasitology
Universiti Sains Malaysia
Health Campus, 16150 Kubang Kerian
Kelantan

Dear Dr..

STATUS OF PATENT APPLICATION

We refer to the above matter. The secretariat for the Intellectual Property Management of Universiti Sains Malaysia (USM) is pleased to confirm that the aforesaid patent application has been accepted and is being processed under reference number USM/ICO/PT/NAN/15060.

TITLE: MULTIPLEX LOOP-MEDIATED ISOTHERMAL AMPLIFICATION LATERAL FLOW DIPSTICK DEVICE AND METHODS, THEREOF.

Should you have any further enquiry, please do not hesitate to contact us for clarification.

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

elopment of multiplex loop mediated isothermal amplification LAMP) label-based gold nanoparticles lateral flow dipstick ensor for detection of pathogenic *Leptospira*☆

Nurul Najian [a], E.A.R. Engku Nur Syafirah [a], Nabilah Ismail [a], Maizan Mohamed [c], Yean Yean [a, b, *]

tment of Medical Microbiology & Parasitology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, , Malaysia

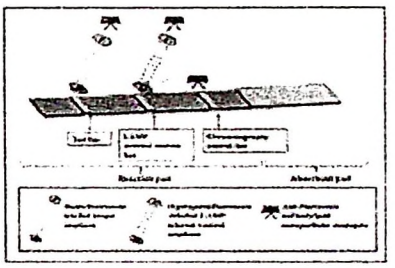
e for Research in Molecular Medicine (INFORMM), Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

of Veterinary Medicine, Universiti Malaysia Kelantan, City Campus, Pengkalan Chepa, Locked Bag 36, 16100 Kota Bharu, Kelantan, Malaysia

HIGHLIGHTS

- develop multiplex LAMP label- lateral flow dipstick biosensor detection of pathogenic *spira*.
- design primers for multiplex targeting the conserved *LipL32* of pathogenic *Leptospira* and internal control.
- ers for both targets are labelled biotin/digoxigenin tag, and escein tag.
- nanoparticles is coupled with fluorescein antibody, to give lize red line on the lateral flow ick.
- ive results show three red lines e negative results show two red on the lateral flow dipstick.

GRAPHICAL ABSTRACT



ICLE INFO

bistory:
d 23 August 2015
d in revised form
ber 2015
d 5 November 2015
le online 24 November 2015

ds:
ased multiplex loop-mediated
mal amplification
flow dipstick

ABSTRACT

In recent years extensive numbers of molecular diagnostic methods have been developed to meet the need of point-of-care devices. Efforts have been made towards producing rapid, simple and inexpensive DNA tests, especially in the diagnostics field. We report on the development of a label-based lateral flow dipstick for the rapid and simple detection of multiplex loop-mediated isothermal amplification (m-LAMP) amplicons. A label-based m-LAMP lateral flow dipstick assay was developed for the simultaneous detection of target DNA template and a LAMP internal control. This biosensor operates through a label based system, in which probe-hybridization and the additional incubation step are eliminated. We demonstrated this m-LAMP assay by detecting pathogenic *Leptospira*, which causes the re-emerging disease Leptospirosis. The lateral flow dipstick was developed to detect of three targets, the LAMP target amplicon, the LAMP internal control amplicon and a chromatography control. Three lines appeared on the dipstick, indicating positive results for all representative pathogenic *Leptospira* species.

s work has been partially presented at Postgraduate Colloquium on Medical Sciences 2015, on 25–26th May 2015, at UiTM Sg Buloh, Selangor, Malaysia.

responding author. Department of Medical Microbiology & Parasitology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kota Bharu, an, Malaysia.
mail address: [illegible] (C.Y. Yean).

