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UTILITY OF LAMP ASSAY IN EARLY DETECTION OF DENGUE VIRUS INFECTION

Prof. Uma Bhardwaj[1], Binish Jawed[2], Mukta Sehgal[2]

[1]*Professor, Biochemistry, MaharajVinayak Global University, Jaipur, Rajasthan, India -302028*
[2]*Ph.D. Scholar, Clinical Biochemistry, Dr. Dangs Lab LLP, New Delhi, India -110016*

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Abstract: In the present day vector born dengue infection has become a major public health concern. Early and accurate detection during the acute phase has crucial for management and prevention of disease. RT-LAMP can be utilized in disease diagnosis as it a highly sensitive and specific method.A genotype specific reverse transcription-loop-mediated isothermal amplification (RT-LAMP) assay was developed in this study and we evaluated its sensitivity and specificity in comparison to NS1 antigen and RT-PCR assay.Serological and RT-LAMP were performed on a total of 343 serum samples of clinically suspected dengue patient. Further RT-LAMP positive serum sample were confirmed by Real Time PCR (RT-PCR) method.A total of 32 % (n=109) dengue positive case, 79 %, 27% and 11% positive for NS1 antigen, IgM and IgG antibody respectively. 83% serum samples were positive for RT-LAMP and 76% was common positive for NS1 Ag and RT-LAMP assay. All RT-LAMP positive samples were also positive for RT-PCR.Developed RT-LAMP assay in this study shows strength of agreement in comparison to others serological assays.It can be utilized in early, rapid, and sensitive detection of dengue infection in resource limited areas of country.

Key words: Reverse Transcription-Loop-Mediated Isothermal Amplification, Dengue shock syndrome, Dengue haemorrhagic fever, Enzyme linked immunosorbent assay**.**

INTRODUCTION:

In current scenario dengue has becomes a major public health problem in tropical and subtropical countries [14,2]. Every year a very large population of India is affected by dengue virus infection transmitted through Aedes mosquito.Antigenically different four genotypes (DEN 1,2,3 & 4) produce either an asymptomatic or a moderate febrile infection such as dengue fever (DF) or severe fetal stage,dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS)[1,3]. In this situation, early and accurate diagnosis play a very important role in disease management and patient relief. High grade fever is a first clinical presentation of infection which is followed by a prompt reduction in platelet count which occurs after complete onset of disease. Unavailability of dengue vaccine and specific medication, precise diagnosis become a very important in preparedness and prevention of disease. Virus isolation, serology, antigen and molecular detection methods are available for dengue infection confirmation[4,5]. Various commercially available tests are quite competitive in detection of dengue antibody in human plasma/serum[6,7]. Suggestive diagnosis can be made on the basis of virus specific captured IgM and IgG enzyme linked immunosorbent assay (ELISA) but it requires continuous monitoring of antibody titer from acute phase to convalescent phase of patient sera[9]. Cross reactivity of dengue antibodies with otherflavivirus specific IgG antibodies is yet again a major drawback. Cell

***Corresponding Author**:
Binish Jawed, Ph.D. Scholar,
Dr. Dangs Lab LLP, C2/1, SDA, New Delhi, India -110016

culture remains a gold standard for virus detection although it requires more than 7 days to complete the test. Recently, two different methods widely adopted for the dengue testing, proven as most consistent. It includesNS1 antigen detection by ELISA and automated real-time PCR assay for dengue genomic detection in patient serum in acute phase of infection[8,10,11]. Many researchers have found the advantage of Real time PCR over conventional RT-PCR in term of quantitation, higher sensitivity, low rate of contamination and performance. Though, all PCR based testing requires a higher accuracy instrument and particularized Standard operating procedure and good financial resources.

The aim of present study is to demonstrate the feasibility of reverse transcription-loop-mediated isothermal amplification (RT-LAMP) assay for clinical diagnosis of dengue virus infection. Originally LAMP technique was introduced by Netomi et al(2000) is very sensitive, efficient, and easy to perform nucleic acid based laboratory diagnostic method without any specific instrument. Continuous amplification under isothermal condition produces large amount of target DNA as well as higher concentration of the by-product magnesium pyrophosphate, which makes reaction volume turbid and easily visible to naked eyes.