[illegible]

Article Title	: A Shelf-stable Fluorogenic Isothermal Amplification Assay for Detection of *Burkholderia pseudomallei*
Journal	: Analyst
Manuscript ID	: AN-COM-08-2015-001741.R1
Status	: Accepted

From: analyst@rsc.org

To: yeancyn@yahoo.com

CC:

Subject: Decision on submission to Analyst - AN-COM-08-2015-001741.R1

Body: 27-Dec-2015

Dear Dr CHAN:

Manuscript ID: AN-COM-08-2015-001741.R1
TITLE: A shelf-stable fluorogenic isothermal amplification assay for detection of Burkholderia pseudomallei

Thank you for submitting your revised manuscript to Analyst. After considering the changes you have made, I am pleased to accept your manuscript for publication in its current form. I have copied any final comments from the reviewer(s) below.

Please note that if you have requested Accepted Manuscript publication we will publish your article shortly and send you an email once it is available online. We will also email you information on how to access your Analyst article proofs shortly.

If you would like to make this article open access or want further information about open access and the services we offer, including our Gold for Gold initiative and discounts, please visit:

http://www.rsc.org/journals-books-databases/open-access/

You can highlight your article and the work of your group on the back cover of Analyst, if you are interested in this opportunity please contact me for more information.

As an author you are entitled to a 25% discount on books published by the Royal Society of Chemistry. To receive this discount, enter the promotional code JLTH25 when purchasing from our online bookshop (pubs.rsc.org/bookshop). Please contact booksales@rsc.org if you have any problems.

Discover more Royal Society of Chemistry author services and benefits here:

http://www.rsc.org/journals-books-databases/about-journals/?author-benefits

Thank you for publishing with Analyst, a journal published by the Royal Society of Chemistry - the world's leading chemistry community, advancing excellence in the chemical sciences.

With best wishes,

Steven A. Soper, PhD
University of North Carolina, Chapel Hill
Chapel Hill, NC 27599
ssoper@unc.edu
(919) 843-5575 (office)
****

REVIEWER REPORT(S):

******
******

Article Title	: Probe-specific Loop-mediated Isothermal Amplification (LAMP) Colorimetric Magnetogenosensor Assay for Rapid and Specific Detection of Pathogenic Leptospira
Journal	: Journal of Microbiological Methods
Ms. Ref. No.	: MIMET-D-15-00882
Status	: Under review

hesday, December 16, 2015 1:29 AM, Journal of Microbiological Methods <ees.mimet.0.35fb9f.a3db736c@eesmail.elsevier.com> wrote:

ef. No.: MIMET-D-15-00882
Probe-specific Loop-mediated Isothermal Amplification (LAMP) Colorimetric Magnetogenosensor Assay for Rapid and Specific Detection of Pathogenic Leptospira
al of Microbiological Methods

Dr. Chan,

submission entitled "Probe-specific Loop-mediated Isothermal Amplification (LAMP) Colorimetric Magnetogenosensor Assay for Rapid and Specific Detection of genic Leptospira" has been assigned the following manuscript number: MIMET-D-15-00882.

nay check on the progress of your paper by logging on to the Elsevier Editorial System as an author. The URL is http://ees.elsevier.com/mimet/.

username is: yeancyn@yahoo.com
need to retrieve password details, please go to:
ees.elsevier.com/mimet/automail_query.asp

k you for submitting your work to this journal.

egards,

al of Microbiological Methods

ther assistance, please visit our customer support site at http://help.elsevier.com/app/answers/list/p/7923. Here you can search for solutions on a range of topics, find rs to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further ance from one of our customer support representatives.

Status : Manuscript writing in progress

A Duplex Loop-mediated Isothermal Amplification (LAMP) Analysis by Laser-induced Fluorescence via Capillary-Gel Electrophoresis for the Detection of Toxigenic *Vibrio cholerae*

Engku Nur Syafirah E. A. R. and Chan Yean Yean*

*Department of Medical Microbiology and Parasitology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

Corresponding author:

1) Chan Yean Yean

Address: Department of Medical M icrobiology and Parasitology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

Tel: +60 97 676 258

E-mail address: yeancyn@yahoo.com

Status : Manuscript writing in progress

An ambient temperature-stable reagents of loop-mediated isothermal amplification (LAMP) biosensing assay for a point-of-care molecular diagnosis of cholera toxin gene *Vibrio cholerae*

Engku Nur Syafirah E. A. R.[a], Nurul Najian A. B.[a], Maizan Mohamed[b] and Chan Yean Yean[a,*]

[a]Department of Medical Microbiology and Parasitology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

[b]Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, City Campus, Pengkalan Chepa, Locked Bag 36, 16100 Kota Bharu, Kelantan, Malaysia.