MATERIAL & METHOD:

Clinical sample

A total of 343 serum sample were collected from clinically suspected patients of dengue infection. All samples undergo the captured IgM, IgG and NS1 antigen testing. Dengue IgM and NS1 positive serum were evaluated for RT-LAMP assay. Age and gender details of patients were not included in presented study. Healthy volunteer samples (n=20) were collected as negative control for RT-LAMP assay.

Serological testing

Panbio Dengue IgM/IgG (Standard Diagnostic Inc, Rebublic of Korea) microwell plate ELISA test kit was used for dengue specific IgM and IgG antibodies estimation and Platelia™ Dengue NS1Ag (Manufacturer, Bio-Rad, France)dengue ELISA kit for NS1 antigen. Procedure followed as per manufactur's protocol.

Nucleic acid extraction and RT-LAMP assay

200 µl serum samples were used for nucleic acid extraction by Roche High Pure Viral Nucleic acid kit (Roche diagnostic GmbH, Germany) following the manufacturer protocol. All nucleic acid extractionwas carried out manually and RNA stored at -70^{0}C until used. The RT-LAMP primer were designed by PREMIER Biosoft LAMP designer 1.14 based on 3' UTR nucleotide sequence of each dengue virus genotype (GeneBank accession number; DENV1- NC_001477, DENV2- NC_001474, DENV3- NC_001475, DEN4- NC_002640) according to described criteria of Notomi et. al. **Table 1**. The RT-LAMP assay were performed in total volume 25 µl of each reaction mixture using Loopamp DNA amplification kit, 25 pmol of each inner primer (FIP, BIP) and loop primers; 3.0 pmol of outer primers; 1.5 µl of fluorescent dye (Eiken chemical Co. ltd. Japan). The reaction tube was incubated at standardized constant temperature (64^{0}C) for 60 minutes in heating block. The positive reaction tube colour turns to green and fluorescent under the UV light as well as negative remains orange and non-fluorescent (**Figure1**). The positive and negative control were put simultaneously with each set of amplification.

Specificity and sensitivity of RT-LAMP assay

Each primer set of DENV RT-LAMP were assessed withChikungunya and Japenese encephalitis virus, which are also endemic at the time of study and sensitivity were evaluated by serial dilution of viral RNA. All NS1 Ag and RT-LAMP positive samples were confirmed by RT-PCR kit Fast track diagnostics, Luxembourg.

Table 1: Dengue primer sets for RT-LAMP assay; Forward outer primer(F3), Backward outer primer (B3), Forward inner primer (FIP), Backward inner primer (BIP), Forward Loop primer (LoopF) and Backward Loop primer (LoopB).

Genotype	Genome Position 3' (UTR)	Primer	Sequence (5'-3')
DENV-1	10274 – 10735(462bp)	F3	AACAAGGCAAGAAGTCAGG
		B3	CCTCTAACCACTAGTCTGCTA
		FIP(F1c+F2)	GGCTTTCGGCCTGACTTCAAGCCATAGCACGGTAAGA
		BIP(B1c+B2)	AAGCCGTGCTGCCTGTAGATGCGTACAGCTTCCATG
		LoopF	GGCTCACAGGCAGCATAG
		LoopB	CTCCATCGTGGGGATGTAAA
DENV-2	10273 – 10723(451bp)	F3	AAACTATGCAGCCTGTAGC
		B3	TTCTGTGCCTGGAATGATG
		FIP(F1c+F2)	TGCTGCGATTTGTAAGGGAGGCATGGAAGCTGTACGCAT
		BIP(B1c+B2)	GATGAAGCTGTAGTCTCGCTGGTTCCCAGCGTCAATATGC
		LoopF	TCTCCTCTAACCGCTAGTCC
		LoopB	AGGACTAGAGGTTAGAGGAGAC
DENV-3	10268 – 10707 (439bp)	F3	CGTTAAAAGAAGAAGTCAGGC
		B3	CAGCAGGATCTCTGGTCT
		FIP(F1c+F2)	GGTCTCCTCTAACCGCTAGTCTGTGGGGACGTAAAACCTG
		BIP(B1c+B2)	CATGACACAACGCAGCAGCGTCTCCTCTAACCTCTAGTCC
		LoopF	TGCGTACAGCTTCCACAG
		LoopB	GGAAGCTGTACCTCCTTGC
DENV-4	10266 – 10649(383bp)	F3	GCGTGGCATATTGGACTAG
		B3	CAACAACACCAATCCATCTTG
		FIP(F1c+F2)	ACCTCTAGTCCTTCCACCAGGCATCACTGATAAAACGCAGC
		BIP(B1c+B2)	AACAGCATATTGACGCTGGGAACTGTGCCTGGATTGATGTT
		LoopF	GTACAGCTTCCTCCTGGC
		LoopB	GAGATCCTGCTGTCTCTGC