Corresponding author:

1) Chan Yean Yean

Address: Department of Medical Microbiology and Parasitology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

Tel : +60 97 676 258

Email address: yeancyn@yahoo.com

UNIVERSITI SAINS MALAYSIA

HICoE iNFORMM

Institut Penyelidikan Perubatan Molekul
Universiti Sains Malaysia (USM
Institute for Research in Molecular Medicine, USM

16150 Kubang Kerian, Kelantan
Malaysia
Telephone +609 767 2402/2403/2404
Facsimile +609 764 8873

11800, USM, Pulau Pinang
Malaysia
Telephone +604 653 4801/4813/4814
Facsimile +604 653 4803

inform~~m~~@kck.usm.my admin_info
www.informm.usm.my

July 14, 2014

Dear Dr. Chan Yean Yean,

I'm pleased to inform you that your 3 book chapters entitled:

1) **The hurdles in making diagnostics accessible to the bottom billions: can lateral flow immunoassays make a difference?**

2) **Cholera diagnosis: current and future technologies for point-of-care diagnosis in less developed countries**

3) **Loop Mediated Isothermal Amplification (lamp): furtherance of assorted detection systems towards point-of-care setting**

have been accepted for publication in the book entitled "**Sustainable diagnostic for low resource settings**".

Received: 4 April 2014
Revision: 6 May 2014
Accepted: 1 July 2014
Expected publish year: 2014

Thank you for submitting your 3 book chapters to "**Sustainable diagnostic for low resource settings**" **(in preparation)**. Your time and effort is greatly appreciated and respected. We look forward to receiving further submissions from you in the future.

The publisher will contact you with the proofs when it is ready for publication.

The hurdles in making diagnostics accessible to the bottom billions: can lateral flow immunoassays make a difference?

Geik Yong Ang, Choo Yee Yu and Yean Yean Chan

Department of Medical Microbiology & Parasitology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

Introduction

Designing diagnostics for the bottom billion is a surmountable task, but aside from identifying the target disease biomarker and investing in research to produce functional test prototype for evaluation, we would first have to understand the targeted population and their predicaments as well as the diseases which greatly afflict their lives. An estimated 2.7 billion impoverished individual worldwide who live on less than US $2 per day suffer from the greatest risk factor for acquiring and succumbing to diseases – poverty [1,2]. In the developing world, transmission of infection can be particularly efficient when one billion people lack access to safe drinking water and 2.5 billion lack access to basic sanitation, not to mention the substandard housing and inadequate vector control [1,3]. Therefore, it is not a coincidence to find that the people in the bottom billion are mostly localized in regions plagued by the "big three global disease" [human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS), malaria, and tuberculosis (TB)] as well as the neglected tropical diseases (NTDs). Poverty not only increases the vulnerability of the poor to communicable disease, the endemicity of various diseases also lead to an increased in co-infection of several diseases, higher level of susceptibility to opportunistic diseases, mistreatment of disease and as a direct consequence, the increased in morbidity and mortality among the bottom billion population [4].

The parasitic and bacterial diseases which are collectively identified as NTDs are regionally endemic and remain as one of the most potent reinforcement of poverty trap that affects the poorest of the poor [4,5]. Being infected by some of the major NTDs would be catastrophic for the patients and families as exemplified by a study in Ghana where the cost of care per patient with Buruli ulcer was reported as an average of 242% of a household annual earnings in the poorest earning quartile [6]. In addition to the heavy direct costs (cost of diagnosis and treatment), household affected by NTDs become poorer through indirect costs such as reduced agricultural productivity as a result of disfigurement or other sequelae of long-term illness, loss of household income and sale of assets such as land and livestock [7]. Not only affected households suffer from the debilitating effects of NTDs; accumulative individual loss of productivity becomes even more significant on a global level where single NTDs alone such as lymphatic filariasis causes almost $1.3 billion a year lost in productivity [6].

(2)

CHOLERA DIAGNOSIS: CURRENT AND FUTURE TECHNOLOGIES FOR POINT-OF-CARE DIAGNOSIS IN LESS DEVELOPED COUNTRIES

Engku Nur Syafirah Engku Abd Rahman, Nurul Najian Aminuddin Baki and Chan Yean Yean

Department of Medical Microbiology and Parasitology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

ABSTRACT

Cholera is caused by the bacterium *Vibrio cholerae*. It remains a problematic disease and is endemic in certain geographical regions, with significant implications in Third World countries, especially those with poor sanitation and sewage systems. The main characteristic of cholera is severe watery diarrhea, leading to dehydration and death, if not treated immediately. The gold standard method for diagnosing cholera is the isolation and identification of the *V. cholerae* bacterium in clinical samples, which is time-consuming and tedious and requires trained personnel to perform the task. Alternative methods for detecting *V. cholerae* have been studied and developed, including molecular tests and recent microfluidic techniques such as the 'lab-on-a-chip', which might alleviate practical diagnostic problems in poor, undeveloped and developing countries, as well as in developed states. This review considers the different methods currently available for cholera diagnosis, and the developments in these technologies needed to assist cholera diagnosis in the developing world.

INTRODUCTION

Despite progress in medicine and biomedical science technologies, cholera remains an epidemic and endemic disease in many regions worldwide, especially in developing areas that currently lack effective sanitation. A cholera epidemic started in the Republic of Haiti, North America, in October 2010. By October 17, 2013, the Ministère de la Santé Publique et de la Population reported 684,085 occurrences with 8,361 deaths since the start of the outbreak, with about 380,846 (55.4%) patients being admitted to hospital (Centers for Disease Control and Prevention, 2013).

(3)

LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP): FURTHERANCE OF ASSORTED DETECTION SYSTEMS TOWARDS POINT-OF-CARE SETTING

Nurul Najian, A.B.*[a], Engku Nur Syafirah, E.A.R.[a] & Chan Yean Yean*[a]

*Corresponding authors

[a]Department of Medical Microbiology & Parasitology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

Email: nurulnajian@yahoo.com, irairaic7@gmail.com, yeancyn@yahoo.com;

Fax: +609 7676289;

Tel: +609 767 6258

INTRODUCTION

Significant developments in molecular detection have been reported over the years. Practical tools for nucleic acid amplification are invaluable in a wide range of applications, including human clinical tests, veterinary molecular tests, as well as identity and forensic tests, among others. Nucleic acid amplification is widely used to diagnose infectious diseases, since it can be performed more rapidly than classical diagnostic methods (Ince and McNally, 2009). Moreover, this process generates several target copies, which significantly increases assay sensitivity.

The development of molecular diagnostic tools has facilitated attempts to achieve the required level of accuracy and reliability to a considerable extent. The ability to detect specific nucleic acid (DNA/RNA) sequences promotes accuracy, sensitivity and specificity in research on clinical-based, forensic and other fields. Readily available rapid and accurate point-of-care diagnostic tests remain an urgent medical need. To this effect, various molecular detection tools have been devised. Each of these amplification methods applies different synthesis principles, with specific advantages and disadvantages.

To date, various isothermal nucleic acid amplification protocols have been developed, including transcription-mediated amplification or self-sustained sequence replication, nucleic acid sequence-based amplification, signal-mediated amplification of RNA technology, strand


UNIVERSITI SAINS MALAYSIA
5th NATIONAL
CONFERENCE
ON INFECTIOUS
Infectious Diseases:
Sustaining The Essentials and
Innovating For The Future
25-26 August 2014
Renaissance Kota Bharu Hotel
Organized by:
Department of Medical Microbiology & Parasitology
School of Medical Sciences
Universiti Sains Malaysia

accompanying symptoms and signs, results of relevant blood investigations and the outcome of meningitis.