RESULT:

A total of 109(32%)dengue infection positive case,87 (79%) NS1 antigen,30 (27%) IgM antibody and 12 (11%) IgG antibodywere found. 11% and 9% samples were only positive for IgM and IgG respectively, 16% were positive for both NS1 Ag and IgM Ab.Both NS1 Ag and IgM positive sample (n=99) were consider as confirmed dengue infection in human serum. Out of 109 samples 91 (83%) were positive for RT-LAMP assay and 83 (76%) were common positive for NS1 Ag and RT-LAMP assay. The sensitivity,specificity, positive predictive value (PPV) and negative predictive value (NPV) of NS1 antigen were 95%, 99.59%, 98% and 98%

with RT-LAMP. All RT-LAMP samples were positive for RT-PCR. Out of 91 RT-LAMP positive; 71% were positive for DENV-2 and 18% positive for DENV-4, which was greater than DENV-1 (9%) and 2(2%).

Table2: No. of dengue serum sample tested positive by different method

Assay		Positive	Negative	Total
ELISA	NS1 Ag	87	256	343
	IgM Ab	30	313	343
	IgG Ab	12	313	343
RT-LAMP		91	252	343
RT-PCR		91	252	343

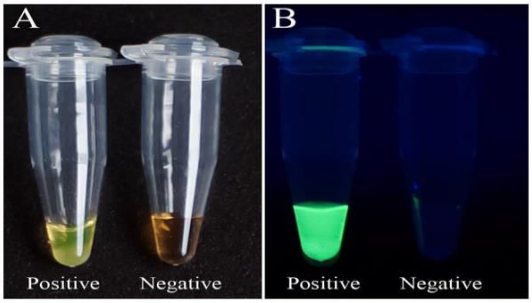

Figure1: Naked eye visualization of RT-LAMP assay result; Positive and negative result in white light (A), UV lamp (B)

DISCUSSION:

In current scenario accuratediagnostic methods play a very imperative role in disease management. Dengue diagnosis in febrile illness has been a challenge for health care service providers. Capture IgM and IgG are most commonly available serological techniques for diagnosis of primary and secondary denguevirus infection buthigh cross reactivity with other flavivirus specific antibodies made difficult definite diagnosis of infection. Dengue NS1 antigen ELISA assay emerge as easy, sensitive and specific serological test for dengue detection over the antibody testingbut somewhere it also compromises the authenticity of testing[12]. Genome based detection i.e. Real Time PCR technique shows improved clinical sensitivity and promised result in case of viral infection[13].Loop mediated isothermal amplification can be a simple, rapid, sensitive and specific alternate method of RT-PCR. In this study, RT-LAMP assay was developed by a set of different primers, targeted 3' untaranslated region (3'UTR) of globally reported four dengue virus strains. No cross reactivity was found with other closely related species. RT-LAMP assay shows higher sensitivity (100%) and specificity (100%) than others serological assays. The RT-LAMP assay can be used in combination with other serological assays in resource limited areas for immediate arbitration and prevention of further spread of dengue.

CONCLUSION:

On the basis of obtained results in this study, RT-LAMP assay can be a potential alternate molecular diagnostic method for routine screening of dengue infection in fibril stage. It can be utilized as an early, rapid, cost effective and highly sensitive tool for dengue diagnosis in resource limited remote areas of country.

REFERENCES:

1. World Health Organization. 2009. Dengue: guidelines for diagnosis,treatment, prevention and control. WHO, Geneva, Switzerland.