Results:
The incidence of postoperative nosocomial meningitis was 67% (84 patients out of 125 patients). The age of patients ranged between newborn and 19 years old (Mean±SD, 74.5±80.6 months). The majority of them were males (65.6%). Fever was the most common presentation (73.6%) followed by poor oral intake (48.0%), seizure (36.0%) and headache (24.8%). The mortality rate was 31.2%. Coagulase negative staphylococcus was the most frequent pathogens isolated (21.6%), followed by *Acinetobacter* spp. (17.6%), *Staphylococcus* aureus (13.6%), *Streptococcus* spp. (11.2%) and *Klebsiella pneumoniae* (6.4%). There were significant association of in-hospital death with age (p=0.020) and conscious level (p=0.001).

Conclusion:
Infectious meningitis is a big health concern, especially among children. The clinical presentation of bacterial meningitis appeared to be similar to that of hospital acquired bacterial meningitis. We found that coagulase negative staphylococcus, Acinetobacter species, *S. aureus*, *Streptococcus* spp and *K. pneumoniae* were prevalent in our hospital. These microorganisms were hospital associated pathogens. The 31% mortality linked to hospital acquired meningitis specifies the need for focused physician attention especially among younger aged patients.

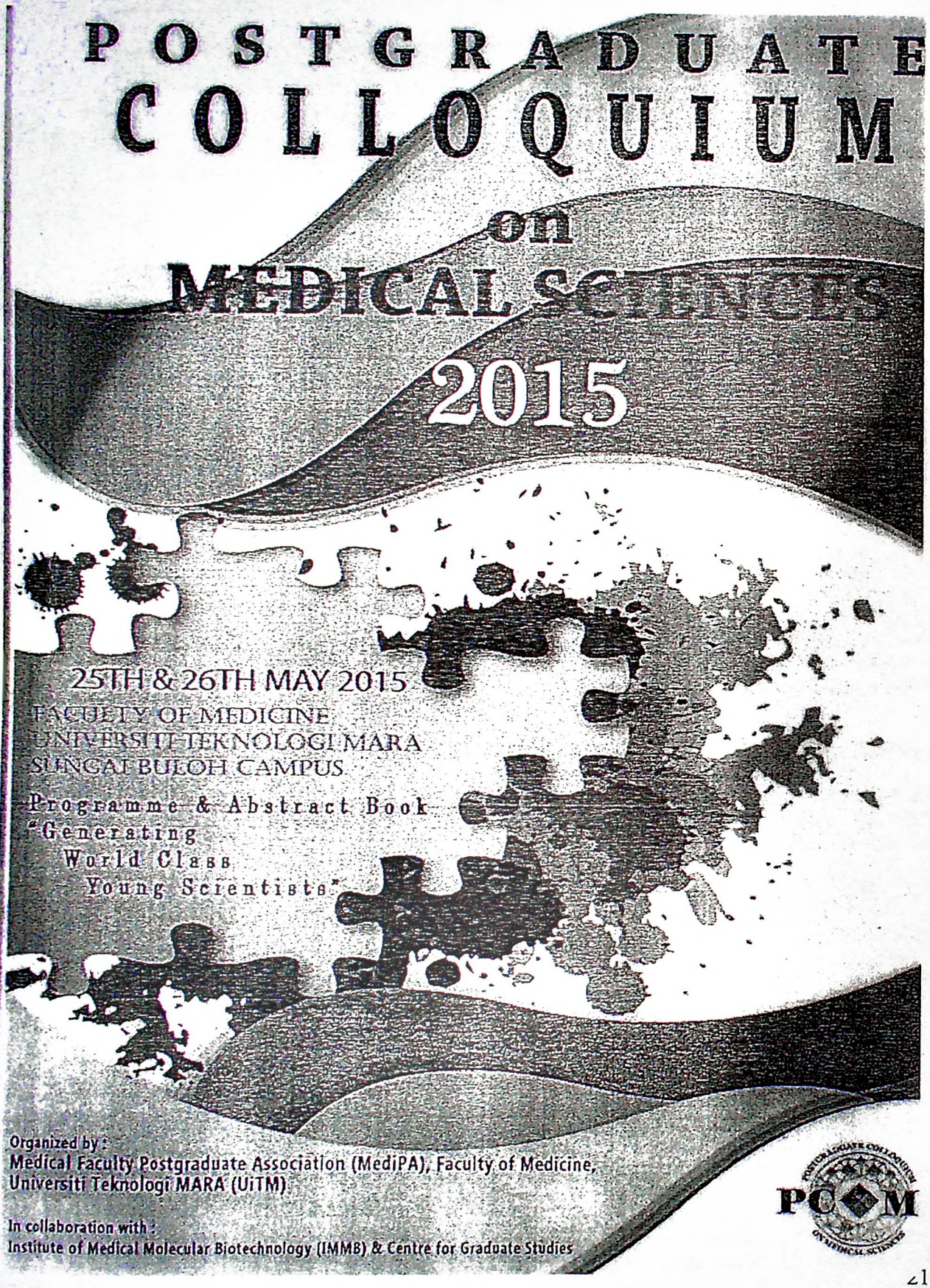
OP05 (15:45-15:55)

DEVELOPMENT OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY FOR A SENSITIVE AND RAPID DETECTION OF TOXIGENIC *Vibrio cholera*

Engku Nur Syafirah bt Engku Abd Rahman and Chan Yean Yean

Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan Darul Naim, Malaysia

Cholera is a life-threatening diarrheal illness caused by *Vibrio cholerae* bacteria via consuming contaminated food and water. Presently, cholera is still problematic in areas known to be cholera hotspot cases especially in undeveloped countries with improper sanitation and sewage system. Current laboratory diagnosis of cholera is time-consuming, tedious and has low sensitivity. Thus, a LAMP assay that amplify specific toxigenic gene to identify *ctxA* for *V. cholerae* was developed in a single reaction. Study showed 8 U/ µl *Bst* DNA polymerase, 6 mM $MgSO_4$, 0.4 M Betaine and 0.6 mM dNTPs mixture were found to be the optimum concentration in a total of 25 µl LAMP reaction. A 1.6 µM inner primers, 0.8 µM loop primer and 0.2 µM outer primers with 1:8 ratio of outer and inner primers has showed the optimal


POSTGRADUATE
COLLOQUIUM
on
MEDICAL SCIENCES
2015
25TH & 26TH MAY 2015
FACULTY OF MEDICINE
UNIVERSITI TEKNOLOGI MARA
SUNGAI BULOH CAMPUS
Programme & Abstract Book
"Generating
World Class
Young Scientists"
Organized by :
Medical Faculty Postgraduate Association (MediPA), Faculty of Medicine,
Universiti Teknologi MARA (UiTM)
In collaboration with :
Institute of Medical Molecular Biotechnology (IMMB) & Centre for Graduate Studies
PCOM

P13

DEVELOPMENT OF INTEGRATED MULTI-LOOP MEDIATED ISOTHERMAL AMPLIFICATION (multi-LAMP) AND LABEL-BASED LATERAL FLOW DIPSTICK FOR DETECTION OF PATHOGENIC *Leptospira* sp.

Nurul Najian Aminuddin Baki[1*], Maizan Mohamed[2], Chan Yean Yean[1]

[1]Department Medical Microbiology & Parasitology, School of Medical Sciences, University Science Malaysia, Health Campus, 16150 Kota Bharu, Kelantan
[2]Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, City Campus, P. Chepa, Locked Bag 36, 16100 Kota Bharu, Kelantan

***Corresponding author:** nurulnajian@yahoo.com

Introduction: *Leptospira* is a causative agent of an emerging disease, Leptospirosis. This disease causes misleading diagnostic since its clinical symptoms are being confused with other diseases. Hence, rapid and specific detection of the presence of this causative agent is very crucial for the need of proper and rapid treatment. This study was aimed to develop a specific multi-LAMP assay for the detection of pathogenic Leptospira with the presence of internal control and coupled with lateral flow dipstick as a point-of-care (POC) device.