2. Monath TP: Dengue: the risk to developed and developing countries. Proc Natl AcadSci USA 1994, 91:2395-2400.

3. Chaturvedi UC, Agarwal R, Elbishbishi EA, Mustafa AS. Cytokine cascade in dengue haemorrhagic fever: implications for pathogenesis. FEMS Immunol Med Microbiol. 2000;28:183e188.

4. 4. Guzman, M. G., and G. Kouri. 2004. Dengue diagnosis, advances and challenges. Int. J. Infect. Dis. 8:69–80.

5. Innis, B. L., A. Nisalak, S. Nimmannitya, S. Kusalerdchariya, V. Chongswasdi, S. Suntayakorn, P. Puttisiri, and C. H. Hoke. 1989. An enzyme-linked immunoassay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. Am. J. Trop. Med. Hyg. 40:418–427.

6. Dussart P, Petit L, Labeau B, Bremand L, Leduc A, et al.: Evaluation of Two New Commercial Tests for the Diagnosis of Acute Dengue Virusn Infection Using NS1 Antigen Detection in Human Serum. PLoSNegl Trop Dis 2008, 2:e280.

7. Lapphra K, Sangcharaswichai A, Chokephaibulkit K, Tiengrim S, Piriyakarnsakul W, et al.: Evaluation of an NS1 antigen detection for diagnosis of acute dengue infection in patients with acute febrile illness. DiagnMicrobiol Infect Dis 2008, 60:387-391.

8. Houng, H. H., D. Hritz, and N. Kanesa-thasan. 2000. Quantitative detection of dengue 2 virus using fluorogenic RT-PCR based on 3-noncoding sequence. J. Virol. Methods 86:1–11.

9. Norma P. Tavakoli, Ellis H. Tobin, Susan J. Wong, Alan P. Dupuis II, Bernadette Glasheen, Laura D. Kramer, and Kristen A. Bernard,Identification of Dengue Virus in Respiratory Specimens from aPatient Who Had Recently Traveled from a Region WhereDengue Virus Infection Is Endemic; JOURNAL OF CLINICAL MICROBIOLOGY, May 2007, p. 1523–1527.

10. Callahan, J. D., S. J. Wu, A. Dion-Schultz, B. E. Mangold, L. F. Peruski, D. M. Watts, K. R. Porter, G. R. Murpgy, W. Suharyono, C. C. King, C. G. Hayes, and J. J. Temenak. 2001. Development and evaluation of serotypeand group-specific fluorogenic reverse transcriptase PCR (TaqMan) assays for dengue virus. J. Clin. Microbiol. 39:4119–4124.

11. Drosten, C., S. Gottig, S. Schilling, M. Asper, M. Panning, H. Schmitz, and S. Gunther. 2002. Rapid detection and quantitation of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift valley fever virus, dengue virus, and Yellow fever virus by real-time reverse transcription-PCR. J. Clin. Microbiol. 40:2323–2330.

12. Pei-Yun Shu, Li-Kuang Chen Shu-Fen Chang, Yi-Yun Yueh, Ling Chow, Li-Jung Chien, Chuan Chin, Ting-Hsiang Lin, and Jyh-Hsiung Huang; Comparison of Capture Immunoglobulin M (IgM) and IgG Enzyme-Linked Immunosorbent Assay (ELISA) and Nonstructural Protein NS1 Serotype-Specific IgG ELISA for Differentiation of Primary and Secondary Dengue Virus Infections;CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, July 2003, p. 622–630.

13. Jesse J. Waggoner,a Janaki Abeynayake,b Malaya K. Sahoo,b Lionel Gresh,c Yolanda Tellez,d Karla Gonzalez,d Gabriela Ballesteros,d Frances P. Guo,b Angel Balmaseda,dKumuduKarunaratne,e Eva Harris,f Benjamin A. Pinskya,b; Comparison of the FDA-Approved CDC DENV-1-4 Real-Time Reverse Transcription-PCR with a Laboratory-Developed Assay for Dengue Virus Detection and Serotyping; Journal of Clinical Microbiology p. 3418–3420.

14. Guzman MG, Kouri G: Dengue: an update. Lancet Infect Dis 2002, 2:33-42.

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