Methods: A total volume of 25 µL LAMP reagents mixture, including 1X buffer, 0.8 M betaine, 6 mM $MgSO_4$, 0.6 mM dNTPs mix, 8U of *Bst* 2.0 Warmstart DNA polymerase, target and internal control primers mixture (5 pmol/ µL of each forward and reverse outer primer, 40 pmol/ µL of each forward and reverse inner primer and 20 pmol/ µL of loop primer), target DNA template and internal control template was prepared in a tube. The reagents mixture was then incubated at 63 °C for 1 hour, followed by termination at 80 °C for 10 min. LAMP amplicons detection was done on lateral flow dipstick. Three lines appeared on the dipstick indicate positive result for pathogenic Leptospira, while two lines indicate negative result.

Result and Discussion: The developed integrated multi-LAMP assay gave positive results for all 15 representatives pathogenic *Leptospira*, while negative results for 2 intermediate *Leptospira*, 1 non-pathogenic *Leptospira* and 10 other bacteria species.

Conclusion: An integrated multi-loop mediated isothermal amplification and label-based lateral flow dipstick was successfully developed for the detection of pathogenic *Leptospira* as a point-of-care device.

Keywords: Loop-mediated isothermal amplification (LAMP), lateral flow dipstick, leptospirosis

UNIVERSITI KUALA LUMPUR

Second International Conference on Tropical Medicine & Infectious Diseases

Diseases Without Borders: Challenges and Preparedness

29th September - 1st October 2015

Sveum Hotel, Ipoh, Perak, Malaysia

Officiated by:

Y. Bhg. Datuk Dr. Hj. Yusof Bin Hj. Yacob

Organised by:

Faculty of Medicine

Universiti Kuala Lumpur Royal College of Medicine Perak

P1 - 5

Development of Rapid Point-of-Care Diagnostic Assay for Detection of Melioidosis

Julien Michelle Wong Tzeling, Abdel Rahman Zueter, Azian Harun, Chan Yean Yean*
Department of Medical Microbiology & Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kota Bharu, Kelantan, Malaysia

Introduction: Melioidosis is an endemic infectious disease caused by the Gram-negative bacteria, *Burkholderia pseudomallei.* Delay in diagnosis is common in rural communities due to laborious and time-consuming routine laboratory diagnosis of *B. pseudomallei* which result in high mortality rate. The aim of the study is to develop a simple and rapid point-of-care diagnostic assay in detection of melioidosis to be used in resource-limited areas.

Methodology: The assay was performed with a set of loop-mediated isothermal amplification (LAMP) primers which was designed to identify conserved DNA sequence of *B. pseudomallei.* Fluorescent Detection Reagent (FDR) was included in all the tubes prior to incubation to allow for rapid visual detection of amplification result using ultraviolet (UV) lamp. Clinical isolates of *B. pseudomallei, Burkholderia cepacia, Burkholderia thailandensis,* and other bacteria were used to determine the specificity of assay. Serial dilution was performed to evaluate the sensitivity of the assay.

Results: A total of 30 *B. pseudomallei* positive tubes showed bright green fluorescence. A *B. cepacia* and *B. thailandensis and 19* other bacteria tubes with dark and less fluorescent colour indicated a negative result. The assay of serially diluted *B. pseudomallei* genomic DNA gave a limit of detection (LOD) of 200 fg/µl.

Discussion: As this developed, rapid and simple LAMP-FDR assay allowed visual discrimination of results using inexpensive laboratory instrument, thus it can be used as an effective point-of-care diagnostic test of melioidosis in field.